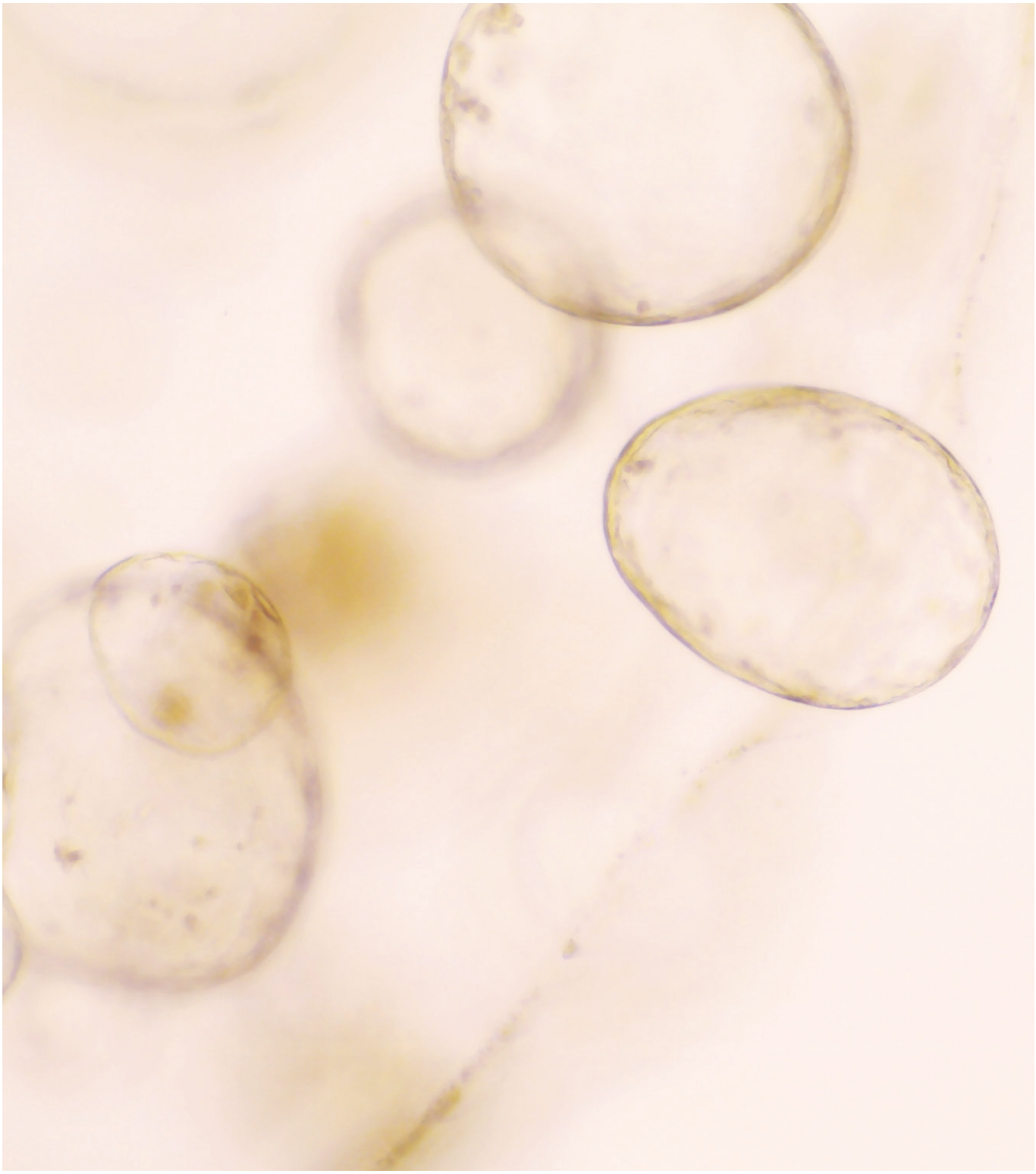
CANCER RESEARCH UK | Manchester Institute

SCIENTIFIC REPORT 2023



cruk.org

COVER IMAGE

PEG hydrogel of 18 kPa stiffness seeded with PDAC organoid fragments then later embedded into a gel of 8 kPa stiffness. Organoids are seen 5 days after initial seeding and 2 days after embedding into a new stiffness, attempting to cross the threshold between the two stiffnesses in different z-positions.

Image supplied by Carmen Rodriguez-Cupello (Systems Oncology)

SCIENTIFIC REPORT 2023

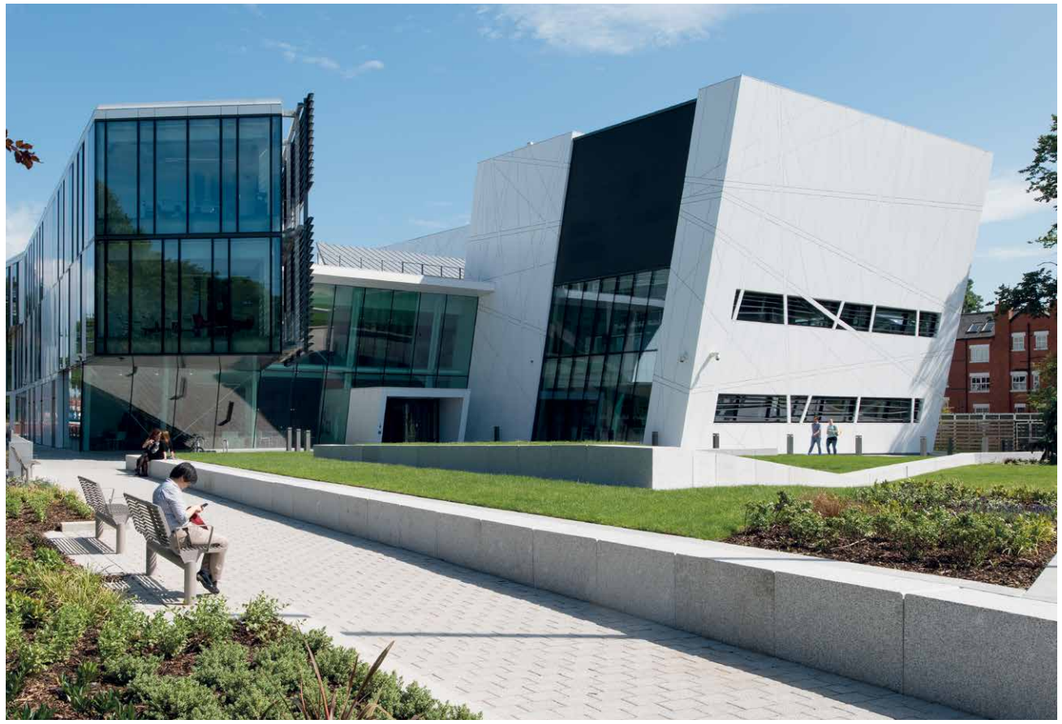
MANCHESTER INSTITUTE

MANCHESTER 1824
The University of Manchester

CANCER RESEARCH UK | Manchester Institute

CONTENTS

The Cancer Research UK Manchester Institute moved to the new Paterson Building on our original site in Withington in June 2023. Some research groups and staff are based in the Oglesby Cancer Research Building.



The Oglesby Cancer Research Building.

DIRECTOR'S INTRODUCTION

Professor Caroline Dive

Director of the Cancer Research UK Manchester Institute

Welcome to the 2023 scientific report of the Cancer Research UK Manchester Institute. This has been a significant year for our Institute as we made the long-anticipated return to the Paterson Building, a brand-new research facility on the Christie NHS Foundation Trust site.

It has been more than six years since the devastating fire that resulted in the Institute having to relocate to Alderley Park in Cheshire. While our hosts at our temporary home were so welcoming and being at Alderley Park allowed us to continue our research, it is truly wonderful to be back and reunited with our colleagues at the Christie and in the Oglesby Cancer Research Building on the Withington campus. The modern ten-storey bespoke building is a fantastic place to work. Everyone is integrating well and capitalising on our reconnections with the wider scientific community within the Manchester Cancer Research Centre.

I would like to recognise the remarkable resilience, patience and enormous efforts of so many individuals across the Institute, together with the determination and hard work of the Paterson Redevelopment Project partners – The Christie, Cancer Research UK, and The University of Manchester – and of course the support of the many generous donors to the new build project.

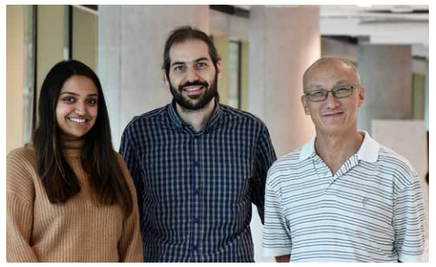
Despite the challenges of the fire, the relocation to Alderley Park, the adjustments swiftly followed by pandemic and then in 2023 the move back to Withington, our staff continue to excel in all aspects of Institute life. I would like to thank everyone who helped facilitate our return to the 'Withington Cancer Campus'. It has certainly been a long journey, but we are now reaping the significant benefits of colocation with researchers, clinicians, allied healthcare professionals and operational staff. In this report we share highlights of the new building and celebrate our return as we deliver our cancer research ambitions in Manchester.

As always, it has been a productive year for our staff, and it is my immense pleasure to spotlight their accomplishments here. Notably, Claus Jørgensen and his Systems Oncology group underwent a successful quinquennial review of their research programme. Claus is a great asset to the Institute, and it is rewarding to see that the calibre of their past research and future plans has been recognised with this impressive result.

This year Iain Hagan stepped down as Interim Deputy Director of the CRUK Manchester Institute. Iain provided tremendous support to me and to the Institute over the past three years and I would like to thank him for all his hard and effective work and generosity with his time during the demanding and challenging five years after the fire, and more recently for his substantial role in organising our return to the Paterson Building. I was also delighted that Claus agreed to be the Deputy Director and as we re-establish ourselves in our new home, I am enjoying working with Claus during this propitious phase and value his strategic insights on important institutional decisions.

I am delighted to welcome Evangelos Giampazolias, who joined us at the start of this year from the Francis Crick Institute as a Junior Group Leader. Evangelos is establishing his new Cancer Immunosurveillance group here, exploring how the immune system recognises and responds to cancer through the integration of cues released by dying cells and commensal microbes. He is already making his mark and I would also like to congratulate Evangelos on securing his first grant from the Royal Society within eight months of his arrival. We look forward to seeing his group flourish in the coming years.

External funding augments the breadth of our research and helps support the development of our researchers. I would like to congratulate Institute Fellow Amaya Virós, who was awarded a prestigious CRUK Advanced Clinical Scientist Fellowship this year. With this fantastic achievement, Amaya and her group can continue their critical research exploring the role lipids play in melanoma metastasis, tropism, and immunotherapy response at different sites in the body. Georges Lacaud also had success, along with Sam Butterworth at The University of Manchester, in securing funds from the charity



The new Cancer Immunosurveillance group: (L-R) Swara, Evangelos and Pengbo.

Claus Jørgensen and his research group Systems Oncology

Blood Cancer UK for his novel work developing KAT6A PROTACS for better and less toxic treatments for AML. This is great news for Georges and his group, and we anticipate exciting results from this project over the next three years.

We continue to publish an impressive collection of scientific discoveries, some of which are presented in our research highlights section. Among those featured is research published in *Cancer Cell* from Tim Somervaille's Leukaemia Biology lab, where Nicosia et al. offer new promise in targeting EP300/CBP – histone acetyltransferases recruited onto chromatin by oncogenic transcription factors control the transcriptional programme via their activity in enhancer areas. This study reports on the positive impact of the small-molecule inhibitor CSS1477 (inobrodib, developed by Cell Centric) in patients with blood tumours and no other therapeutic options. Joint author Luciano Nicosia received the Institute's Award for the Best Young Scientist of 2023, The Edith Paterson Prize, for his significant contribution to understanding the mechanism of inobrodib.

Georges Lacaud and his team worked with former Group Leader Esther Baena on a new group of proteins that could be used to improve survival in patients with prostate cancer (*Cell Reports* 2023, 42(4), 112377). Notably they demonstrated that conditional deletion of PTEN in the murine prostate epithelium caused an expansion of transformed LY6D+ progenitor cells without impairing stem cell properties.

New research from Iain Hagan and his Cell Division lab has looked at how we could repurpose an existing drug to fight cancer. They show how metformin – a drug commonly used to treat the disease diabetes – can dramatically slow the growth of cancer cells with hyperactivated AMPK. Published in *Open Biology*, this study unveils new options for clinical trials in cancer therapy.

As an Institute, we know from experience how connected communities are critical for the advancement of science. Taking part in conferences enables researchers to share findings, exchange ideas, and to build networks for collaboration and career development. This is especially important for early career researchers at the vanguard of cancer research, so I am pleased that many of our students attended the International PhD Student Science Conference this year. The conference was a great success and saw two of our PhD students win first and second place prizes for their posters. Bradley Revell, from the Leukaemia Biology group, was recognised for his work on the molecular mechanisms of transcription factors IRX3 and FOXC1, and Parsa Pirhady from the Translational Oncogenomics group was selected for his poster on the DNA damage repair pathway in prostate cancer. I would also like to congratulate Federica Spaggiari from the CRUK Cancer Biomarker Centre who won a poster prize at the North West Cancer Research Scientific Symposium.

We had further successes from our early career researchers who presented their research at the Division of Cancer Sciences Postgraduate Research Showcase. Two PhD students from the Cancer Inflammation and Immunity group won prizes at the event - Maria Koufaki won first prize for her poster and Charles Earnshaw won an award for his oral presentation.

Our early career researchers continue to enjoy connecting with members of the local community. It was great to see such enthusiasm and dedication behind the creation of a well-designed and inspiring outreach experience, 'The Biomarker Lab' that they took to several schools in the area during British Science Week. Helping to inspire the next generation of cancer researchers is an important engagement activity and I would like to thank all who took part.

Lastly, as we become more settled in our new home, I am looking forward to further strengthening our existing partnerships, forging new collaborations and maximising the incredible opportunities we have on site as we drive forwards in our new facility, integrating our basic discovery, translational and clinical research towards improved patient outcomes.

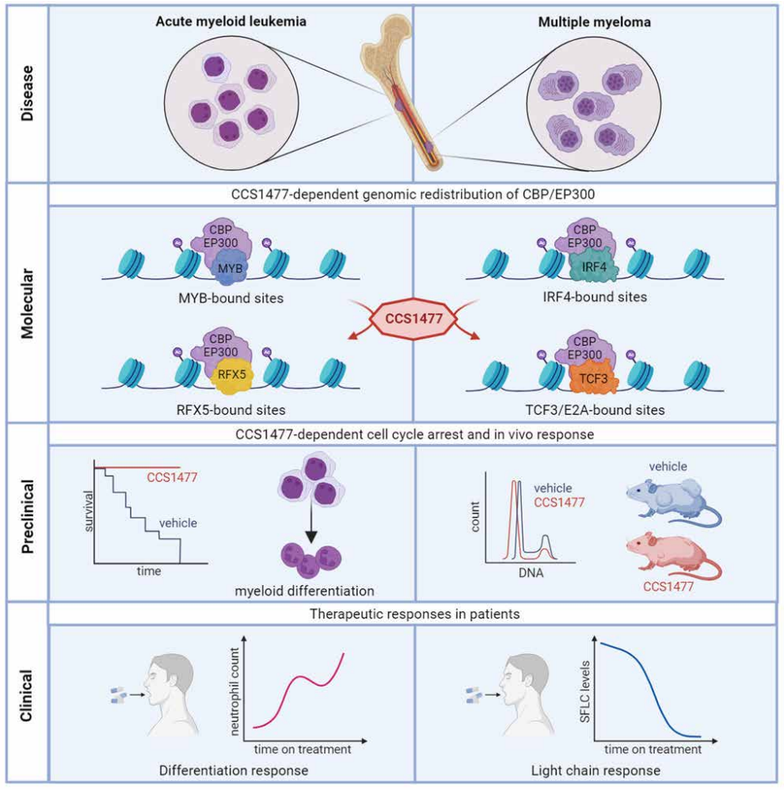
Professor Caroline Dive, CBE., FMedSci.
Interim Director, Cancer Research UK Manchester Institute

RESEARCH HIGHLIGHTS

In this section we highlight some research publications from 2023 which report significant advances in specific areas. The selected papers demonstrate the breadth and the quality of the research being undertaken by the groups at the Cancer Research UK Manchester Institute.

Nicosia L, Spencer GJ, Brooks N, Amaral FMR, Basma NJ, Chadwick JA, Revell B, Wingelhofer B, Maiques-Diaz A, Sinclair O, Camera F, Ciceri F, Wiseman DH, Pegg N, West W, Knurowski T, Frese K, Clegg K, Campbell VL, Cavet J, Copland M, Searle E, Somervaille TCP. (2023)
Therapeutic targeting of EP300/CBP by bromodomain inhibition in hematologic malignancies.
Cancer Cell 41(12):2136-2153.

Graphical abstract shows that CCS1477 (inobrodib), an EP300/CBP bromodomain inhibitor, induces cell-cycle arrest and differentiation in haematologic malignancy models through disrupting EP300/CBP recruitment to enhancer networks occupied by critical transcription factors. In patients with relapsed/refractory disease, CCS1477 monotherapy induces differentiation responses in AML and objective responses in myeloma.



While treatment options in blood cancers like acute myeloid leukaemia (AML) and multiple myeloma (MM) continue to develop, there remains an unmet need for better, less toxic therapies. EP300 and CREBBP are paralogous genes coding for multi-domain acetyltransferases targeting diverse cellular proteins. They have long been considered strong targets for therapeutic intervention in blood cancer. Development of clinical-grade acetyltransferase inhibitors has been challenging but targeting the EP300/CBP bromodomains has been more fruitful. CCS1477 (also known as inobrodib) is a highly selective EP300/CBP bromodomain inhibitor, initially evaluated in castration-resistant prostate cancer. In collaboration with colleagues at the biotech company CellCentric, the Leukaemia Biology group report here their pre-clinical and initial early phase clinical data. They found that in AML cells, CCS1477 promotes rapid eviction of EP300/CBP from an enhancer subset marked by strong MYB occupancy and high H3K27 acetylation, with downregulation of the subordinate oncogenic network and redistribution to sites close to differentiation genes. In myeloma cells, CCS1477 induced eviction of EP300/CBP from FGFR3, the target of the common (4; 14) translocation, with redistribution away from IRF4-occupied sites to TCF3/E2A-occupied sites. In the team's early phase clinical trial, in a subset of patients with relapsed or refractory disease, CCS1477 monotherapy induced differentiation responses in AML and objective responses in heavily pre-treated multiple myeloma. In vivo preclinical combination studies further revealed synergistic responses to treatment with standard-of-care agents, which are currently being evaluated in the ongoing early phase trial. In summary, CCS1477 exhibits encouraging preclinical and early-phase clinical activity by disrupting recruitment of EP300/CBP to enhancer networks occupied by critical lineage-specific transcription factors.

Camera F, Romero-Camarero I, Revell BH, Amaral FMR, Sinclair OJ, Simeoni F, Wiseman DH, Stojic L, Somervaille TCP. (2023)
Differentiation block in acute myeloid leukemia regulated by intronic sequences of *FTO*.
iScience 26(8):107319.

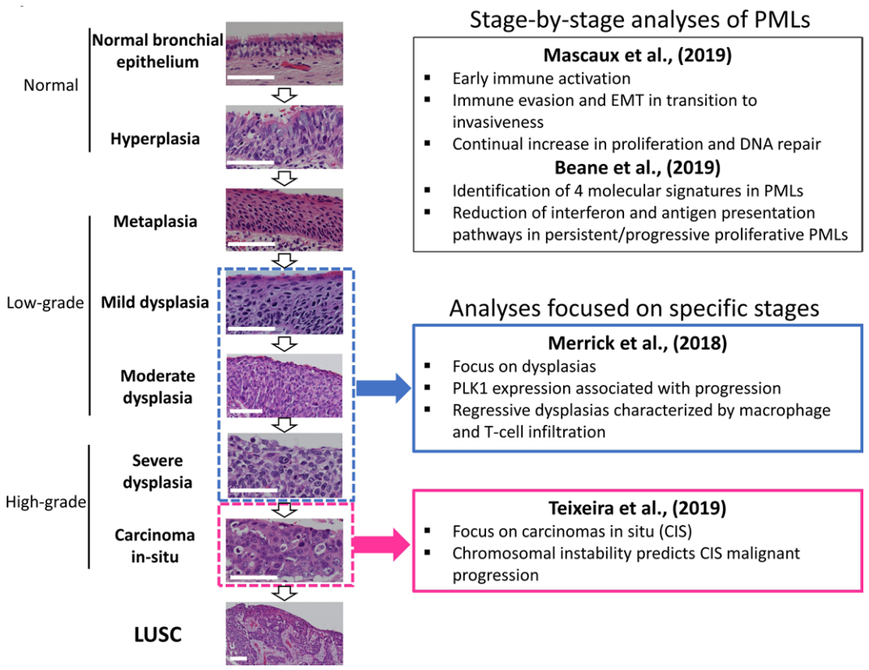
While in normal haematopoiesis the transcription factor gene IRX3 (for Iroquois Homeobox 3) is minimally expressed, by contrast in acute myeloid leukaemia (AML) it is highly expressed in 20–30% of patients. In the Leukaemia Biology group's prior functional work (Somerville et al., 2018, Cell Reports), they have demonstrated that mis-expressed IRX3 contributes to the myeloid differentiation block, which is pathognomonic for the disease. It has not been clear, however, how IRX3 expression is upregulated in AML. They discovered that sequences in intron 8 of a neighbouring gene called fat mass and obesity associated (FTO) located ~220kB downstream of IRX3 exhibit histone acetylation, DNA methylation and physical contacts with the IRX3 promoter. The extent of each of these correlates with IRX3 expression in AML cells. Deletion of these FTO intron 8 enhancer elements confirmed their role in positively regulating IRX3 expression. Furthermore, RNA sequencing revealed the presence of long non-coding (lnc) transcripts arising from this locus in AML cell lines and primary samples from patients with NPM1-mutated AML. Knockdown of these FTO-lncAML transcripts induced differentiation of AML cells, loss of clonogenic activity and reduced FTO intron 8:IRX3 promoter contacts. While both FTO-lncAML knockdown and IRX3 knockdown induced differentiation, FTO-lncAML knockdown but not IRX3 knockdown led to HOXA downregulation, suggesting long non-coding transcript activity in trans regulated HOX gene expression. In keeping with this, FTO-lncAML^{high} AML patient samples expressed higher levels of HOXA and lower levels of differentiation genes. Thus, a regulatory module in FTO intron 8 consisting of clustered enhancer elements and a long non-coding RNA is active in human AML, impeding myeloid differentiation.

Roberts M, Ogden J, Hossain ASM, Chaturvedi A, Kerr ARW, Dive C, Beane JE, Lopez-Garcia C. (2023)
Interrogating the precancerous evolution of pathway dysfunction in lung squamous cell carcinoma using XTABLE.
Elife 12:e77507.

The transformation of a normal tissue into a tumour is a multistep process that comprises intermediate phases called premalignant or preinvasive lesions. In lung squamous cell carcinoma (LUSC), up to six premalignant stages have been identified. Understanding the biology of these lesions in LUSC is necessary to prevent cancer, to detect them before they become cancer, and to distinguish those that are going to progress to cancer from those that aren't.

Being able to interrogate how the expression of genes change during the transition between premalignant stages is crucial to acquire this understanding. However, not every laboratory has the resources to interrogate publicly available data from LUSC premalignant lesions.

RESEARCH HIGHLIGHTS (CONTINUED)



Histological images depicting the developmental stages of lung squamous cell carcinoma (LUSC) and a summary of the four studies included in XTABLE. Roberts, Matthew et al. "Interrogating the precancerous evolution of pathway dysfunction in lung squamous cell carcinoma using XTABLE." *eLife* vol. 12 e77507. 9 Mar. 2023, doi:10.7554/eLife.77507. https://doi.org/10.7554/eLife.77507. Shared under Creative Commons license.

Publicly available datasets contain information on tens of thousands of genes but only information on a handful of genes would be presented in the original article. This limitation therefore, restricts the reanalysis of the data and the generation and/or validation of new hypotheses.

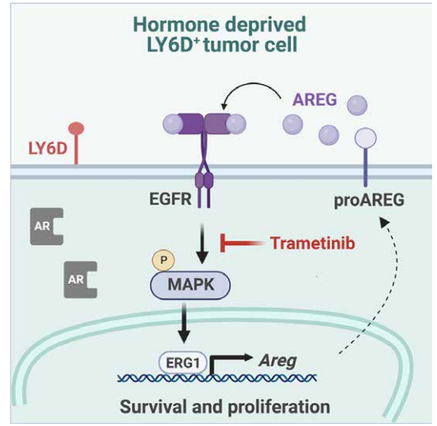
By building XTABLE, the Translational Lung Cancer Biology group aimed to facilitate access to and the analysis of all the available gene expression studies on preinvasive LUSC samples from patients in a versatile and adaptable manner. This new capability will shed light on the processes that make premalignant LUSC lesions become cancer and help identify proteins uniquely present in each type of premalignant lesion that can aid the development of biomarkers for diagnosis.

XTABLE contains multiple functions and tools that enable the classification of samples in groups using multiple characteristics and can identify which genes are different between the groups, analyse the expression of single genes or groups of genes involved in the same biological processes. This comprehensive collection of bioinformatic tools enabled the group to identify how important processes such as SOX2 target regulation, PI3k/Akt pathway, Nrf2 pathway and CDK2/4 activity, known to be important for LUSC, evolve in premalignant lesions.

Steiner I, Flores-Tellez TDNJ, Mevel R, Ali A, Wang P, Schofield P, Behan C, Forsythe N, Ashton G, Taylor C, Mills IG, Oliveira P, McDade SS, Zaiss DM, Choudhury A, Lacaud G, Baena E. (2023)
Autocrine activation of MAPK signaling mediates intrinsic tolerance to androgen deprivation in LY6D prostate cancer cells.
Cell Reports 42(4):112377.

Prostate cancer is the second most common cancer type in men and affects over one million people globally each year. When caught early enough, most patients survive for at least 10 years. Treatment usually consists of surgery followed by radiotherapy. However, if this approach does not cure the cancer, some men receive a hormone therapy known as androgen deprivation therapy (ADT). This dramatically reduces the level of male hormones such as testosterone, which can help slow tumour progression. Sadly, some patients stop responding to this hormone treatment and become castration resistant (CR). CR-PCa is usually incurable with a high mortality rate. Around half of patients with CR-PCa have alterations in a gene called PTEN, which is important for stopping cellular growth. This mutation turns off PTEN, allowing tumour cells to continue growing.

Researchers – from the former Prostate Oncobiology group, along with Stem Cell Biology – found that a protein on the surface of cells, called LY6D, is increased in prostate tumour



Graphical abstract showing conditional deletion of PTEN in mouse prostate epithelium caused an expansion of transformed LY6D+ progenitor cells without impairing stem cell properties. *© 2023 The Authors. Reprinted under the terms of the Creative Commons CC-BY license.*

cells. They found that cells with increased LY6D produce more tumours in castration resistant mice. The team discovered that a network of interacting proteins (known as the EGFR-MAPK-ERK pathway) was increased. This pathway governs cell growth and survival and is likely responsible for the increased growth in LY6D-high tumours. The researchers therefore treated LY6D-high cells with drugs – such as trametinib and erlotinib – that are known to inhibit this pathway. Notably, this treatment decreased the growth of the LY6D-high cells.

Overall, this important new data shows the potential for treating prostate cancer patients that have a PTEN mutation with these drugs, alongside hormone therapy. There is hope for this combination therapy to reduce the occurrence of castration resistance and improve future outcome for these patients.

Pearsall SM, Williamson SC, Humphrey S, Hughes E, Morgan D, García Marqués FJ, Awanis G, Carroll R, Burks L, Shue YT, Bermudez A, Frese KK, Galvin M, Carter M, Priest L, Kerr A, Zhou C, Oliver TG, Humphries JD, Humphries MJ, Blackhall F, Cannell IG, Pitteri SJ, Hannon GJ, Sage J, Dive C, Simpson KL. (2023)
Lineage Plasticity in SCLC Generates Non-Neuroendocrine Cells Primed for Vasculogenic Mimicry.
J Thorac Oncol. 18(10):1362-1385.

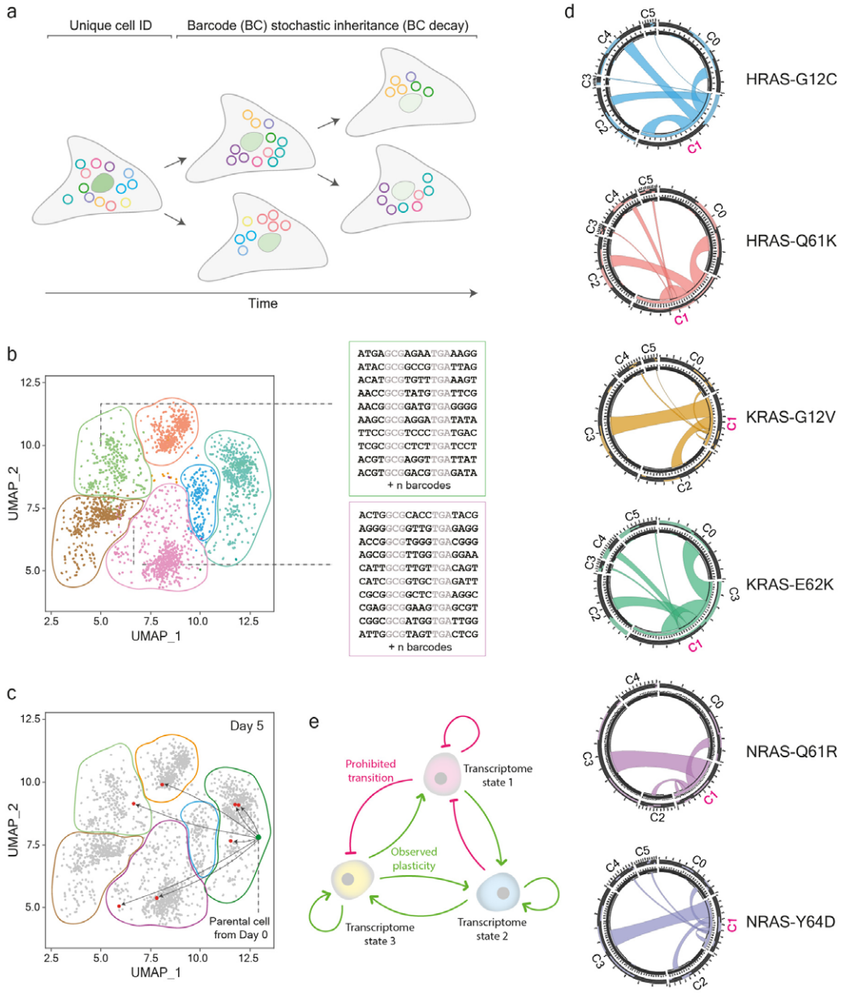
Small cell lung cancer is a highly vascularised, aggressive neuroendocrine (NE) cancer associated with high mortality. SCLC treatment has remained unchanged in decades, with the recent introduction of immunotherapy benefiting a minority of unselected patients. There is increasing understanding of SCLC heterogeneity, including SCLC molecular subtypes based on expression of NE and non-NE transcription factors (TFs). Additionally, SCLC plasticity *via* NE to non-NE phenotype transition is linked with metastasis, chemoresistance, and immune evasion. Whilst personalised medicine approaches attempt to exploit molecular subtype differences, an incomplete understanding of the functional significance of NE to non-NE plasticity creates additional complexity. Using SCLC circulating tumour cell (CTC)-derived tumour explant (CDX) models, the Cancer Biomarker Centre and colleagues report the first functional evidence of the role of NOTCH-driven NE to non-NE plasticity in SCLC, facilitating vasculogenic mimicry (VM). VM is an angiogenesis-independent epithelial-to-endothelial transition of tumour cells to acquire endothelial properties and form *de novo* vessels. Immunohistochemistry showed co-localisation of VM vessels with the non-NE marker REST *in vivo* whilst *ex vivo* cultures of isolated non-NE and NE cell populations showed only non-NE cells could form hollow tubules on Matrigel (the gold-standard surrogate assay for VM competency). Moreover, intravenous injection of lectin to CDX-bearing mice revealed that VM vessels are functionally perfused *in vivo*.

RNAseq of NE and non-NE cell populations showed that non-NE cells are transcriptionally primed to undergo tubule formation and are enriched for hypoxic, vascular endothelial and ECM remodelling gene signatures, hinting at hypoxia being a putative VM driver. *Ex vivo* studies with different growth substrates, dye-quenched collagen and integrin blocking antibodies revealed that tubule formation by non-NE cells is regulated by β1-integrin-mediated collagen remodelling. Overall, these data highlight the need to target both NE and non-NE cells to effectively inhibit VM and have prompted further studies to understand its role in supporting tumour growth and metastasis.

Shlyakhtina Y, Bloechl B, Portal MM. (2023)
BdLT-Seq as a barcode decay-based method to unravel lineage-linked transcriptome plasticity.
Nature Commun 14, 1085.

The field of genetics revolutionised how scientists approach biological queries and, though its relevance cannot be contested, an overwhelming number of observations suggest that the non-genetic compartment is of equal significance to define life as we know it. Along those lines, despite being rather clear that non-genetically encoded cell plasticity plays a fundamental role in every aspect of biology, the lack of adequate technologies to query this phenomenon in a reliable and quantitative manner hindered the study of its underlying molecular foundations.

To alleviate this vacuum in knowledge, the former Cell Plasticity & Epigenetics group developed a technological charter designed to study the molecular underpinnings of cell plasticity and discovered that transcriptome heterogeneity is not randomly established upon cell division but follows predetermined inheritance and plasticity patterns, which are perpetuated for several generations suggesting the existence of a non-genetically encoded molecular memory. Importantly, within the diverse scenarios in which transcriptome heterogeneity is thought to play a central role,



Development of Barcode decay Lineage Tracing (BdLT-Seq) to explore plasticity. Blue, pink, green, orange, purple and yellow circles represent episomes which are enclosed inside a cancer cell and give it a 'unique ID'. The cancer cell divides over time, giving rise to new cancer cells that inherit some episomes from the parent cell. This property of inheritance can be used to identify individual cells and trace them back to their roots. This forms the basis of BdLT-Seq. Source: Shlyakhtina, Bloechl & Portal, (2023).

the study of its involvement in cancer onset, evolution and the response to anticancer therapies has recently gained substantial momentum. Following this line of thought, the team demonstrated that lineage-linked restricted plasticity dictates the phenotypic outcome of each cell in response to intracellular and extracellular cues such as oncogene activation (cell death vs senescence vs transformation) and therapeutic challenges (death vs resistance).

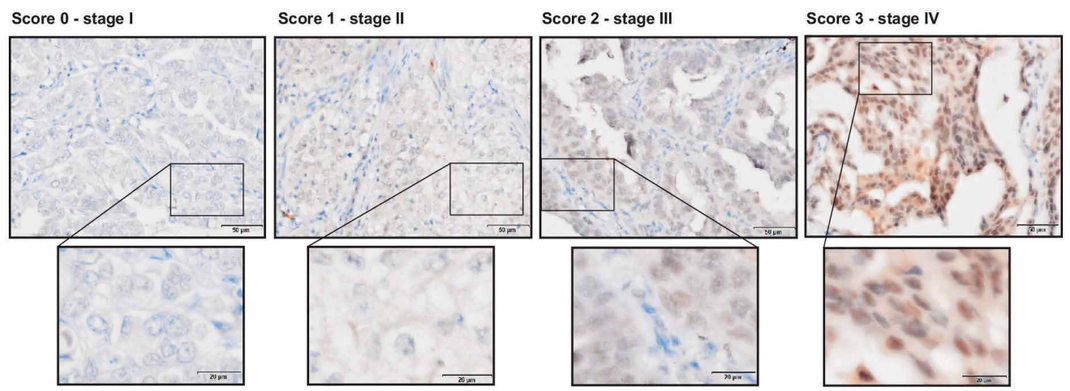
Their data supports a model where the genetic and non-genetic compartments are interlinked by yet unknown molecular machineries that drive phenotypic output. Therefore, they postulate that integrating these two crucial biological concepts – genetics and non-genetics – and deciphering their interplay will drive forward our understanding of non-genetically supported cell plasticity in cancer evolution, which in time may lead to the conceptualisation of more tailored and effective therapies.

Hornigold K, Baker MJ, Machin PA, Chetwynd SA, Johnsson AK, Pantarelli C, Islam P, Stammers M, Crossland L, Oxley D, Okkenhaug H, Walker S, Walker R, Segonds-Pichon A, Fukui Y, Malliri A, Welch HCE. (2023)
The Rac-GEF Tiam1 controls integrin-dependent neutrophil responses.
Front Immunol. 14:1223653.

Neutrophils are essential white blood cells that function as one of the body's first lines of defence against pathogens. For neutrophils to function properly, they need to recognise a stimulus released by a pathogen or distressed cell and adhere to a blood vessel wall before actively migrating towards the source of that stimulus. Once at the site of infection or damage, neutrophils will release reactive oxygen species and engulf pathogens to destroy them. A greater understanding of how neutrophils perform this function is required to better understand diseases involving inflammation. The small GTPases RAC1 and RAC2 are essential for these functions of neutrophils. The Cell Signalling group has previously focused their work on the exchange factor, TIAM1, which functions to activate RAC. In this study, they collaborated with Dr Heidi Welch at The Babraham Institute by providing TIAM1 knockout mice so that the role of TIAM1 in neutrophils could be investigated. Work in Dr Welch's lab showed that mice lacking TIAM1 had a reduced ability to clear bacteria from the lungs and reduced recruitment of neutrophils to the peritoneum in a thioglycolate-induced sterile peritonitis system, demonstrating that TIAM1 plays an important role in neutrophil function. Upon further investigation, it was found that TIAM1 is required for efficient release of cytotoxic antimicrobial compounds from neutrophils, as well as the production of extracellular traps (release of DNA that binds and captures pathogens). As a result, TIAM1-deficient neutrophils displayed impaired bacterial killing. This study concluded that TIAM1-deficient neutrophils may have altered spatiotemporal distribution of activated RAC1, leading to ineffective neutrophil migration and antimicrobial action. Therefore, TIAM1 has an important role in the complex regulation of migration and bacterial killing by neutrophils.

Ginn L, Maltas J, Baker MJ, Chaturvedi A, Wilson L, Guilbert R, Amaral FMR, Priest L, Mole H, Blackhall F, Diamantopoulou Z, Somervaille TCP, Hurlstone A, Malliri A. (2023)
A TIAM1-TRIM28 complex mediates epigenetic silencing of protocadherins to promote migration of lung cancer cells.
Proc Natl Acad Sci U S A 120(40):e2300489120.

Abundance of nuclear TIAM1 correlates with NSCLC progression. Representative examples of strong (score 3), moderate (score 2), weak (score 1), and negative (score 0) immunohistochemical staining of TIAM1 expression in a panel of LUAD tumors from stage I-IV patients. (Scale bars, 50 µm and 20 µm.)



Lung cancer is the leading cause of cancer deaths. Non-small cell lung cancer (NSCLC), the most common subtype of lung cancer, is mostly diagnosed at advanced stages of the disease, characterised by epithelial-to-mesenchymal transition (EMT) of transformed lung cells. EMT is associated with enhanced migration and invasion of these cells but is also a mechanism of resistance to therapeutics and thus has multiple effects on disease progression and treatment outcomes. At present, we have an incomplete understanding of how this dramatic reprogramming of cell phenotype is achieved. In this study, Ginn et al. demonstrated that TIAM1, an activator of the small GTPase RAC1, promotes EMT and migration of NSCLC cells. They also showed that TIAM1 performs this function through an unexpected nuclear function. They found that nuclear TIAM1 interacts with TRIM28, a transcriptional co-repressor that recruits histone deacetylase and histone 3 Lysine 9 (H3K9) methyltransferase to gene promoters, promoting EMT. This occurs through H3K9me3-induced silencing of protocadherins and by decreasing E-cadherin expression, thereby antagonising cell-cell adhesion. Consistently, TIAM1 or TRIM28 depletion suppresses the migration of NSCLC cells, while the simultaneous depletion of protocadherins restores migration. Importantly, the authors found that high nuclear TIAM1 in clinical specimens is associated with advanced-stage lung adenocarcinoma, decreased patient survival, and inversely correlates with E-cadherin expression.

Morrison KR, Wang T, Chan KY, Trotter EW, Gillespie A, Michael MZ, Oakhill JS, Hagan IM, Petersen J. (2023)
Elevated basal AMP-activated protein kinase activity sensitizes colorectal cancer cells to growth inhibition by metformin.
Open Biol. 13(4):230021.

Despite the adage that you cannot teach an old dog new tricks, there is growing interest in asking whether there are any ways or cancer contexts in which we can repurpose drugs that are currently used to treat other conditions for the fight against cancer. It was therefore with considerable excitement that the collaboration

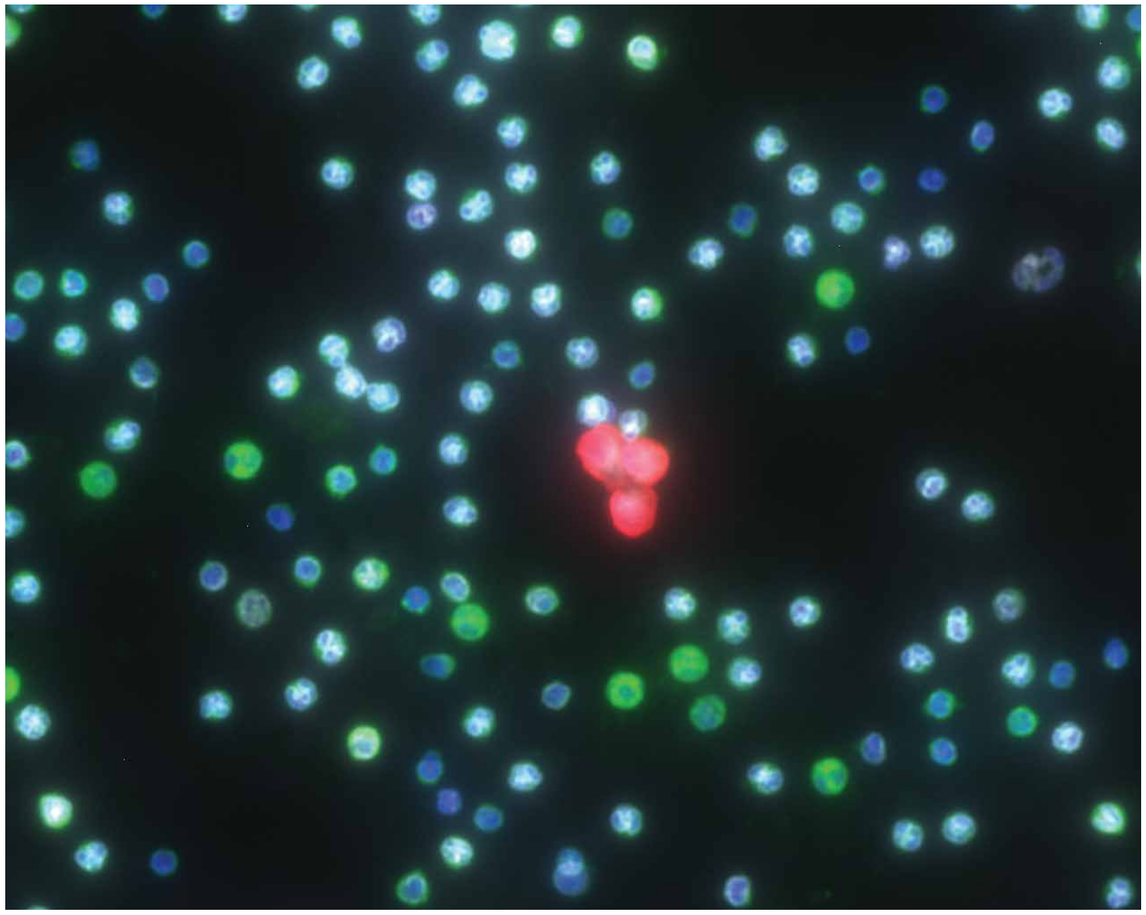
between the Institute's Cell Division group and Prof. Janni Petersen's group in Flinders University, Adelaide, Australia saw a strong inhibitory impact of the diabetes drug, metformin upon the growth of colorectal cancer models.

In their study published in *Open Biology*, the impact emerged when a key metabolic signalling network had been perturbed in ways that naturally occur in a subset of patients with colorectal cancer. Metformin blocked cancer cell proliferation when the signalling molecule, AMPKa, was mutated to render it insensitive to repression by a second key growth control network, TOR signalling. They complemented their genetic approach with pharmacological stimulation of AMPKa signalling in two different cancer lines. In each case, they found that stimulating AMPKa signalling enabled them to use metformin to block cancer cell proliferation.

Although the molecular basis for this phenomenon awaits characterisation, these are important findings because metformin is the most common oral medication used to treat type 2 diabetes. Because it is so well tolerated and widely used, it has become apparent that metformin use reduces the incidence of colorectal cancer and increases survival rates for pancreatic and endometrial cancer patients. One key appeal for the use of metformin in the context of colorectal cancers identified in the Manchester/Adelaide collaboration is that the drug accumulates up to 300 times normal blood levels in the target cells that line the colon.

Having established the principle that altered AMPKa signalling generates a vulnerability to metformin in this cancer, the teams will now seek funding to work with the Cancer Biomarker Centre to develop tests that can identify patients in which to assess the predictions of this exciting basic science in clinical trials.

RESEARCH GROUPS



Immunofluorescence image (x40) of cells with epithelial and mesenchymal phenotype (stained red) from the blood of a patient with non-small cell lung cancer. Red cells are suspected tumour cells; the other cells are white blood cells. Markers: Blue (DAPI), Green (CD45/CD31), White (Vimentin), Red (Cytokeratins).

Image supplied by Joseph Alexandrou (Cancer Biomarker Centre)

www.cruk.manchester.ac.uk/Our-Research/Cancer-Biomarker-Centre

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This will be the final CRUK Manchester Institute Cancer Biomarker Centre report as the 'CRUK National Biomarker Centre' will be formally launched in 2024. It has been a wonderful adventure and now almost 20 years since the first biomarker projects were initiated in the former Paterson Institute. We will of course remain fully aligned with the Institute, working in tandem and our researchers studying small cell lung cancer biology will remain as an Institute group.

In 2023 we developed new liquid and tissue biopsies and are validating them in large clinical cohorts; we launched new biotech/Pharma partnerships, tested novel therapies in our patient derived models, developed new ex vivo platforms to discover predictive biomarkers for immunotherapy and deployed digital approaches in clinical trials. We also welcomed two new team leads, Dr Harriet Unsworth who took up the reins of the digital Experimental Cancer Medicine Team and our new Nucleic Acids Biomarker team lead, Dr Florent Mouliere. Highlights from each team are summarised here.

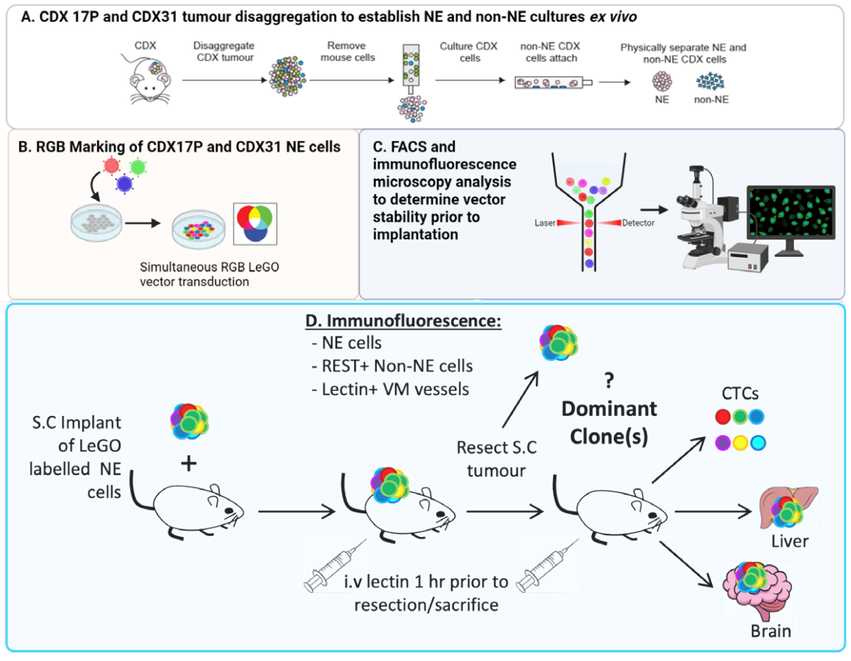

Figure 1. LeGO RGB lentiviral barcoding approach to investigate clonal dominance of NE to Non-plasticity in relation to VM and metastasis. **A**, physical separation of NE cells from bulk tumours containing NE and Non-NE cells. **B**, lentiviral transduction of LeGO vectors (up to 96 hues) and **C**, QC to establish vector stability. **D**, in vivo implantation of S.C tumours, followed by resection and harvesting of organs and CTCs for metastasis. Deconvolution of fluorescent labels to determine clonal origin. Prior to sacrifice mice will receive i.v. lectin in order to mark perfused, VM vessels and REST staining will determine presence of Non-NE cells.

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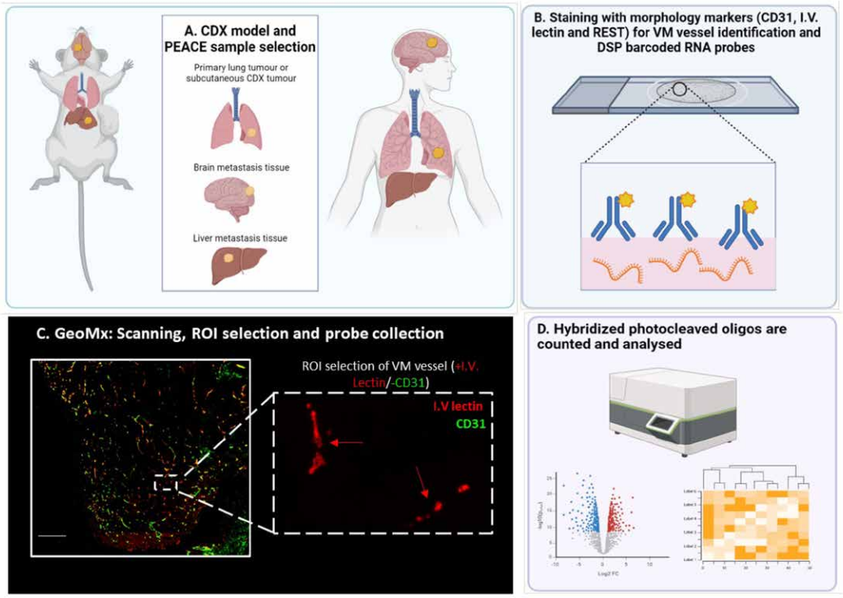

Figure 2. Spatial transcriptomics to profile inter- and intra-tumoural heterogeneity of SCLC in CDX and patient samples where multiple metastases are observed. **A**, CDX models selected based on frequency of metastasis from subcutaneously implanted tumour to brain and/or liver, with patient samples from the PEACE protocol containing primary SCLC tumours and metastatic lesions from liver and brain. **B**, Regions of interest (ROI) selected by staining with CD31 and lectin (following intravenous lectin prior to animal sacrifice to reveal perfused vessels) to identify perfused VM (lectin+/CD31-) and endothelial vessels (lectin+/CD31+), and REST to identify Non-NE cells. Digital Spatial Profiling (DSP)-barcoded RNA probes applied for transcriptomics. **C**, Image scanning and photocleavage of DSP probes. **D**, Gene expression analysis to identify transcriptomic profiles associated with VM and endothelial vessels, and non-NE SCLC cells.

The Preclinical Pharmacology Team (PP)

Patient derived preclinical models reveal novel biology of SCLC. Small cell lung cancer (SCLC) is an aggressive neuroendocrine (NE) cancer with <1-year median overall survival. We continue to characterise SCLC molecular subtypes using our biobank of patient-derived circulating tumour cell explant models (>65 CDX) that reflect the genetic heterogeneity, phenotypic plasticity and metastatic proclivity of SCLC. Phenotypic cellular plasticity in the form of NOTCH-driven NE to non-NE transition is associated with several SCLC behaviours, including acquired chemoresistance, metastasis and immune evasion. We showed that the rarer, non-NE cells within CDX tumours undergo further phenotypic transition via Vasculogenic Mimicry (VM), adopting endothelial cell characteristics with *de novo* formation of vessel-like structures that are associated with poorer patient outcomes (Williamson et al *Nature Communications*, 2016; Pearsall et al *Journal Thoracic Oncology*, 2023). VM is frequently observed in CDX models, and we hypothesise that it enables tumour growth under conditions of limiting oxygen/nutrients and supports metastasis. Integrated projects are underway to understand the impact of this plasticity. We are tracking NE to non-NE transition *in vivo* using CDX models using LeGO vectors, a stable lentiviral vector-mediated fluorescent Red/Green/Blue (RGB) colour marking approach. Viral integration of combinations of these primary colours in NE cells results in up to 96 hues that can be imaged. Immunofluorescence on tissue will establish whether cells undergoing VM and those that metastasise result from non-NE transition of a dominant NE clone(s) and from which defined regions of a subcutaneously (s.c.) implanted tumour (Figure 1). Spatial transcriptomics are underway in CDX models with different metastatic tropism to complement these studies (Figure 2).

We previously discovered a molecular subtype driven by the transcription factor ATOH1 (Simpson et al *Nature Cancer* 2020). We then demonstrated that ATOH1 promotes cell survival and supports CTC dissemination to the liver (manuscript submitted, Catozzi et al). To explore ATOH-1 dependent stages in the cascade resulting in liver metastasis we are applying luciferase labelling of CDX cells prior to implantation coupled with enabling live animal imaging with single cell RNA sequencing to CTCs, early founders in the liver and macro-metastases (Figure 3). We are also exploiting our large CDX biobank to explore the efficacy of a range of novel therapeutics in partnership with pharmaceutical companies.

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[2]Left in 2023
[3]Employed by The University of Manchester's IT Services or Department of Computer Science and fully or partly funded by CRUK Manchester Institute. The wider team includes Dr Andre Freitas (Department of Computer Science) and Dr Donna Graham (The Christie NHS Foundation Trust)

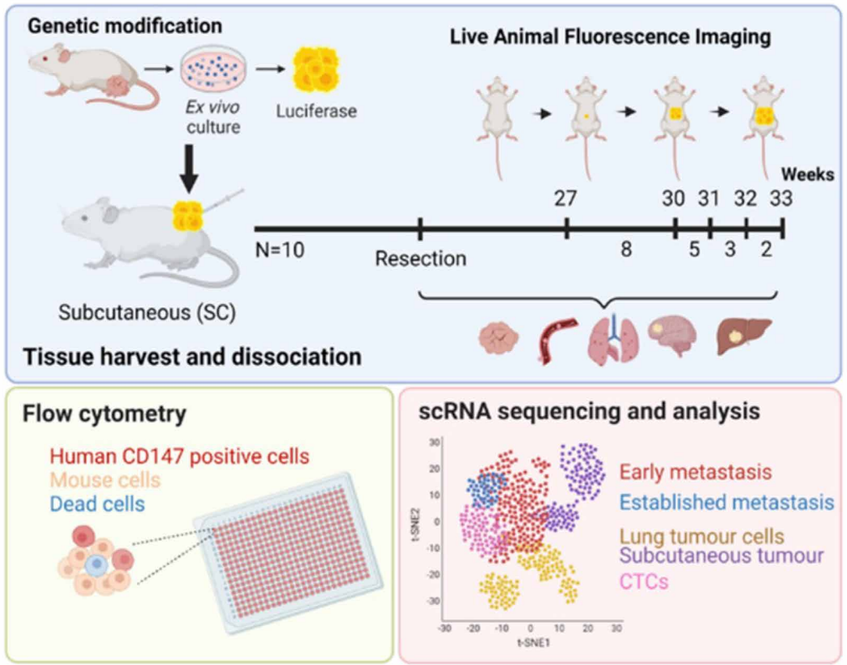


Figure 3. Study of SCLC liver metastasis using CDX models. Upper panel, Workflow to isolate CTCs, liver founder tumour cells and established macro-metastases from CDX. **Lower panels**, FACS-mediated isolation of tumour cells and scRNAseq analysis.

The Rare Cells Team (RC)

This team provides a highly specialised capability within CBC focusing on the identification, enumeration and molecular characterisation of circulating cancer-associated cells using several technology platforms with the goal of developing novel cell-based biomarkers.

Development of the High Definition Single Cell Analysis (HDSCA) Platform This marker independent platform examines all nucleated cells within a blood sample and identifies abnormal cellular phenotypes (Figure 4). HDSCA is being used in several ongoing studies including our early lung cancer detection programme (see NAB team) and the COMPASS (COMmunity based blood testing to monitor PAtientS after lung cancer Surgery) trial both co-led by Prof Phil Crosbie (MFT/UoM). HDSCA is also being used to investigate rare cell populations, including CTCs, in a cohort of early-stage non-small cell lung cancer (NSCLC) patients (n=30) compared to cancer negative individuals (n=30) and those with indeterminate pulmonary nodules (n=30) in the MISIL-1 (Multiparametric Stratification of Indeterminate Lung nodules) trial with collaborators in Cambridge, aiming to identify cell-based biomarkers that discriminate between cancer free, benign nodules and cancer-positive samples.

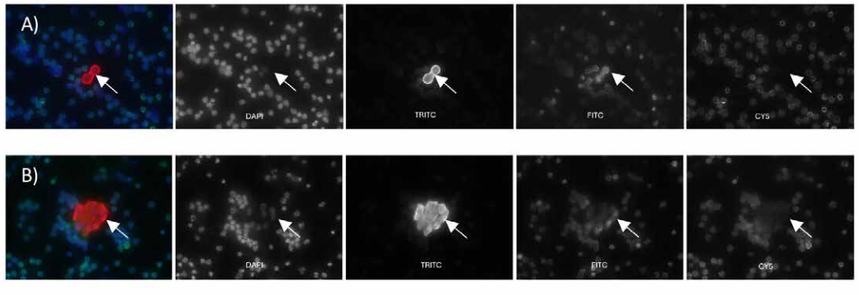


Figure 4. Examples of rare events detected in HDSCA samples from 2 COMPASS patients. Samples were stained for Cytokeratins (TRITC), Vimentin (FITC), CD31 / CD45 (CY5) and DAPI. **A**) 2 cytokeratin positive, vimentin positive cells (white arrow) in a background of leukocytes. **B**) a large cluster of cytokeratin positive, vimentin positive cells (white arrow) in a background of leukocytes.

In collaboration with the NAB team, we also established workflows for collection and genomic characterisation of cells with abnormal phenotypes at the single cell level, developing single cell isolation protocols on both the DEPArray PLUS (Menarini), which is a rare cell sorter with single cell resolution and microcapillary single cell picking platform (ALS CellCelector).

The Translational Immunology Team (TI)

The absence of a robust predictive biomarker for immunotherapy (IO) is a pressing challenge in oncology. In collaboration with external industry and academic partners, we are profiling the tumour immune microenvironment (TME) of a range of tumour types using several omics-based platforms seeking to discover, develop and validate IO predictive biomarkers. In 2023, we established two *ex-vivo* platforms to enable pre-clinical drug testing and biomarker discovery. With CRUK MI Senior Group Leader Santiago Zelenay (page 22) we are optimising patient derived tumour fragments (PDTFs) that retain immunological features of the patient of origin including *in situ* leukocyte infiltration state, lymphocyte activation or exhaustion phenotype, and the secretion of cytokines as a platform to measure IO responses. We also established a robust co-culture platform to assay autologous cytotoxic T-lymphocyte (CTL) responses to antigens presented on the cell surface of SCLC CDX or organoid models from other tumour types (Figure 5).

We are currently developing a biobank of organoid models and PDTFs to accommodate patient heterogeneity for novel IO therapy testing with parallel biomarker discovery. We also seek to discover novel cancer immunobiology, for example examining the function of chromatin remodelling enzymes in regulation of tumour responses to IFN-γ JAK-STAT1 pathway modulation and CTL mediated anti-tumour immunity. With Zelenay (page 22), we are currently validating a gene signature based on his studies of cyclooxygenase-2 (COX-2) inflammation biology as a predictor of relapse in early stage, resected NSCLC.

The Nucleic Acids Biomarker Team (NAB)

We are validating our T7-MBD-Seq cfDNA methylation workflow across multiple liquid biopsy projects and cancer types, examining the cfDNA methylome and harnessing multiple data layers to derive multi-modal biomarkers.

Multi-modal cfDNA assays: Our methylation approach (Chemi et al *Nature Cancer*, 2022), is undergoing validation in >10 independent clinical cohorts across different clinical scenarios (Figure 6). A multi-modal liquid biopsy study, combining cfDNA methylation, cfDNA mutation (collaborator Prof Max Diehn, Stanford), and rare circulating cells (using the HDSCA platform with Prof Peter Kuhn, USC) funded by a CRUK Early Detection Programme grant, is underway aiming to combine these parameters in a cohort of 200 stage I/II screened cancers and 200 cancer negative risk matched controls in collaboration with Prof Philip Crosbie (MFT, UoM).

Cancer of Unknown Primary (CUP): Patients with CUP have limited treatment options and poor outcomes, lacking a primary tumour diagnosis which makes selection of a beneficial treatment challenging. With the BBS team and Drs Natalie Cook and Alicia-Marie Conway (UoM/CFT), we further developed cfDNA methylation profiling to generate a tissue-of-origin classifier (TOO), across 29 cancer types to support treatment

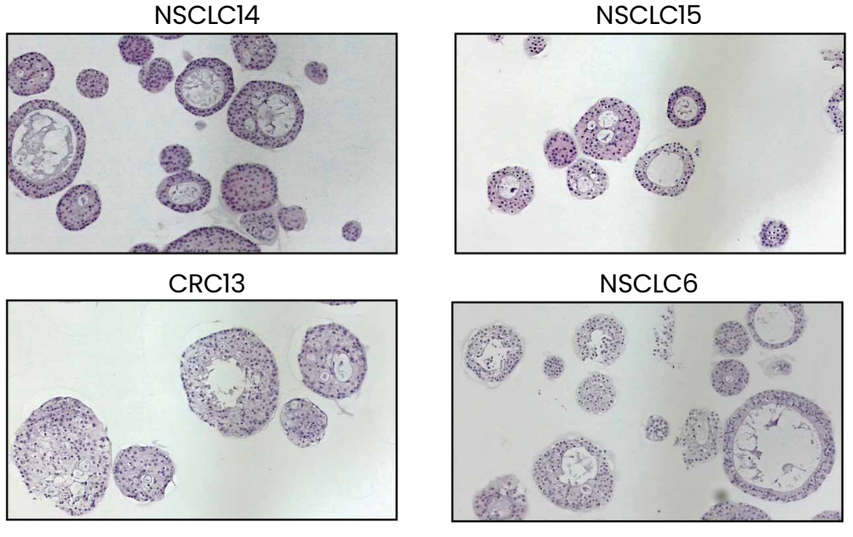


Figure 5. Representative haematoxylin and eosin images of organoid models derived from patient tumours. Non-small cell lung cancer (NSCLC) or colorectal cancer (CRC). NSCLC6 and NSCLC14 are derived from a lung adenocarcinoma (LUAD) tumour. NSCLC15 is derived from a lung squamous cell carcinoma (LUSC) tumour. All NSCLC tumours shown are from patients with a history of smoking. CRC13 is derived from a CRC adenocarcinoma.a background of leukocytes.

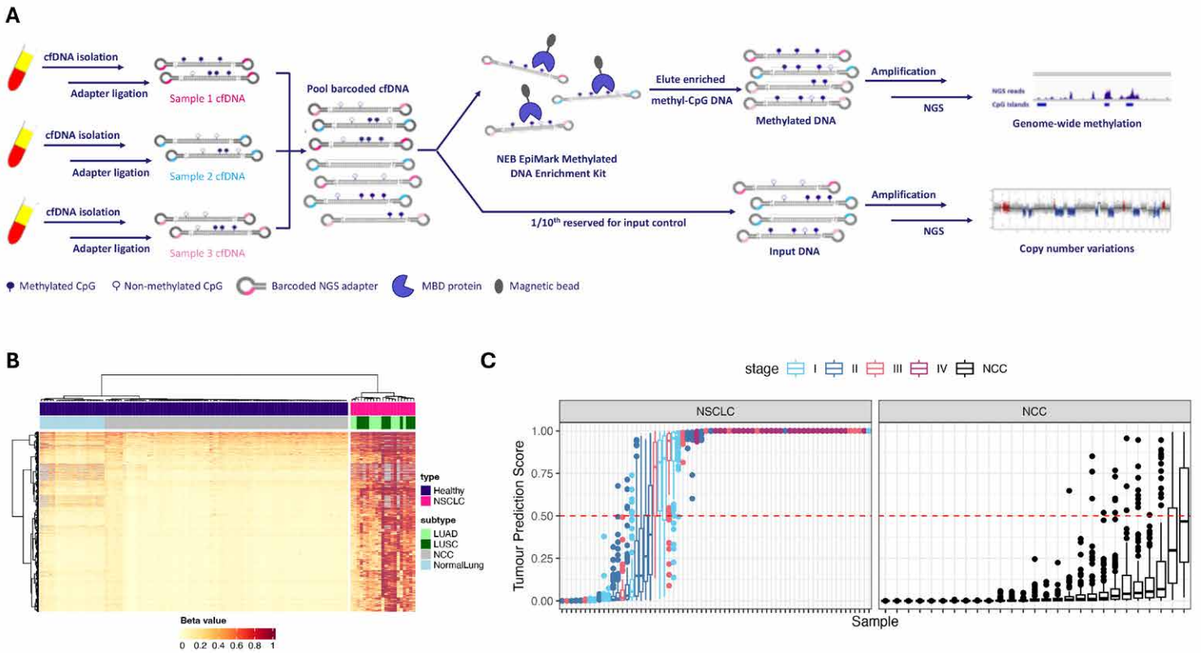


Figure 6. Methylation sequencing of plasma cfDNA using T7-MBD seq. A. experimental workflow of T7-MBS sequencing. **B.** Differentially Methylated Regions (DMRs) for a cohort of NSCLC samples and healthy individuals calculated from the T7-MBS seq data. **C.** Pilot data for NSCLC detection depending on the disease stage.

decisions (Conway et al *Nature Communications, accepted*). Application of the classifier to 143 cfDNA samples from patients with 13 different tumour types showed 97% TOO accuracy and 85% sensitivity. In a pilot of 41 patients with CUP, tumour class predictions were made retrospectively from cfDNA in 78% (32/41) of cases; 72% of predictions (23/32) were consistent with clinically confirmed or suspected diagnoses and 19% of these (6/32) were for patients with no diagnostic suspicions. Importantly, our predictions were for tumour types with radically different treatment options to SOC chemotherapy recommended for CUP and validation studies are underway in larger patient cohorts.

ctDNA mutation profiling and ddPCR-based primary assay to direct therapy decisions in melanoma. We optimised our ctDNA profiling assays using NGS-based gene panels and deployed these in clinical studies with pharmaceutical collaborators. We also developed highly sensitive unique molecular indexed (UMI) based assays, which can detect somatic mutations at low tumour fractions (<0.1% VAF). With the support of our Quality Assurance team, we broadened our portfolio of GCP-compliant liquid biopsy assays in collaboration with Prof Paul Lorigan and Dr Rebecca Lee (UoM), completing validation of ddPCR assays to monitor ctDNA levels for the upcoming DYNAMIC trial to optimise therapy scheduling in melanoma.

Bioinformatics and Biostatistics Team (BBS)

This team plays an essential role across many CBC projects with expertise in bioinformatic and statistical method development, software engineering, machine learning and experimental design.

cfDNA Methylation analysis: With the NAB Team we are optimising pipelines for analysis and interpretation of cfDNA methylation data. Machine learning based classifiers capable of determining SCLC molecular subtypes (Chemi et al *Nature Cancer* 2022) and tissue of origin (TOO) in CUP (Conway et al *Nature Communications, accepted*; patent (GB2317261.2)) underwent additional optimisation and validation with increased clinical cohorts. The bioinformatic analysis of cfDNA methylation data also diversified into studies looking at treatment associated toxicity in collaboration with clinical colleagues on the TORPEDO trial and continued development of our early detection (ED) of lung cancer study (see NAB team) which requires integration of multi-omics data. Additional bioinformatics tools are being developed include fragmentomic analysis of ctDNA in collaboration with Dr Florent Mouliere (NAB Team Lead) to improve both sensitivity and specificity of our liquid biopsy approaches.

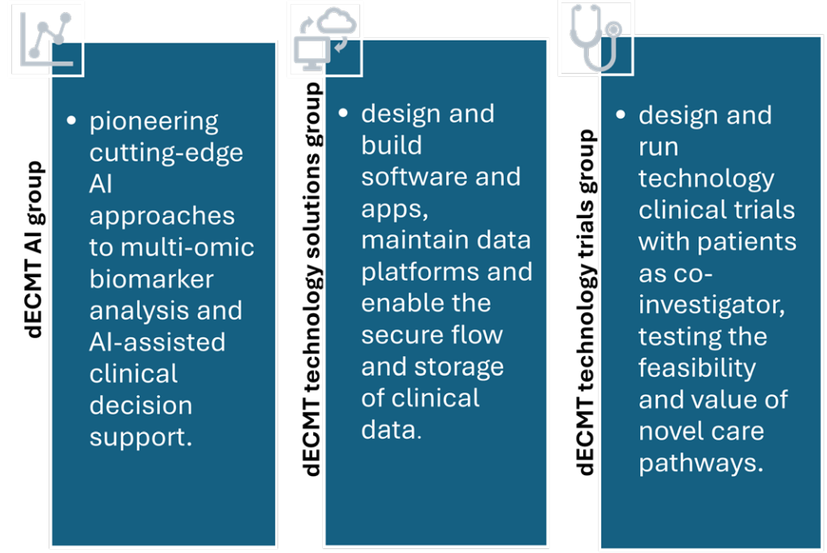
Single cell transcriptomic analysis: Working with the PP Team we aim to understand intra-tumoural cellular heterogeneity in SCLC brain metastasis. Using single cell RNASeq data (10X Genomic scRNASeq) and established analytic workflows (Seurat, SCRAN), we are comparing cellular transcriptomes between s.c. tumours and brain metastases in SCLC CDX models with emerging data suggesting adaptive changes in cellular metabolism within the brain TME.

Immune-based informatics: With the TI Team, BBS has analysed combined bulk RNA-Seq and Assay for Transposase-Accessible Chromatin using Sequencing (ATAC-Seq) data to investigate an epigenetic mechanism of immune evasion in SCLC aiming to understand transcriptional deregulation induced by experimental drugs and identify therapeutic strategies to re-sensitise SCLC to immunotherapy.

The digital Experimental Cancer Medicine Team (dECMT)

Our remit is to create and evaluate innovative digital, AI, and in-home technologies to support cancer pathways. The 3 subgroups within the dECMT each contribute specific expertise (Figure 7).

Figure 7. Structure of the digital Experimental Cancer Medicine Team (dECMT).



Five highlights from this year include:

1. Use of the eTARGET data visualisation tool, created and maintained by the dECMT, is now being expanded across the TARGET National trial. This trial will recruit 6000 patients with advanced cancer. We now support over 200 eTARGET users nationally.

2. We opened our first multi-national trial, the APACE study. APACE uses activity and sleep monitoring watches to record patient function and physical ability before and after starting a phase 1 study of a new cancer therapy. The Christie site has completed recruitment and we have started to receive data from participants at the Leicester site. Sites in Italy and Spain will begin recruitment in 2024.

3. We developed a machine learning approach to create a meta-review informed method for identifying cytokine release syndrome in patients having chimeric antigen receptor therapies.

4. We have developed and prototyped the QA tool, a unique tool that allows clinicians and trial sponsors oversight of protocol deviations in clinical trials. We will be integrating NLP into this tool to aid automated data categorisation.

5. We released the PROACT 2.0 patient-reported outcomes tool, developed with our UpSMART partners in Italy, as an open-source product. PROACT 2.0 won two health innovation awards in Italy and will be evaluated in a clinical trial due to start in 2024.

The Quality Assurance, Portfolio and Laboratory Management, and Project Delivery and Administration Teams

These teams provide critical support of all our research across the CBC.

Publications listed on page 56

CANCER IMMUNOSURVEILLANCE

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The immune system serves as the body's innate defence against pernicious threats. While traditionally studied in the context of infectious diseases, it is now well-established that the immune system can also prevent cancer formation by identifying and eliminating malignant cells. T cells, a component of adaptive immunity, often play a leading role in anti-cancer immunity. Immune checkpoint blockade (ICB) therapy, which reinvigorates T cell immunity against cancer cells, stands as a pinnacle of success in treating patients with advanced cancers. Despite remarkable success in clinical care, only a fraction of patients undergoing ICB therapy achieve lasting responses. Resistance to this therapy often stems from a lack of pre-existing anti-tumour T cell responses. What initiates T cell immunity against cancer? When does it fail to initiate? Can we predict and restore T cell-mediated cancer immunity? Unravelling the origins of T cell-mediated cancer immunity is crucial to overcoming immunotherapy resistance.

Dendritic cells (DCs) are innate immune sentinel cells that play a crucial role in instructing T cell immunity. Similar to other innate immune sensor cells, DCs express a diverse array of receptors that equip them with the ability to recognise microbial presence and tissue damage. The precise contributions of these functions to anti-cancer immunity are not fully understood. However, there is evidence linking dying cancer cells and specific commensal bacterial taxa to the induction of T cell-mediated anti-cancer immunity and improved responses to immunotherapy. In the Cancer Immunosurveillance group, we integrate genetically modified mouse models and tumour engineering techniques to unravel the intricate interactions between tumours and the host, which form the foundation of T cell-mediated cancer immunity. Our ultimate vision is to contribute to the fundamental understanding of cancer immunity and to pave the way for therapeutic strategies aimed at overcoming resistance to immunotherapy.

Cell death sensing in cancer immunity

Cell death often occurs within the tumour microenvironment (TME) as a result of tumour suppressor mechanisms and anti-cancer therapies. Cell death elicits $CD8^+$ T cell immunity by acting as a source of antigens and immunostimulatory molecules. We have previously shown that immune detection of dying tumour cells can elicit anti-cancer immunity (Giampazolias *et al. Nature Cell Biology*, 2017; Giampazolias *et al. Cell Cycle*, 2018; Giampazolias *et al. Cell*, 2021; Lim KHJ *et al. JITC*, 2022). Dendritic cells (DCs) possess the ability to detect cell debris and can frequently induce antigen specific CD8+ cytotoxic T lymphocyte (CTL) responses by integrating and processing molecular cues associated with dead cells. In both mice and humans, DNGR-1 (also known as CLEC9A), a receptor primarily expressed on type 1 conventional dendritic cells (cDC1), binds to F-actin exposed by dying cells, facilitating antigen-specific CTL responses. Our previous research in mice has demonstrated that secreted gelsolin (sGSN), an ample plasma protein that binds to and severs F-actin, impedes DNGR-1 activation, thereby suppressing anti-cancer immunity and diminishing the effectiveness of various anti-cancer therapies including immunotherapy, radiotherapy, as well as immunogenic chemotherapy and targeted therapy. Interestingly, we have observed that tumours engineered to express an antigen linked to the actin cytoskeleton (LifeAct-OVA) are preferentially controlled in sGSN deficient mice in comparison to wild-type hosts,

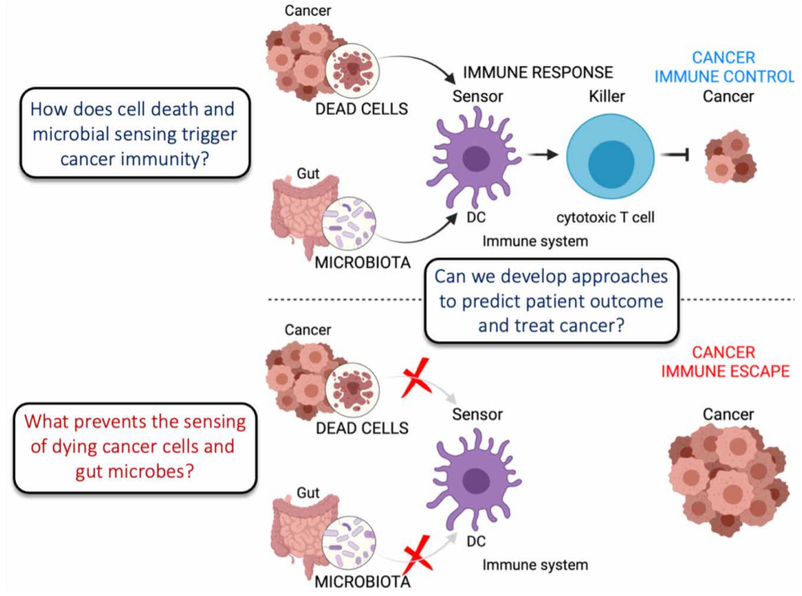

Figure. Cell death and microbial sensing trigger immunity to cancer. In the Cancer Immunosurveillance group we investigate the cells, molecules and pathways involved in the recognition of dying cancer cells or intestinal commensal microbes and study their functional consequences on immune components (e.g. DC and T cells) that determine anti-cancer immunity and immunotherapy success.

suggesting that DNGR-1 signalling could potentially favour the cross-presentation of cytoskeletal antigens to T cells (Giampazolias E *et al. Cell*, 2021). Similarly, in humans, intratumoural levels of sGSN transcripts inversely correlate with signatures of anti-cancer immunity and improved patient survival in subcohorts of patients that express nonsynonymous mutations to F-actin binding proteins (Giampazolias *et al. Cell*, 2021).

Although DCs can couple cancer cell death to cancer immunity, not all dying cells are good instigators of T cell responses. We have previously shown that the mechanism by which a cell commits to death is a critical determinant of its immunogenicity. Indeed, apoptotic cells, which are frequently found in the TME, do not always elicit robust anti-tumour $CD8^+$ T cell responses. Apoptosis is often triggered by caspase activation downstream of mitochondrial outer membrane permeabilisation (MOMP). MOMP serves as a point-of-no-return, ensuring cell demise even if caspase activity is inhibited. We have found that cells undergoing MOMP still die, via a pathway termed caspase-independent cell death (CICD) resulting in the release of proinflammatory mediators (e.g. type I interferons) that are necessary for T cell activation and concomitant tumour control *in vivo* (Giampazolias et al. *Nature Cell Biology*, 2017). Collectively, we have identified caspases and sGSN as natural barriers to cancer immunity dampening the immunogenicity of dying tumour cells and the sensing of associated signals by DCs that are necessary for the generation of anti-tumour T cell immunity.

Microbial sensing in cancer immunity

Numerous studies in mice and humans have underscored a correlation between the abundance of certain intestinal commensal microbes and the efficacy of T cell-based immunotherapies against extraintestinal tumours. Therefore, the interaction between dying cancer cells and the immune system is necessary; it alone is insufficient to elicit cancer immunity due to the necessity of immunologically permissive environments dictated by the gut microbiome. However, the lack of commonalities in the microbiota composition across cohort studies currently confounds our understanding of how host-microbiome interactions dictate protective immunity to cancer. Importantly, these inconsistencies could be attributed to genetic and/or environmental factors, such as dietary habits that can impact not only the composition but also the function of the microbiome. Currently, we are focusing on characterising the mechanisms governing host-microbiome interactions that permit systemic immune responses to cancer using mouse models and dietary interventions. Our goal is to evaluate the predictive and therapeutic significance of gut microbiome in cancer immunity as well as uncovering novel strategies for therapeutic intervention.

CANCER INFLAMMATION AND IMMUNITY

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[1]Left in 2023
[2]Joined in 2023

The local and systemic inflammatory response to cancer constitutes an established hallmark intimately linked to aggressive tumour growth, dismal prognosis, and resistance to treatment. By contrast, the remarkable efficacy of immunotherapy using immune checkpoint inhibitors (ICIs) has brought to light a distinct type of inflammatory response with potent tumour-inhibitory properties.

Since its inception in 2015, our group at the Cancer Research UK Manchester Institute has been investigating the signals and pathways that govern the initiation and establishment of these seemingly antagonistic tumour-promoting or tumour-restrictive inflammatory responses. Anchored in fundamental science, our research efforts have expanded to bridge the translational gap between fundamental laboratory findings and their clinical applications. For this, we have leveraged the rich cancer research ecosystem of Manchester developing close collaborations with the Cancer Biomarker Centre and clinical colleagues at The Christie NHS Foundation Trust.

Immunotherapy utilising immune checkpoint inhibitors (ICIs) has revolutionised cancer treatment and is emerging as the standard of care for various cancer types including melanoma, lung, kidney, head and neck or bladder cancer. Despite its transformative impact, the beneficial effects of immunotherapy are restricted to a minority of patients, even when administered alongside chemotherapy or targeted therapies. Many patients across all cancer types fail to respond to ICI therapy, and for those who do, eventual disease progression remains a major challenge. Preclinical work and analysis of cancer patient samples have demonstrated that the baseline cellular and molecular immune landscape when treatment commences constitutes a major determinant of immunotherapy outcome. Associated to ICI response is a type of inflammatory profile, less prevalent in clinically apparent tumours, characterised by heightened infiltration by select immune cells such as cytotoxic T cells, natural killer (NK) cells and conventional dendritic cells (cDCs). Our past and recent research efforts have focused on dissecting the signals orchestrating the formation of this type of tumour restrictive tumour microenvironment. In doing so, we have uncovered the cyclooxygenase-2 (COX-2)/prostaglandin E2 (PGE2) pathway, often upregulated in multiple malignancies, as an inflammatory axis that enables immune escape, thus enabling cancer development, progression and therapy resistance. We have shown that genetic manipulation of this pathway in animal models can turn an aggressive tumour into a spontaneously regressive one, or sensitised unresponsive tumour types responsive to ICI treatment (Bonavita et al, *Immunity.* 2000;53(6): 1215-1229; Pelly et al, *Cancer Discov.* 2021;11(10):2602-2619). Furthermore, we have generated proof of concept data demonstrating that pharmacological inhibition of this pathway with widely used anti-inflammatory drugs or state-of-the-art PGE2 receptor antagonists can boost the response to ICIs or combinations of ICIs and chemotherapy (*Cancer Discov.* 2021;11(10):2602-2619; Bell et al*, Nat Commun.* 2022;13(1):2063).

Our research hinges on a fundamental premise: to elucidate the mechanisms of immune evasion in cancer. To do this, we need first an in-depth understanding of the signals and processes that underly an effective anti-tumour immune response. It is working under this premise and through a comprehensive analysis of the tumour microenvironment in various cancer models that we have found a non-redundant pivotal function for the NK cells/cDC axis in natural and therapy-induced T cell-mediated tumour control (Bonavita et al, *Immunity.* 2000;53(6): 1215-1229; Pelly et al, *Cancer Discov.* 2021;11(10):2602-2619). With an interest in the processes that drive cDC activation, we have identified an enigmatic cluster of cDCs through single cell RNA sequencing. This seemingly distinct cDC population has been also found infiltrating mouse and human tumours by others, but knowledge of their supporting or restraining contribution to immune cell priming and tumour immunity is currently lacking.

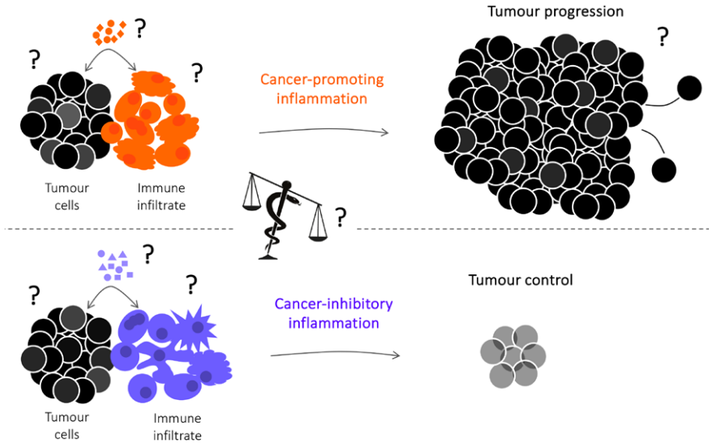

Figure 1. The figure depicts the current research interrogation points of the Cancer Inflammation and Immunity group aimed at elucidating the mechanisms that control spontaneous and therapy-induced tumour immunity, focused on the cellular and molecular mediators that regulate the balance between cancer-promoting and cancer-inhibitory inflammation. The snake of the Greek god of medicine Asclepius, winding around and tipping the balance towards cancer-inhibitory inflammation, represents our search for pharmacological interventions to improve the efficacy of cancer therapies that harness the anti-cancer functions of the immune system.

Harnessing the comprehensive single cell transcriptome profiling of these and other tumour-infiltrating leucocytes, we have generated new genetically engineered mouse strains that allow to selectively track, isolate, image and, crucially, ablate these cells. By exploiting these new mouse strains, we have made very significant progress in our quest to define the function of cDCs in T cell-mediated tumour immunity.

Our past efforts have been largely focused on the study of immunity to solid cancer at the primary site. We have now turned our attention to the analysis of the immune control of metastatic disease. Building on the lessons from our past work, we are examining the extent to which previously identified cellular and/or molecular mediators similarly influence immunity to metastatic cancers. Our analysis indicates that while some of the key players are conserved, intriguing qualitative differences exist between the inflammatory and immune response to the very same cancer cells, depending on whether the tumours are developing at their primary site or disseminating to distant sites. In light of these findings, our current working hypothesis is that immune therapies effective to fight cancer at the primary site are less effective to control metastatic disease. We further speculate that this reduced efficacy is because the central players of immunity to disseminating cells varies substantially. Additionally, the systemic inflammatory response that ensues during cancer progression further subverts the tumour-restrictive arms of the immune system. To test this central hypothesis, we are leveraging and refining various experimental approaches to model and monitor metastatic disease characterising side by side the immune response to primary or disseminated tumours. In research activities more directly centred on improving the efficacy of cancer treatment, we are searching for translatable pharmacological strategies to turn tumour inflammatory profiles from 'cold' to 'hot'. We are focusing our efforts on hard-to-treat preclinical models which do not respond to the current standard of care ICIs. We are examining ways to target basal or therapy-induced pro-tumourigenic inflammation with particular interest in approaches already successfully used for managing inflammatory diseases in non-cancer settings. We reason that re-purposing these drugs to treat cancer is an attractive path forward since we already have advanced knowledge about their pharmacology, including, crucially, their safety profiles. For these approaches we continue to exploit and refine versatile experimental cancer models of various levels of immunogenicity and immunotherapy responsiveness. We have also expanded our arsenal of approaches, establishing the pioneering patient-derived tumour fragment platform recently developed by Daniela Thommen and colleagues from The Netherlands Cancer Institute. This culture system uses surgically resected patient tumour material from patients and provides an unprecedented opportunity to rapidly evaluate and perturb the response of tumour-infiltrating immune cells to treatments such as ICI, representing a useful approach to design and run *ex vivo* clinical trials.

Finally, we have made substantial progress testing the hypothesis that monitoring COX-2-associated pro-tumourigenic inflammation represents a powerful biomarker to predict patient overall outcome and response to ICI. Having computationally refined our original signature mining large cancer patient datasets, we found that the new signature predicts worse prognosis and ICI unresponsiveness. Notably, the signature patented last summer by CRUK Horizons, is also showing great promise as a predictor of relapse in earlier-stage cancer. Given these encouraging findings, we have also developed and validated what we argue constitutes a new clinically compatible assay for inflammatory molecular profiling of archival tumour samples. We anticipate that rapid molecular profiling of the type of inflammation before treatment commences could help guide treatment choices.

By understanding how inflammatory mediators influence spontaneous or treatment-induced immune control to primary or metastatic disease, assessing their prognostic and predictive value, and evaluating how their manipulation impacts cancer treatment outcomes, we aim to help design better therapeutical approaches that harness anti-cancer properties of the immune system, whilst also supporting accurate prediction to guide treatment selection.

Publications listed on page 58

CELL DIVISION

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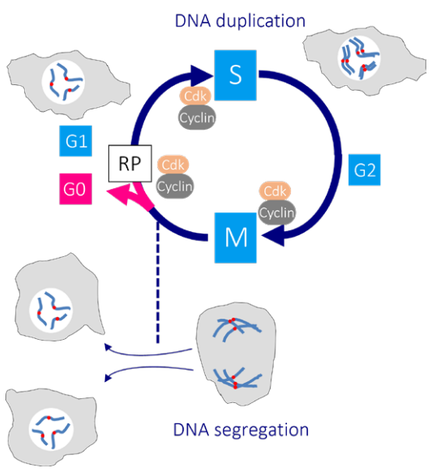
The inappropriate proliferation of cancer cells can arise from unchecked cell division, a failure to engage cell death pathways, or simultaneous changes in both. Understanding how the diverse cues are integrated to co-ordinate cell division and death is therefore key to understanding the biology of cancer. We study cell cycle controls that determine when a cell commits to the physical process of genome segregation, mitosis.

Because the regulatory networks that control cell division are highly conserved, we use both unicellular fission yeast and human cells in our investigations as the yeast work identifies core principles with which to frame questions to ask of the more complex context of human cell division. Our yeast work addresses how signals from the broad range of pathways are integrated by regulatory relays on neighbouring scaffolds on the centrosome to generate a single signal to trigger division when the time is right. Complementary studies are asking whether similar controls operate in human cells and characterises one of the key cell cycle checkpoint molecules that determines when mitosis begins, PKMYT1.

In a typical cell division cycle the G1 gap phase precedes DNA replication in S phase, before a second gap phase, known as G2, separates S from genome segregation with the mitotic spindle in Mitosis, or M phase (Figure 1). Growth, developmental and environmental cues determine whether and when a cell leaves the non-cycling G0 state to enter the cell cycle by passing through a decision point of no return in G1 phase called the "Restriction point" (denoted by RP in Figure 1). Successive waves of CDK-cyclin activities then drive different events as cells transit the cycle. Defects in DNA integrity activate cell cycle checkpoints that block progression through key cell cycle transitions until the damage/deficiency is restored. As the mutations that enable cancer cells to bypass normal growth controls lead to the accumulation of DNA damage and changes to the chromosome number, cancer cells become more reliant upon these checkpoints than their normal neighbours. Consequently, agents that enhance DNA damage are widely used in the clinic as they increase the level of damage in the already stressed cancer cells to a point where checkpoint defenses are unable to prevent catastrophic division. By contrast, their normal neighbours simply extend their cell cycle times to accommodate the elevated level of damage. We are therefore asking how these checkpoints operate to find ways to manipulate checkpoint controls in a manner that will selectively eliminate cancer cells.

Figure 1: The human cell cycle with Cdk1-Cyclin B control of the G2/M transition. Passage through the restriction point (RP) in G1 phase commits a cell to passage through the cell division cycle. DNA replication in S phase is separated from mitosis by a gap phase, G2. Transition through the major rate limiting commitment steps into the cycle, DNA replication (S) and genome segregation (M) is driven by CDK-Cyclin activities.



The transition from G2 phase into mitosis is driven by activation of the CDK1-Cyclin B protein kinase. CDK1-Cyclin B activity is restrained through inhibitory phosphorylation by the WEE1 family kinases WEE1 and PKMYT1. When the time is right, the inhibitory phosphate is removed by CDC25 phosphatases and cells enter mitosis (Figure 2A). The checkpoint pathways that block mitotic commitment when DNA is damaged, or replication is incomplete, do so by boosting the activity of WEE1 family kinases and repressing CDC25 (Figure 2B). As cancer cells harbour more damage than normal tissue, they are more reliant upon these checkpoints than their normal neighbours, making targeting the checkpoint pathways a major focus in drug discovery at present (Figure 2B). WEE1 control of the CDK2-Cyclin Complexes that control the timing and execution of DNA replication alongside its inhibition of CDK1-Cyclin B means that clinical application of WEE1 inhibitors is proving problematical. In contrast, because PKMYT1 only regulates CDK1-Cyclin B and PKMYT1 can be completely removed from untransformed cells without affecting viability, the PKMYT1 inhibitor Lunresertib is generating great excitement as its excellent pre-clinical efficacy is being matched by performance with minimal toxicity in early clinical trials. We want to guide and refine the use of PKMYT1 inhibitors in the clinic by finding more about the basic biology of the molecule. We want to know how, when, and why PKMYT1 is used to regulate mitotic commitment.

Figure 2: PKMYT1 in Cdk1-Cyclin B regulation in checkpoint control A) CDK1-Cyclin B activity is held in check in interphase as a consequence of phosphorylation of CDK1 by WEE1 family kinases. CDC25 removes the inhibitory phosphate to trigger mitosis. B) DNA damage or incomplete DNA replication triggers checkpoint pathways that boost inhibitory phosphorylation of CDK1 and reduces counteracting CDC25 activity. Lunresertib inhibition of PKMYT1 abolishes this restraint to initiate division before DNA integrity is restored, leading to death.

Figure 3: Mitotic commitment control from the fission yeast spindle pole body
Signalling from a range of environmental cues and spindle integrity converge on the dialogue between the SPB scaffolds Cut12 and Sid4 to determine the timing at which the decision to enter mitosis is triggered. Eviction of PP1 from Cut12 is a key rate determining step upon which all these signals converge.

The observation that active CDK1-Cyclin B appears on human centrosomes before propagating throughout the cell has been consolidated by other data to suggest that the centrosome provides a specific microenvironment for the activation of CDK1-Cyclin B to trigger the G2/M transition. Our studies of the fission yeast centrosome equivalent, the spindle pole body (SPB), provide molecular insight into how this switch may operate. Simply blocking the recruitment of protein phosphatase 1 (PP1) to the SPB scaffold Cut12 enables cells to live without Cdc25. Furthermore, PP1 eviction from Cut12 is the only essential function for Cdk1-CyclinB^{Cdc13} in driving cells into division. The means by which the Cut12/PP1 switch regulates mitotic commitment appears to involve the mitotic kinase Polo, as Polo activity and recruitment to the SPB shows a direct, inverse correlation with PP1 recruitment to the SPB, and artificial elevation of Polo activity at the SPB drives cells into division. As Polo kinase activity is regulated by nutritional status and stress responses, Polo kinase engagement in this switch couples division timing to the specific demands of any given environmental context (Figure 3).

The threshold for Cut12 signalling that must be passed before the cell enters division is set by a signalling relay on a neighbouring scaffold molecule, Sid4. This relay regulates the SPB residence of a Cdk1-Cyclin B counteracting phosphatase called Cdc14^{Flp1}. Thus, it is the dialogue between Cut12 and Sid4 that determines when division will be initiated. Sid4 also anchors the cytokinesis regulating Septum Initiation Network to the SPB. This anchorage is essential to ensure the SIN signalling can drive the events of mitotic exit and cytokinesis. Thus, signalling from these two neighbouring SPB signalling platforms acts like the central processing unit of a computer. Converging signals from multiple pathways are integrated to generate a coherent signal that sets the flux through outgoing signalling cascades that tells the cell when to enter and exit mitosis. We are currently seeking the mechanistic basis for Polo and Cdc14^{Flp1} engagement in these networks and the function of similar centrosomal scaffolds in human cells.

A highlight for our group this year was the discovery of a novel target in colorectal cancer. Working with Janni Peterson and collaborators from Flinders University (Adelaide, Australia), we found that elevated basal AMP-activated protein kinase activity sensitises colorectal cancer cells to growth inhibition by metformin. This discovery could lead to the repurposing of an existing drug – metformin is used to treat diabetes – to treat cancer.

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Figure 2

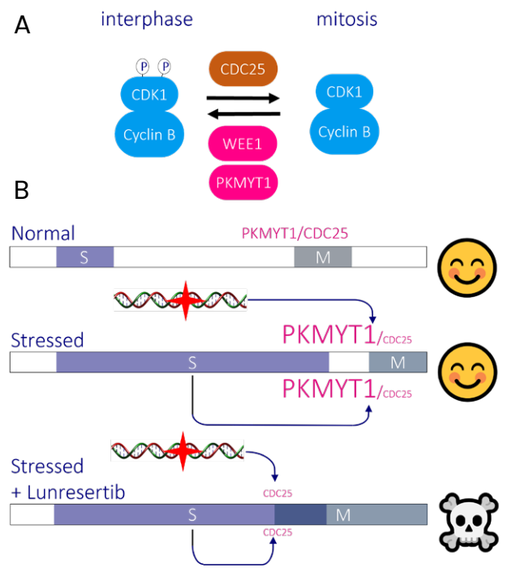
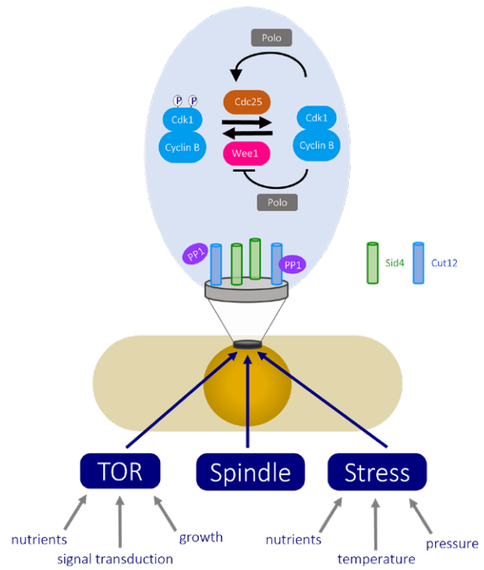


Figure 3



CELL SIGNALLING

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[1]Left in 2023

The main focus of the Cell Signalling group is the identification of therapeutic targets in lung cancer, the most common cause of cancer-related deaths worldwide. Lung cancer is divided into non-small cell lung cancer (NSCLC, approximately 85% of cases) and SCLC (~15% of cases). The most common histological subtype of NSCLC is adenocarcinoma, of which driver mutations in KRAS are the most common. KRAS mutant lung adenocarcinoma (KRASm-LUAD) and SCLC treatments lag behind other lung cancer types, for which targeted therapies offer additional treatments prolonging patient survival. No approved targeted therapies exist for SCLC, and until recently, nor did they for KRASm-LUAD. However, direct inhibitors against the most common KRAS mutation in LUAD (KRASG12C) have recently entered the clinic, but resistance is rapid and common in relapsing patients. Therefore, current drug development efforts focus not only on KRAS itself, but also on downstream targets. One such downstream target under investigation in our laboratory is the small GTPase RAC1.

Our laboratory's focus during 2023 has been the investigation of therapeutic targets in KRASm-LUAD. We have been interested in understanding whether different KRAS mutations lead to the activation of different signalling pathways in LUAD and consequently to different therapeutic vulnerabilities. This is a project in close collaboration with Dr Colin Lindsay, a clinician colleague based at the Christie NHS Foundation Trust in Manchester. We have been investigating tumour formation, signalling and therapeutic vulnerabilities of LUAD initiated with either the most prevalent *KRASm* allele, $KRAS^{G12C}$ or $KRAS^{G12D}$, another common KRAS mutation. Important differences in $KRAS^{G12D}$- and $KRAS^{G12C}$-driven LUAD have been documented: $KRAS^{G12D}$ mutation is enriched in never-smokers, whereas $KRAS^{G12C}$ is associated with smoking, tobacco-carcinogen mutational profiles and higher tumour mutation burden. Moreover, the frequency of associated co-mutations differs between *KRASm* alleles, indicative of divergent trajectories to neoplasia. We discovered that $KRAS^{G12D}$ is much more potent than $KRAS^{G12C}$ in initiating LUAD in mouse and identified unique combination treatment vulnerabilities in $KRAS^{G12D}$ vs $KRAS^{G12C}$ mutant LUAD (McDaid *et al.*, in revision).

Apart from investigating the role and signalling differences of KRAS mutations in LUAD, we have also been interested in investigating whether the RAC1 signalling pathway (which is activated downstream of KRAS) could be a good therapeutic target in KRAS mutant lung cancer either on its own or in combination with specific KRAS inhibitors. RAC is a member of the RHO-like family of GTPases and cycles between a GDP- and GTP-bound state. When GTP-bound, it interacts with effector molecules that regulate several cellular processes including proliferation and migration. Multiple mechanisms control RAC activity including control of nucleotide binding and hydrolysis by guanine nucleotide exchange factors (GEFs) and GTPase Activating Proteins (GAPs) respectively, regulation of subcellular localisation and modulation of RAC protein levels (reviewed in Porter *et al.*, Small GTPases 2017). Moreover, several studies using recombinant RAC and RAC GEF mice have shown that RAC is required for the formation and growth of tumours. RAC is required for the formation of KRASm-LUAD in mice. However, RAC1 performs several physiological roles and interestingly its activation can have contrasting effects on cell migration and invasion. Studies from our laboratory, for example, have shown that activation of RAC1 can in some cases promote migration (Rooney *et al.*, EMBO Rep. 2010; Castillo-Lluva *et al.*, Nat Cell Biol 2010; Castillo-Lluva *et al.*, Oncogene 2013; Marei *et al.*, Nat Comm 2016; Woroniuk *et al.*, Nat Comm 2018), whereas in other cases inhibit migration (Malliri *et al.*, J Biol Chem 2004; Woodcock *et al.*, Mol Cell 2009; Mack *et al.*, Nat Cell Biol 2012; Vaughan *et al.*, Cell Rep 2015; Marei *et al.*, Nat Comm 2016; Diamantopoulou *et al.*, Cancer Cell 2017). Therefore, for RAC to be a good therapeutic target in cancer, it is important to identify the factors that influence whether its activation will promote or inhibit migration. One such factor we have identified is the GEFs that activate RAC. RAC GEFs are multi-domain proteins with many binding partners. We showed that TIAM1 and another RAC GEF, P-REX1, have diametrically opposite effects on cell migration through RAC in certain epithelial cells and fibroblasts: TIAM1 promotes cell-cell adhesions to oppose cell migration while P-REX1 promotes migration (Marei *et al.*, Nat Comm 2016). We therefore hypothesise that inhibiting the activation of RAC by particular GEFs would be better therapeutically in KRASm-LUAD than inhibiting RAC itself. In this regard we have been evaluating the role of the RAC GEF TIAM1 in KRASm-LUAD formation and progression because TIAM1 is a GEF specific for RAC, it is non-essential in mouse, is a known RAS effector required for the activation of RAC by RAS (Lambert *et al.*, Nat Cell Biol 2002) and is required for RAS-induced skin tumours in mouse (Malliri *et al.*, Nature 2002).

To investigate the role of TIAM1 in LUAD we probed a human LUAD tumour microarray for TIAM1. We revealed not only cytoplasmic but also nuclear TIAM1. Interestingly nuclear TIAM1 prevalence was associated with advancing lung cancer stage (Figure 1A) and with decreased survival of patients, suggesting a pro-malignancy role for nuclear TIAM1 in LUAD. To investigate the role of TIAM1 in malignant progression of LUADs, we depleted TIAM1 in several LUAD cell lines which were also inducibly expressing nuclear TIAM1. Interestingly, TIAM1-depletion suppressed the migration of LUAD cells, an effect rescued by nuclear-localised TIAM1 (NLS-TIAM1) (Figure 1B). To determine the mechanism by which TIAM1 stimulates LUAD cell migration, we performed two proteomic screens for nuclear TIAM1 interactors. TRIM28, a transcriptional co-repressor that recruits histone deacetylase and histone 3 Lysine 9 (H3K9) methyltransferase to gene promoters, was identified in both screens and subsequently validated as a TIAM1 interactor. Interestingly, TIAM1-depletion reduced repressive histone methylation marks (H3K9me3). To further explore how TIAM1 promotes migration, we determined the effect of TIAM1-depletion on gene expression. RNA-sequencing and gene set enrichment analysis revealed significant upregulation of genes associated with cell-cell adhesion in TIAM1-depleted LUAD cells, particularly protocadherin (PCDH) family members and E-Cadherin, indicating that TIAM1 mediates epithelial-to-mesenchymal transition (EMT). Moreover, ChIP-seq demonstrated an overlap between genomic sites occupied by TIAM1, TRIM28 and H3K9me3 at regulatory regions of PCDH clusters, consistent with their repressed expression. Importantly, both TIAM1 and TRIM28 knockdown increased E-Cadherin at cell-cell junctions explaining the decreased migration following TIAM1 depletion. Furthermore, protocadherin depletion reversed the reduced migration due to TIAM1 or TRIM28 downregulation. We therefore conclude that TIAM1 promotes LUAD cell migration and invasion by suppressing cell-cell adhesion thereby contributing to EMT (Ginn *et al.*, PNAS 2023).

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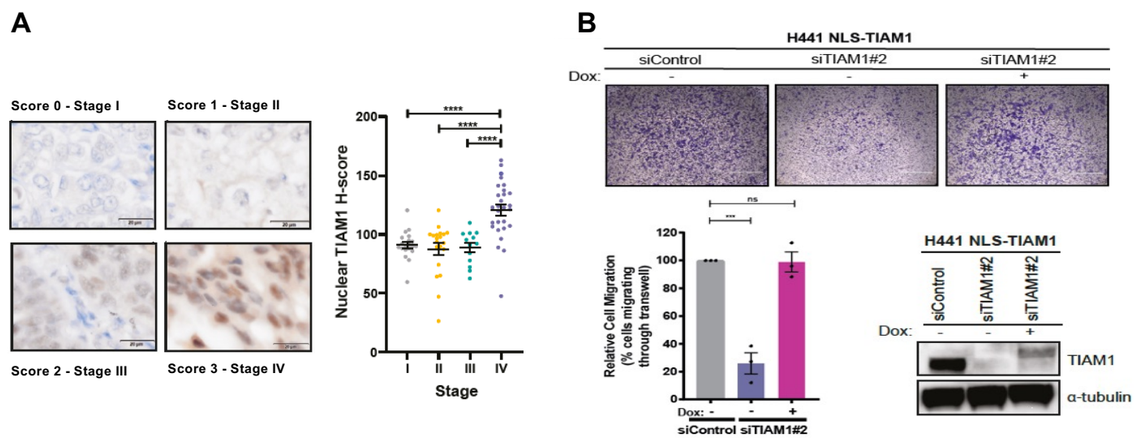

Figure 1
(A) Representative examples of strong (score 3), moderate (score 2), weak (score 1), and negative (score 0) immunohistochemical staining of TIAM1 expression in a panel of LUAD tumours from stage I-IV LUAD patients. Scale bars, 50 µm & 20 µm. (B) Representative images of H441 cells migrating through transwell inserts, transfected with TIAM1 or control siRNAs and inducibly expressing NLS-TIAM1 following the addition of doxycycline. Scale bar, 1000 µm. Graph shows average percentage of migrating cells where control cell migration is set to 100%. Western blot shows the expression of endogenous or exogenous TIAM1 (following the addition of doxycycline).

LEUKAEMIA BIOLOGY

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Recent years have seen significant progress in the development of better therapies for people with blood cancer, with concomitant improvements in response. However, there remains a substantial unmet need for more effective and less toxic treatments.

For example, outcomes in acute myeloid leukaemia (AML) are particularly poor in older adults and those with relapsed or refractory disease and malignancies, such as multiple myeloma, are incurable for the great majority. The overarching goal of the Leukaemia Biology group is to deliver a bench-to-bedside programme of blood cancer research.

Much of our effort is focused on understanding how transcription factors and their associated chromatin cofactors sustain myeloid blood cancers such as AML. In keeping with this, in 2023 we reported our discovery of how a small molecule bromodomain inhibitor of the acetyltransferases EP300 and CBP induces cell cycle arrest and cellular differentiation in blood cancer, as well as our preliminary data from the early phase clinical trial evaluation of CCS1477, where we see promising signs of clinical activity across a range of haematological malignancies.

EP300 and *CBP* are paralogs coding for essential transcription factor coactivators that occupy DNA enhancer elements. The proteins are large with multiple domains that mediate scaffolding interactions with dozens of proteins in a cell context dependent manner. Both have promiscuous acetyltransferase activity acetylating diverse targets including histones, transcription factors, chromatin remodelling proteins and transcriptional coactivators. Evidence of the attraction of EP300/CBP as a target in haematological malignancy includes (i) its critical role in promotion of cellular growth and cell cycle progression, (ii) chromosomal translocations in AML such as *MLL-EP300* and *MOZ-CBP*, and (iii) the presence of frequent mutations in lymphoid malignancies such as lymphoma.

Our colleagues at CellCentric, a UK-based biotechnology company, developed CCS1477 (also known as inobrodib), which is a potent and selective, first-in-class inhibitor of the bromodomains of EP300 and CBP. In our pre-clinical analyses, we found that CCS1477 induces cell cycle arrest and cellular differentiation in a range of haematological malignancy models including cell lines, murine models and patient samples. Indeed, in two separate retroviral transduction and transplantation murine AML models, treatment of mice with oral CCS1477 for 42 days was sufficient to cure them of the disease. To explore the mechanism of action, we treated both AML cells and multiple myeloma cells with CCS1477 in time-course experiments and performed detailed analysis of the transcriptome, chromatin accessibility and chromatin occupancy by selected factors. In AML cells CCS1477 induced rapid eviction of EP300/CBP from an enhancer subset marked by strong MYB occupancy and high H3K27 acetylation, with downregulation of the subordinate oncogenic network and redistribution of EP300/CBP to RFX5-occupied enhancers controlling differentiation genes. In a similar manner, in multiple myeloma cells we found that CCS1477 induced eviction of EP300/CBP from *FGFR3*, the target of the common (4;14) translocation, as well as redistribution away from IRF4-occupied and to TCF3/E2A-occupied sites.

As the laboratory work was progressing, we were able to evaluate in parallel initial results from our Phase 1 clinical trial where we are evaluating the safety and efficacy of CCS1477 across a range of haematological malignancies. Notably, in patients with relapsed AML, CCS1477 monotherapy induced differentiation responses and in heavily pre-treated multiple myeloma and T-cell lymphoma patients there were long lasting objective responses, which is truly remarkable for a novel agent in early phase development (Figure 1). Thus, CCS1477 exhibits encouraging preclinical and early phase clinical activity by disrupting recruitment of EP300/CBP to enhancer networks in blood cancer cells occupied by critical lineage-specific transcription factors.

It is generally the case that treatments in blood cancer are delivered in combination to maximise the chances of disease remission and cure. We have evaluated CCS1477 in combination with standard-of-care agents in xenograft models of AML and myeloma. We discovered that in AML azacitidine and venetoclax exhibit additive or synergistic activity, as do bortezomib, lenalidomide and

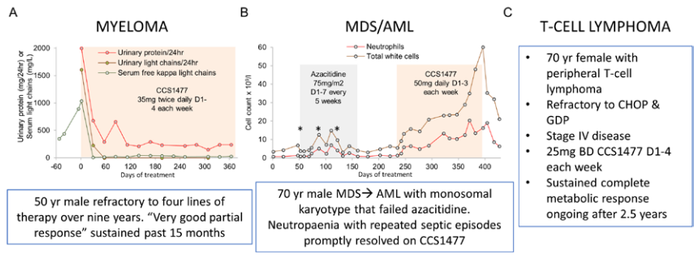

Figure 1. Summary of exemplar case histories from Nicosia et al. (Cancer Cell, 2023) showing disease and treatment response trajectories in patients given oral CCS1477 monotherapy at the indicated dose. In (B), asterisks indicate timing of azacitidine cycles. Orange boxes indicate duration of CCS1477 therapy.

vorinostat in myeloma. Such experiments have already informed on the combinations being tested in the next phase of our early phase clinical trial, and with encouraging results. Specifically, addition of CCS1477 to venetoclax/azacitidine can induce remission in relapsed/refractory AML patients who had exhausted standard-of-care options. In myeloma, addition of CCS1477 to pomalidomide (which promotes cereblon-mediated degradation of IKZF1/3) can resensitise pomalidomide-refractory patients resulting in objective responses. Thus, there is already clear evidence that combining CCS1477 with additional agents has a positive impact in multiple blood cancers.

Based on the totality of this data the US FDA recently granted CellCentric "Fast Track designation" for treatment of patients with relapsed or refractory multiple myeloma and Orphan Drug designation. CellCentric has also received strategic investment from Pfizer to drive forward the exciting next steps in clinical development.

In 2023 we also reported the findings of our investigation of the mechanisms by which a transcription factor gene called Iroquois Homeobox 3 (*IRX3*) gets upregulated in acute myeloid leukaemia. *IRX3* is normally expressed in the developing nervous system, limb buds and heart, and transcript levels specify obesity risk in humans. Its expression levels in normal haematopoiesis are minimal. In a previous study (Somerville et al., 2018) we reported a functional role for *IRX3* in human acute leukaemia: tissue-inappropriate derepression of *IRX3* contributes significantly to the block in differentiation, which is the pathognomonic feature of human acute leukaemias. High *IRX3* expression is found in ~30% of patients with acute myeloid leukaemia (AML), ~50% with T-acute lymphoblastic leukaemia and ~20% with B-acute lymphoblastic leukaemia, frequently in association with high-level HOXA gene expression. Expression of *IRX3* alone is sufficient to immortalise a hematopoietic stem and progenitor cells (HSPCs) in myeloid culture and induce lymphoid leukaemias *in vivo*. *IRX3* knockdown induced terminal differentiation of AML cells and combined *IRX3* and *Hoxa9* expression in murine stem and progenitor cells impeded normal T-progenitor differentiation in lymphoid culture and substantially enhanced the morphologic and phenotypic differentiation block of AML in myeloid leukaemia transplantation experiments through suppression of a terminal myelomonocytic programme. Likewise, in cases of primary human AML, high *IRX3* expression is strongly associated with reduced myelomonocytic differentiation.

It has not been clear, however, how IRX3 expression is upregulated in AML. Through making use of transcriptomics, ChIP sequencing, DNA methylation analysis and assessments of DNA contacts in cells through 4C-sequencing, we discovered that sequences in intron 8 of a neighbouring gene called fat mass and obesity-associated (*FTO*) located ~220kB downstream of IRX3 exhibit histone acetylation, DNA methylation and physical contacts with the IRX3 promoter. The extent of each of these correlates with *IRX3* expression in AML cells. Deletion of these *FTO* intron 8 enhancer elements confirmed their role in positively regulating *IRX3* expression. Furthermore, RNA sequencing revealed the presence of long non-coding (lnc) transcripts arising from this locus in AML cell lines and primary samples from patients with NPM1-mutated AML.

Knockdown of these *FTO*-lncAML transcripts induced differentiation of AML cells, loss of clonogenic activity and reduced *FTO* intron 8:*IRX3* promoter contacts. While both *FTO*-lncAML knockdown and *IRX3* knockdown induced differentiation, FTO-lncAML knockdown but not IRX3 knockdown led to HOXA downregulation, suggesting long non-coding transcript activity *in trans* regulates HOX gene expression. In keeping with this *FTO*-lncAML^{high} AML patient samples expressed higher levels of HOXA and lower levels of differentiation genes. Taken together, this study led by former PhD student Francesco Camera, demonstrates that a regulatory module in *FTO* intron 8 consisting of clustered enhancer elements and a long non-coding RNA is active in human AML, impeding myeloid differentiation (Figure 2).

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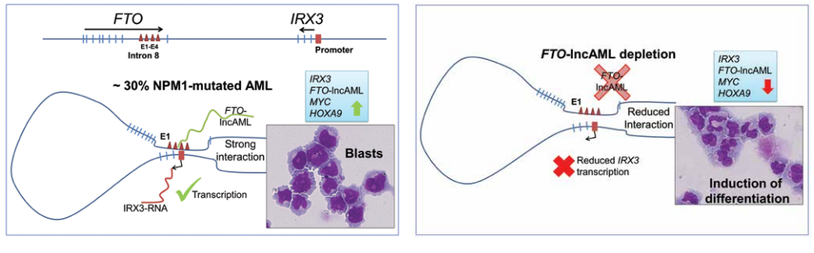

Figure 2. Summary of findings from Camera et al. (iScience, 2023). A regulatory module in FTO intron 8 consisting of clustered enhancer elements and a long non-coding RNA is active in human AML, impeding myeloid differentiation.

LEUKAEMIA IMMUNOLOGY & TRANSPLANTATION

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For most patients with acute myeloid leukaemia (AML) allogeneic haematopoietic stem cell transplantation represents the only prospect of cure. The therapeutic effect of transplant depends on the ability of donor immune cells to eliminate residual disease, but despite >50 years of clinical experience, we still don't understand why some patients relapse and others do not. Our group aims to elucidate mechanisms of immune evasion and devise novel therapeutic strategies to treat or prevent AML recurrence.

Inducing leukaemic differentiation to augment donor T-cell responses

A common finding at post-transplant AML relapse is transcriptional downregulation or genomic loss of major histocompatibility complex class II (MHCII) molecules. MHCII allows professional antigen presenting cells (APCs) to display extrinsic peptides to CD4+ T cells and these observations suggest that interactions between AML and CD4+ T cells are key to establishing successful donor immune responses. AML is a cancer of the progenitors that normally gives rise to APCs, such as macrophages, and the cardinal pathologic feature is a block to differentiation. Attempts to overcome this block have yielded a series of compounds that induce leukaemic differentiation and show promise in clinical trials. We are investigating the potential of drugs that induce leukaemic differentiation to drive expression of MHCII, enable robust CD4+ T-cell activation and promote successful disease clearance (Figure 1A). This is a very appealing strategy in the context of stem cell transplantation, where treatments that promote generalised donor immune cell activation generally cause unacceptable toxicity.

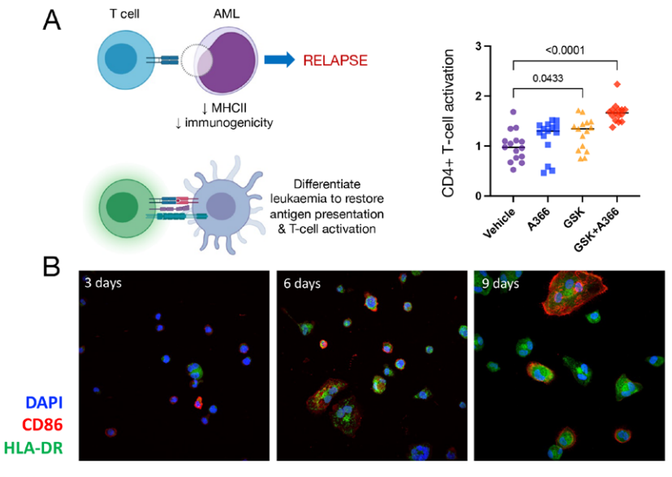

Figure 1. (A) Diagram depicting the hypothesis that inducing leukaemic differentiation enhances antigen presentation and T-cell activation. (B) Immunohistochemistry demonstrating pharmacological induction of differentiation in human leukaemia. (C) Chart showing increased CD4+ T-cell activation upon culture with human AML treated with various compounds (5 AML cell lines cultured with T-cells from 3 healthy donors).

Postdoc Teresita Flores has been exploring various compound combinations that induce leukaemic differentiation and increase expression of MHCII and CD86, the latter being a co-stimulatory molecule that is essential for effective T-cell activation (Figure 1B). Drug-induced differentiation produces cells that display many morphological, transcriptional and phenotypic similarities with APCs whilst retaining leukaemic transcriptional features; we therefore term these cells leukaemia-derived APCs (LD-APCs). These cells are able to process extrinsic antigen and augment CD4+ T-cell activation (Figure 1C). PhD student Florentia Mousoullou has developed murine models of inducible leukaemic differentiation enabling us to study *in vivo* immune phenomena, such as T-cell priming, tumour rejection and the formation of immunological memory.

Identifying leukaemia-associated T-cell populations

Another core aim of our group is to understand the mechanisms that drive T cell dysfunction and permit leukaemia immune escape. However, we must first identify the T-cell populations that are relevant for disease pathogenesis and control. Myeloid malignancies are yet to benefit from the immunotherapy revolution and a likely explanation is that the immunobiology of the bone marrow in general, and these diseases in particular, remains critically understudied. Compared to solid tumours, AML immunology is complicated by the fact that bone marrow is both a primary and secondary lymphoid organ and also serves as a reservoir for multiple immune populations. Recent studies of AML immunology have tended to exploit single cell RNA sequencing approaches but given that leukaemia-reactive T cells typically represent <1% of bone marrow T cells, these studies are often critically underpowered. To address this problem, postdoc Vicky Smith has developed a mass cytometry panel that provides deep profiling of T cells with a particular focus on T-cell dysfunction/exhaustion. Her protocol includes control samples to correct for batch effects and permits the profiling of hundreds of millions of cells from hundreds of patients. This approach allows us to identify rare T-cell populations and correlate their abundance with disease features, patient characteristics and clinical outcomes. Early data has identified several novel AML-associated T-cell populations, which are not found in healthy bone marrow and display differential abundance depending on disease genotype (Figure 2A&B).

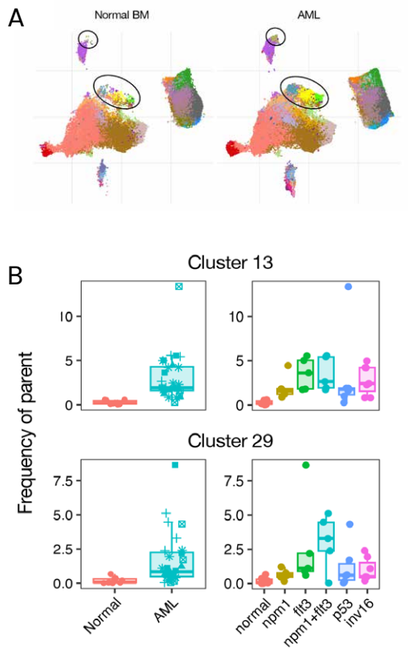

Figure 2. Mass cytometry data comparing healthy (n=10) to AML (n=28) bone marrow. (A) UMAP of non-naïve CD8+ T cells from healthy (~155,000 cells) and AML (~160,000 cells) bone marrow. Leukaemia-associated populations are highlighted. (B) Box plots showing cluster abundance by disease status (left) or leukaemia genotype (right).

Developing biomarkers of immune dysfunction to predict post-transplant AML relapse

Currently, we have no way of knowing whether a transplant is working or not. Minimal residual disease assessments based on molecular or flow cytometric evaluation provide early warning of impending relapse but contain no information regarding the establishment or loss of a donor immune response. Several small studies have detected exhausted T cells in the blood and bone marrow of patients who go on to experience post-transplant relapse. We will use our T-cell dysfunction mass cytometry panel to establish whether T-cell exhaustion can be used to identify patients who have failed to establish a durable anti-leukaemic immune response. These patients could potentially be salvaged by giving early donor lymphocyte infusions. To this end we have opened a clinical study called Precision Medicine for Stem Cell Transplantation (PM-SCT) that will collect peripheral blood samples at 8 timepoints from 300 transplant recipients (Figure 3). The study opened in June 2023 and has now recruited 65 patients.

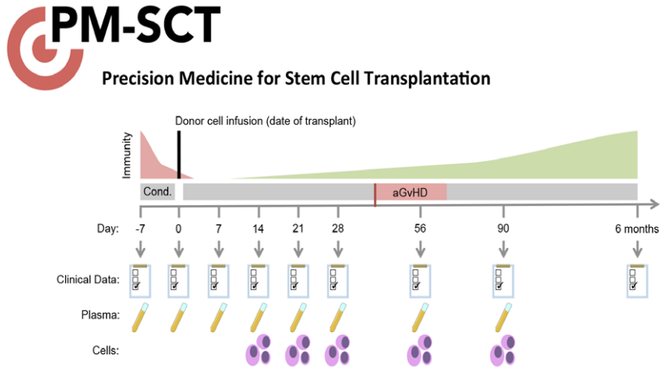

Figure 3. Schematic depicting the study design and sample collection schedule for PM-SCT.

Predicting transplant complications

Our laboratory is also interested in developing biomarkers that predict the onset of graft-versus-host disease (GvHD), a devastating transplant complication and common cause of death for recipients. Healson Ihuoma, a clinical haematology trainee, has been supporting PM-SCT as an NIHR associate PI. He is planning to join our group within the next 18 months and begin analysing study samples. Several tissue leakage proteins can be found in plasma following transplantation, reflecting sub-clinical organ damage that may occur weeks before the onset of clinically apparent GvHD. Healson will be applying MS- and affinity-based proteomic methods to longitudinal blood samples to discover novel biomarkers that predict GvHD onset. He will also be exploring the utility of cell free DNA methylation to detect sub-clinical inflammation and identify the tissue of origin. Our ultimate ambition is to combine our studies of relapse and GvHD, developing multi-modal signatures and algorithms capable of predicting multiple transplant outcomes.

Experimental transplantation

Another exciting development has been the formation of a collaboration with Professor Rob Wynn of the Royal Manchester Children's Hospital at the Manchester University NHS Foundation Trust. Professor Wynn has been pioneering a highly innovative transplant protocol that has shown promising results in children with highly refractory leukaemias. In the coming months we will begin analysing samples from Rob's trial, aiming to understand how his approach supports donor immune responses and mitigates the leukaemic immune evasion mechanisms we have been characterising.

SKIN CANCER AND AGEING

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[1]Joined in 2023
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The Skin Cancer and Ageing lab studies the mechanisms that impact the efficiency of melanoma metastasis by age. Aged individuals accumulate genetic damage and epigenetic changes, leading to alterations in cellular pathways that drive the development and progression of cancer. Additionally, ageing has a profound impact on the immune system and on the metabolic energy system that can also affect cancer incidence and progression. Skin cancer mostly affects the aged population and has unique clinical and epidemiological presentation in this subset of patients. Aged patients more frequently present melanomas that arise at chronically sun exposed sites, like the head and neck, and are more likely to present solid organ metastasis.

In contrast, younger patients are more likely to report sunburns in childhood. Critically, younger melanoma patients more often present brain metastases, in contrast to older patients, who more frequently have metastases in other visceral organs like the lungs and the liver. Our lab studies why melanoma is more metastatic in aged patients, why it seeds visceral organs, and how the response to therapy differs in young and aged patients.

Melanoma and the tumour microenvironment

The incidence and mortality rates in melanoma continue to rise, as does the proportion of the population over 60 years old in the UK. Older patients with melanoma are also at higher risk of other cancers and specifically at very high risk of developing other skin cancers, which complicates their plan of care and prognosis. Most skin cancer deaths and skin cancer complications affect the elderly, and mortality due to skin cancer is specifically increasing in this group of the population.

We are studying how the lipids that normally nourish the skin (stromal lipids), where primary cutaneous melanoma arises, differ in young and aged skin and affect patient survival. Stromal lipids are used physiologically in the skin to maintain homeostasis, but cancer cells that develop in the skin can co-opt these nutrients and use them to grow. Our preliminary results on how skin lipids are used by melanoma cells have now expanded to look at how solid organs colonised by melanoma may also be affected by lipid quantities and lipid species.

In November 2023, our lab started a new research programme, entirely funded by Cancer Research UK, to continue this work. This grant will support our lab over the next five years. We have started research to discover how tissue specific factors, including lipids, within the organs of melanoma metastasis (lung, brain, liver and lymph nodes) impact melanoma cell growth once melanoma cells seed their metastatic organ of destiny. Furthermore, we have preliminary work showing that the tissue specific nutrients, which vary by organ, can impact the way patients respond to therapy. We are studying specifically whether secondary organ nutrients affect the organ-specific immunotherapy rate of response, as in clinical practice we see lower rates of response for metastatic melanoma that seed the brain and the liver.

Melanoma and diet

With ancillary funding from Rosetrees and the Melanoma Research Alliance, we are expanding the focus of our stroma research to study how obesity in humans affects the response to immunotherapy. We have new preclinical melanoma models indicating that dietary factors and medication can synergise and enhance immunotherapy response in overweight animals. We are currently looking at how we can implement dietary interventions in humans in clinical practice to improve the response to therapy.

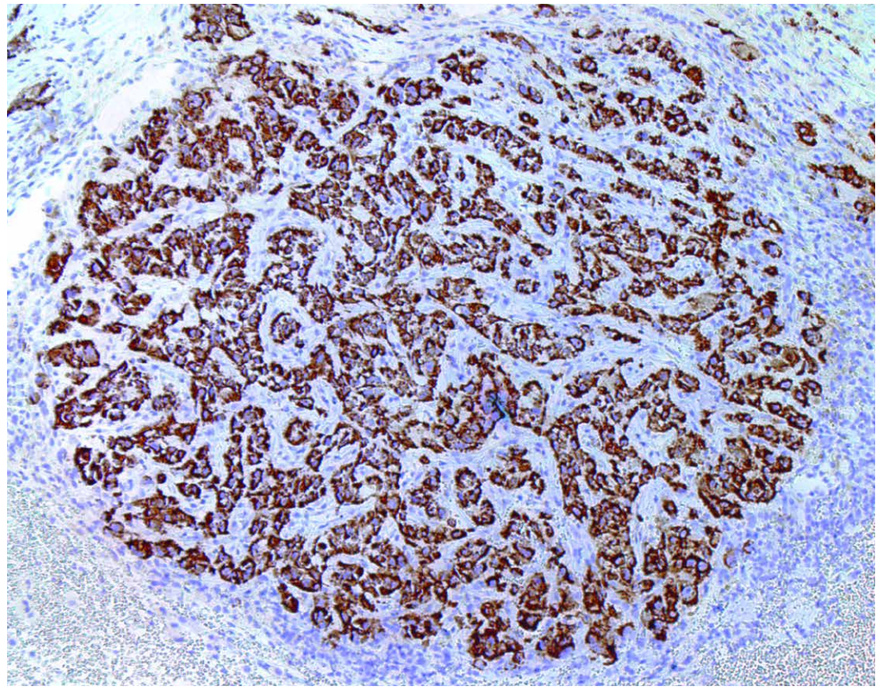

Figure 1. Histological slice showing cutaneous squamous cell carcinoma. Tumour cell mitochondria are stained brown.

Image supplied by Tim Budden.

Epithelial cancer invasion and the TME

Our lab has been contributing to the CRUK early detection initiative by studying tissue microenvironment factors that are in human epithelia and impact cancer initiation and invasion. Epithelial tissues are critical in physiology, as they are the primary interface between the internal and external environments, lining the surfaces of organs and cavities in the body. Their main role is to form a protective barrier against external injury. Squamous cell carcinomas (SCC) are tumours that arise from epithelia and have distinct clinical courses depending on the epithelium of origin. SCCs of the skin often affect aged patients in multiplicity and are the second most common human cancer. Although they are extremely common, they have a low rate of mortality (2%). In contrast, SCCs that arise from lung, head and neck, and the oesophagus have a very high rate of mortality. Our lab has found that there are differences in epithelia by anatomic site that underpin a different disease progression from *in situ* disease to invasive, deadly carcinoma. We are now collaborating with clinical partners to identify early-stage patient samples to confirm our findings.

UV light and melanoma immunotherapy

Melanoma incidence is strongly associated with ultraviolet (UV) light exposure, and excessive UV is linked to higher melanoma incidence. Both UV-driven inflammation and DNA damage to melanocytes have been linked to melanoma initiation. In our lab we have a research programme looking at how UV radiation can impact melanoma survival and the response to immunotherapy in cancer patients.

Group developments

In September 2023, two new PhD students joined our lab. Ms Vanessa Parietti is working on melanoma liver metastases, and Ms Charlotte Russell is exploring how ageing affects the rate of melanoma development in the brain.

Dr Tim Budden, our senior Postdoctoral Fellow has successfully secured a principal investigator lecturer position at the University of Liverpool. He will start this new job in the coming year and focus his research on lung and other epithelial cancers. He will remain linked to our team as we plan to pursue common research interests in epithelial tumours and the role of the stroma. His work in the past year has focused primarily on early detection and how the extracellular matrix affects melanoma cell invasion and immunity in the skin.

Dr Shilpa Gurung, who obtained her PhD with us in 2022, continued with a one-year postdoctoral position with the lab and has now taken up a postdoctoral research associate position studying brain metastases at The University of Manchester.

Publications listed on page 60

STEM CELL BIOLOGY

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[3]Joined in 2023

Cytotoxic therapy has stood as the mainstay treatment for acute myeloid leukaemia (AML) for decades, yet its efficacy remains limited, with a five-year survival rate hovering around 20%. This sobering statistic underscores the urgent need for more precise and efficient therapeutic strategies.

To this end, our laboratory undertakes two integrated research programmes: a) investigating mechanisms driving initiation and maintenance of leukaemia; and b) extending the understanding of normal haematopoietic system development. Through these efforts, we aim to identify and validate new therapeutic targets for leukaemia treatment while also refining protocols for the *in vitro* production of clinical-grade blood cells, particularly for adoptive cancer immunotherapies.

MOZ and AMLs

Acute myeloid leukaemia (AML) is a devastating clonal haematological neoplasm with vast genetic and clinical heterogeneity but uniformly high morbidity and mortality and a limited therapeutic arsenal. The disease emerges when mutations in human haematopoietic stem cells (HSCs) give rise to leukaemic stem cells, whose large, immature blast cell progeny cannot differentiate, resulting in infiltrative bone marrow failure and death.

Central to our investigation is KAT6A, also known as MOZ or MYST3, a member of the MYST family of lysine acetyltransferases (KATs) crucial for HSC development and maintenance. The histone/lysine acetyl transferase (HAT/KAT) activity of KAT6A plays a pivotal role in haematopoietic stem cell maintenance. The co-activations of the haematopoietic specific transcription factors RUNX1 and PU.1 through interaction with KAT6A serine/methionine (SM)-rich domain are also likely contributory. Additionally, KAT6A interaction with the mixed lineage leukaemia (MLL) protein, a histone methyl transferase, through its catalytic KAT domain (also called MYST domain) regulates HOXA gene expression in HSCs. Notably, HOXA proteins are implicated in maintaining self-renewal and repressing senescence in leukaemia, two cellular functions impaired in KAT6A-deficient foetal cells. Moreover, aberrant HOX gene expression has long been acknowledged in AML, with a recent analysis of 776 patients reporting that KAT6A is one of the three 'most significant upstream regulators' of the HOXA cluster in AML. It is, therefore, unsurprising that KAT6A was identified as a genetic vulnerability in AML and, specifically, MLL rearranged (MLLr) AML in two separate CRISPR screens, a finding corroborated by the DepMap consortium.

Mechanistically, it has been shown in an MLL-AF9 AML cell line that KAT6A acetylates H3K9 at *MYC*, *MYB* (and other) promoters enabling binding of ENL, which recruits transcriptional activation and elongation machinery for these genes, thereby upregulating stemness-associated genes. Therefore, targeting the catalytic activity of KAT6A in MLL-AF9 AML is a promising therapeutic strategy. *KAT6A* is also over-expressed in a variety of tumour types, including breast, prostate, ovarian and cervical cancers, lung, colon and rectal adenocarcinomas, and medulloblastoma. The KAT6A inhibitor WM-1119, the first-in-class of the MYST family acetyltransferases, has entered Phase 1 clinical trials (NCT04606446) in breast, lung and prostate cancers.

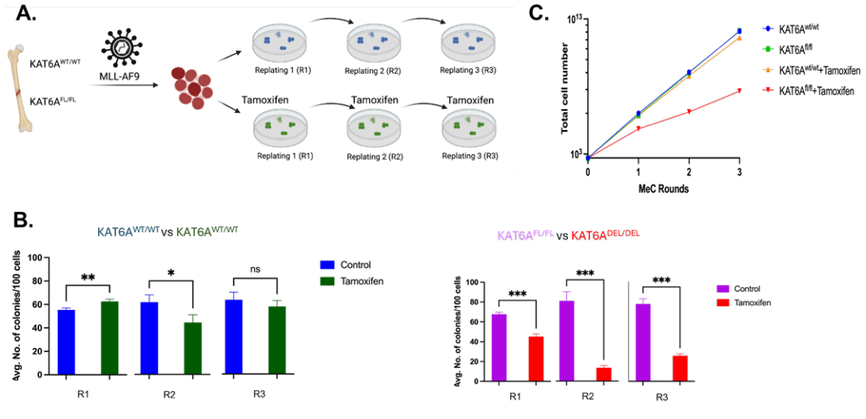

Figure 1. Deletion of the entire KAT6A protein reduces leukaemogenicity of a murine MLL-AF9 AML. A. Scheme for serial replating colony forming assays to investigate the role of the catalytic site of KAT6A. cKIT$^+$ cells from the bones of mice with a tamoxifen inducible Cre-loxP system and the listed genotypes were infected with MLL-AF9 virus. The resulting AML cells were replated in semi-solid media with or without tamoxifen. B. Tamoxifen does not affect colony forming ability but deletion of the KAT6A protein does. C. Total number of leukaemic cells are decreased KAT6A deletion. * p<0.05, ** p<0.005, *** p<0.0005.

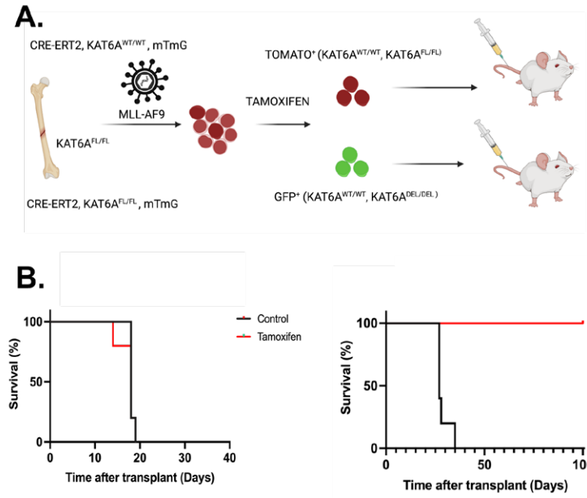

Figure 2. Deletion of the entire KAT6A protein improves survival of a murine MLL-AF9 AML model. **A.** Scheme detailing *in vivo* assessment. Primary leukaemia was generated by lentiviral infection of cKIT$^+$ cells from mice with floxed KAT6A or wild type controls. Primary leukaemia cells were then treated *ex vivo* with tamoxifen, sorted by FACS for those cells in which Cre was active (GFP$^+$) or inactive (Tomato$^+$) and transplanted into sub lethally irradiated C57/Bl6 mice. **B.** Deletion of KAT6A abolishes the leukaemogenicity of murine MLL-AF9 *in vivo*.

Whilst the importance of the catalytic activity of the KAT domain has been shown, this does not exclude the importance of KAT6A's scaffolding function, given it binds BRPF1, which is itself implicated in leukaemogenesis, and MLL, through this KAT domain, and interacts with other key haematopoietic transcription factors by its SM-rich domain. We performed studies employing genetically engineered mouse models (GEMMs) to compare the relevance of both the catalytic activity and scaffolding function of KAT6A in leukaemogenesis. These models contain a floxed functional KAT6A (FL) that can be deleted (DEL) upon CRE activation with tamoxifen or a KAT6A mutant allele (MUT) in which two amino acid substitutions result in a catalytically inactive KAT domain. These two GEMMs were coupled with tamoxifen inducible Cre and the two-colour fluorescent Cre reporter allele mTmG.

We generated primary MLL-AF9 AML with our different GEMMs. Serial replating colony-forming assays were then performed. We found a highly significant reduction in colony-forming ability across all rounds of replating in cells with deleted KAT6A (KAT6A$^{fl/fl}$ +Tamoxifen) (Figure 1). In contrast, the *in vitro* colony-forming activity was not significantly affected when the KAT activity of KAT6A was abrogated by genetic (KAT6A$^{mut/fl}$ +Tamoxifen) (not shown). This highly significant reduction of proliferation of MLL-AF9 in the absence of KAT6A was translated *in vivo* to complete survival (Figure 2). ShRNA and CRISPR KAT6A knockdown and knockout experiments further confirmed that KAT6A is a genetic vulnerability in several human MLLr-AML cell lines bearing different chromosomal translocations (not shown). The differentiation phenotype seen upon knockdown or knockout of KAT6A was more marked than that seen upon treatment of cells with the KAT6A inhibitor WM-1119 (not shown). Altogether, our results suggest that targeting the full KAT6A protein could be a better therapeutic strategy than inhibiting its KAT activity.

Cellular immunotherapies

Cell-based cancer immunotherapy has revolutionised the treatment of haematological malignancies. Specifically, autologous chimeric antigen receptor-engineered T (CAR-T) cell therapies have received approvals for treating leukaemia, lymphoma, and multiple myeloma following unprecedented clinical response rates. Despite their unprecedented clinical responses, their autologous nature poses significant challenges related to cost and manufacturing complexity. In contrast, allogeneic cell products offer scalability and ease of administration but are hindered by concerns such as graft-versus-host disease (GvHD) and rejection.

Stem cell-derived immune cells could represent potential alternatives to offer off-the-shelf therapies. These stem cell-engineered allogeneic cell therapies could include conventional αβ T cells as well as unconventional T (iNKT and γδ T) cells, natural killer (NK) cells and myeloid cells. Most of these cells could be generated either from haematopoietic stem cells (HSCs). Alternatively, they could also be produced directly from a subtype of endothelial cells, haemogenic endothelial (HE) cells, through an endothelial-to-haematopoietic transition (EHT). Understanding the generation process of these cells is paramount to establishing scalable platforms for therapeutic blood cell production.

In conclusion, our multifaceted research efforts encompass elucidating the molecular mechanisms underlying leukaemia pathogenesis, identifying therapeutic targets such as KAT6A, and advancing cellular immunotherapies to improve treatment outcomes for patients with AML and other haematological malignancies. Through these efforts, we strive to translate scientific discoveries into clinical interventions that offer hope and benefits to patients battling these devastating diseases.

Publications listed on page 60

SYSTEMS ONCOLOGY

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Luke Taylor[2,4]

[1]Left in 2023
[2]Joined in 2023
[3]Joint with Juan Valle, Christie NHS FT and Lucy Foster, MFT
[4]Joint with clinicians at the Christie NHS FT and MRI FT, and the MCRC Biobank

Tumours are complex ecosystems where cancer cells are embedded within an intricate stromal microenvironment comprising multiple infiltrating cell types and pathological changes to the extracellular matrix. The aim of the Systems Oncology laboratory is to determine and define how tumour cells conscribe host cells to support tumour development and resistance to therapies. Understanding these rules will enable the development of rational combination therapies targeting both tumour cell intrinsic dependencies as well as their extrinsic dependencies on stromal reciprocal signals.

Pancreatic ductal adenocarcinoma

Pancreatic ductal adenocarcinoma (PDA) is a dismal disease with an average five-year survival rate of 12%. PDA is the 11th most common cancer in the UK but the fourth largest contributor to cancer related deaths. Due to the limited treatment options and propensity for late detection, PDA is projected to be the second largest contributor to cancer related deaths by 2023. A characteristic feature of PDA is an extensive desmoplastic reaction, which makes up 85% of the tumour volume on average. Here, an abundant and pathological remodelled extracellular matrix increases tissue stiffness and interstitial pressure, which results in decreased therapeutic efficiency. Moreover, the microenvironment contains an abundant fibroblast and myeloid cell infiltrate, which reduces immune surveillance and confers resistance to therapy.

Mapping the tumour microenvironment of PDA

Due to the abundant tumour microenvironment much emphasis has been given to mapping of signalling pathways by which tumour cells conscript host cells. Pre-clinical studies have further demonstrated that these pathways can be successfully targeted to improve therapeutic response. However, in some cases, therapeutic and genetic targeting of the microenvironment has resulted in accelerated disease progression rather than retardation. Collectedly, these results suggest that interactions between tumour and host can be tumour restrictive as well as tumour promoting, yet the molecular mechanisms governing these pro- and anti-tumour effects remain poorly defined.

We recently used single cell mass cytometry to annotate the microenvironmental composition in a commonly used murine model of PDA (pdx-1 Cre; $KRas^{LSL\text{-}G12D/Wt}$; $p53^{LSL\text{-}R172H/Wt}$; KPC). The advantage of an antibody-based approach is that individual cell populations of interest can subsequently be purified and analysed functionally. We observed that PDA tumours contain two separate populations of cancer associated fibroblasts (CAFs) distinguished by the expression of CD105 (Endoglin). Moreover, we identified both $CD105^{pos}$ and $CD105^{neg}$ fibroblasts in all normal and tumour-bearing tissues analysed.

Isolation and characterisation of both $CD105^{pos}$ and $CD105^{neg}$ pancreatic fibroblasts revealed that the two stromal subsets express CD105 in a noninterchangeable manner and respond differentially to most exogeneous signals tested. This suggests that the subsets may have distinct functional roles in the tumour microenvironment. Indeed, tumour cells co-implanted with $CD105^{pos}$ fibroblasts grew slightly faster than tumour cells implanted in isolation, suggesting a tumour permissive role of $CD105^{pos}$ fibroblasts. In contrast, co-implanted $CD105^{neg}$ fibroblasts restricted tumour growth. This effect is dependent on functional immunity. Collectedly, these data demonstrate that tumour permissive and restrictive fibroblasts co-exist throughout PDA development.

To subsequently interrogate how $CD105^{neg}$ fibroblasts might engage the immune system to regulate tumour growth, we aimed to identify functionally important transcriptional programmes and the upstream transcription factor(s) regulating these. To this end, we undertook a computational predictive analysis to identify regulators of differentially regulated genes from $CD105^{pos}$ and $CD105^{neg}$ CAFs isolated from the KPC mouse and $CD105^{neg}$ fibroblasts co-cultured with tumour and immune cells *in vitro*. This identified C/EBPb as a putative transcriptional regulator. Notably, analysis of publicly available single cell gene expression data revealed that C/EBPb expressing CAFs were also enriched for immune regulating signals. Moreover, C/EBPb was bound to immune regulating genes and genetic deletion of C/EBPb in fibroblasts blunted expression of immune-regulating genes. Importantly, in contrast to the tumour suppressive effect observed from co-implanted $CD105^{neg}$ wild-type fibroblasts, $CD105^{neg}$ C/EBPb^{null} fibroblasts were significantly blunted in their ability to control tumour growth. Remarkably, this was correlated with decreased immune cell infiltration in developing tumours. Subsequent systematic analysis of C/EBPb regulated genes identified that C3 and G-CSF were individually required for $CD105^{neg}$ fibroblast control of tumour growth *in vivo*. Together, these results demonstrate an intricate relationship between tumour suppressive fibroblasts and the immune system and provide molecular insights into the signals that govern tumour development.

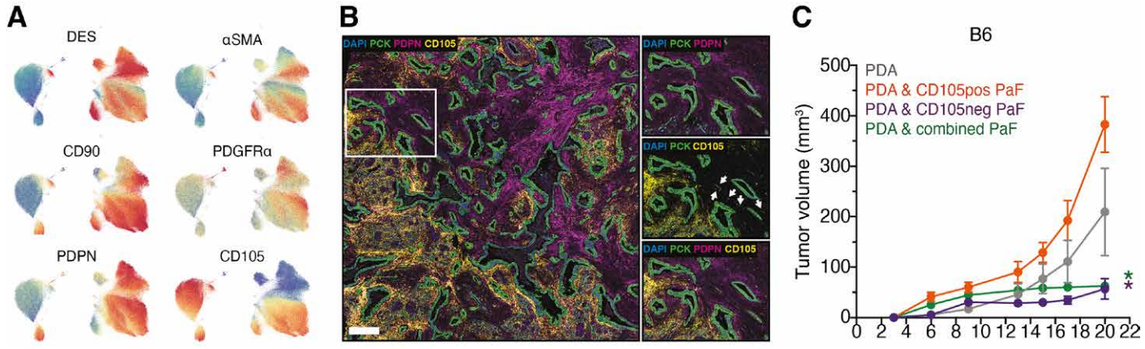

Figure 1. Identification and characterisation of CD105 positive and CD105 negative fibroblasts and their function. A. Mass cytometry analysis of murine PDA tumours. Selected markers of cancer-associated fibroblasts are shown. **B.** Multiplexed immunofluorescence of human resected PDA validates the existence of CD105 positive and CD105 negative fibroblast subsets. **C.** Functional analysis of CD105 positive and CD105 negative fibroblasts. Whereas CD105 positive fibroblasts are permissive for tumour growth, CD105 negative fibroblasts are tumour restrictive.

Modelling human pancreatic cancer

Tumour cells are embedded in a biophysically stiff environment and constitute less than 15% of the tumour volume in patients, yet most *in vitro* models do not replicate these aspects. In an ongoing collaboration with Prof Linda Griffith (MIT) and Prof Martin Humphries (UoM) we have adapted a fully synthetic scaffold to support the growth of both tumour and host cells. Peptide ligands were used to mimic adhesive signals found in the tumour microenvironment of pancreatic cancer, which enabled growth of both normal and tumour cells. Moreover, tumour cells grown in these scaffolds produce their own extracellular matrix, which we found engages integrin ligands in a similar manner to what is observed *in vivo*. Due to the synthetic nature of these scaffolds, they can be modified to recapitulate the entire stiffness range of patient tumours. Notably, tumour cells exhibit different growth morphologies and signalling depending on the scaffold stiffness, suggesting that incorporation of these models will be important to further address the impact of the environment on tumour cell function and to functionally interrogate stromal targeted therapies in patient derived models. Ongoing work in the laboratory is seeking to fully explore the application of these models to interrogate tumour cell dependencies.

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TRANSLATIONAL LUNG CANCER BIOLOGY

Institute Fellow
Carlos Lopez-Garcia

Scientific Officer
Anthony Oojagger

Graduate Student
Julia Ogden

Lung squamous cell carcinoma (LUSC) is an aggressive type of lung cancer that originates in bronchial basal cells with limited therapeutic options. Only chemotherapy and immunotherapies result in marginal improvement of survival in LUSC patients. Targetable genetic alterations are infrequent in LUSC, resulting in a lack of targeted treatments that can only be overcome by unravelling new dependencies in this disease.

Early detection is currently the most effective tool to prevent deaths by LUSC. Screening programmes by CT-scanning in high-risk populations have overwhelmingly confirmed this benefit. However, 40% of patients diagnosed with early-stage disease still die within five years, having failed to detect preinvasive lesions. These precancerous bronchial lesions show high-risk of malignant progression but can be easily removed with minimally invasive procedures. However, detection of these high-risk premalignant lesions is rare as scalable methods to detect them in screening programmes have not been developed.

Preventing death in LUSC patients requires improving therapeutic modalities and identification of new early detection biomarkers. These improvements depend heavily on more ambitious, innovative, and patient-relevant preclinical models that recapitulate the intra-tumour and inter-patient heterogeneities so frequent in this disease as well as developmental stages of LUSC progression. However, existing LUSC models do not recapitulate those complexities, which is a barrier to reversing the dismal landscape of LUSC.

Modeling the complexity of LUSC

Lung squamous cell carcinoma has been historically difficult to model using genetically engineered mouse models (GEMMs), and to date are not sufficiently developed. In addition, there has been comparatively less research focus on LUSC than other lung cancer types. The identification of *SOX2* (frequently amplified in LUSC and a component of the squamous differentiation pathways) as the most important LUSC driver and its incorporation in LUSC modelling strategies has made LUSC models more patient relevant. However, multiplatform characterisation of large patient cohorts has revealed a complex landscape of molecular subtypes with and without *SOX2* amplification, with obscure biological origins and unknown vulnerabilities that are not represented by any existing experimental models.

Moreover, there is no single targetable pathway that dominates the genomic landscape of LUSC, unlike lung adenocarcinoma that is dominated by alterations in the Ras pathway. Instead, the most frequently altered pathways in LUSC are PI3K/Akt pathway (47%), squamous differentiation pathway (44%) and oxidative stress response (34%) (Figure 1A). Furthermore, analysis of LUSC genomes has not shown co-occurrence or mutual exclusivity in these dysregulated pathways. This suggests that none of the pathways are indispensable in driving LUSC, but also that they can cooperate. Deciphering the biology of this complex inter-patient diversity requires individual interrogation using appropriate models to address several key questions:

- Can these pathways drive LUSC tumourigenesis per-se or there is an obligate cooperation between them?
- Are LUSC cells addicted to these pathways?
- Are these pathways mutually dependent?
- Are these pathways relevant in all LUSC molecular subtypes and if not, what processes drive LUSC in those subtypes?

Answering these key questions requires intensive research programmes that involve the manipulation of multiple loci. Approaches to avoid a large cost in mouse lives and distress is a responsibility of the scientific community, especially in the field of LUSC, where the new availability of more relevant mouse models will increase the number of projects involving animal research. Genetic manipulation of human bronchial epithelial cells (HBECs) is an alternative to GEMMs, that can facilitate the modelling of LUSC heterogeneity and developmental stages. However, a proof of principle for this alternative LUSC modelling strategy is required.

Current methodologies permit efficient expansion of HBECs, genome editing and development of organoids mimicking bronchial morphology. Using these methodologies, we

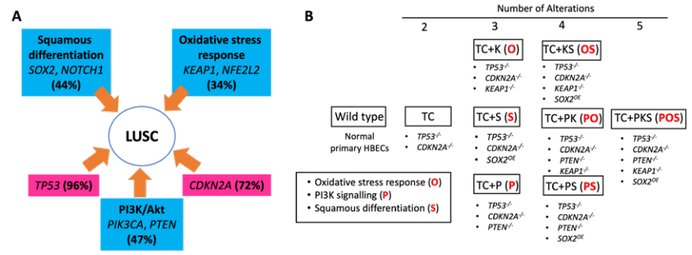

Figure 1. A. Summary of the most relevant tumour suppressors (pink boxes) and pathways (blue boxes) involved in LUSC development, with examples of pathway components altered in LUSC and the percentage of cases with at least one alteration targeting the pathway. **B.** Summary of the mutant HBECs that we have developed in this project. All mutants contain *TP53* and *CDKN2A* mutations (almost universally inactivated in LUSC) and all possible combinations of the three alterations targeting the most frequently dysregulated pathways in order to assess individual and combined effects. Red characters show the pathways targeted in each mutant.

can disentangle how driver alterations induce epithelial perturbations indicative of LUSC initiation and progression. Additionally, HBECs reflect human diversity better than mouse models, constitute a more adequate system to investigate the effect of exposures, mainly smoking, and predisposition.

Genetic manipulation of HBECs

In the TLCB lab, we have designed, implemented and characterised a genome engineering strategy whereby, using genetically modified HBCs, we intend to establish a proof-of-principle for the use of HBECs to model LUSC (Figure 1B). To do this, we have generated increasingly complex mutant HBECs bearing inactivating mutations in the tumour suppressors *TP53* and *CDKN2A* (ubiquitous alterations in LUSC) and combinations of alterations in components of the squamous differentiation, PI3K/Akt and oxidative stress response pathways, namely, *SOX2* overexpression, *PTEN* truncation and *KEAP1* truncation respectively (Figure 1B). With this strategy we intend to capture the essential components of LUSC development.

Analysis of air-liquid interface (ALI) organotypic cultures showed that *SOX2* overexpression induces epithelial morphologies indicative of the transition from normal to low-grade premalignant stages (Figure 2) characterised by a prominent squamous differentiation. Addition of *PTEN* and *KEAP1* mutations results in complete loss of epithelial polarity and expansion of p63-positive cells consistent with transition to high-grade preinvasive stages. Importantly, invasion assays revealed that the dysregulation of the three pathways was necessary for acquisition of invasiveness. Our observations have enabled us to define a genetic roadmap that describes the LUSC developmental stages (Figure 2), from a normal bronchial epithelium to high-grade premalignant stages, and confirm that activation of the three pathways was necessary for complete transformation of HBECs into invasive LUSC cells. These obligate requirements were consistent with the classical LUSC subtype, in which there is a co-occurrence of alterations targeting the squamous differentiation, oxidative stress response and PI3K/Akt pathways.

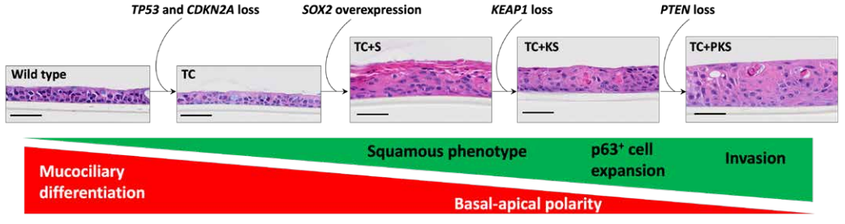

Figure 2. Haematoxylin–eosin stained sections or air-liquid interface (ALI) HBEC cultures from wild-type and the mutants HBECs that follow the most likely evolutionary trajectory of LUSC inferred from our results. The diagram below shows the phenotypic changes associated with this evolutionary trajectory.

SOX2 overexpression and PI3K/Akt activation

The main advantage of our strategy is the possibility to analyse the phenotypes associated with concomitant activation of pathways and therefore, identify pro- and anti-tumourigenic interactions between pathways. These interactions can guide the design of synthetically lethal treatments or to redefine existing therapeutic modalities for personalised medicine.

We detected that simultaneous *SOX2* overexpression and *PTEN* truncation led to negative selection of SOX-overexpressing cells in organotypic cultures. However, this negative selection was not observed in mutants with *SOX2* overexpression, *PTEN* truncation and *KEAP1* truncation. These results explain the reason for concomitant activation of the three pathways occurring in the classical subtype and indicate that therapies currently in clinical trials to inhibit the oxidative stress response in LUSC should be targeted to patients with *SOX2* amplification.

SOX2 overexpression and the oxidative stress response pathway

Our approach to model LUSC also enabled us to explore the effect of pathway dysregulation in the transcriptome of mutant HBECs and the overall downstream processes relevant for LUSC progression perturbed by such pathways. SOX2 overexpression resulted in the expected dysregulation of biological processes related to epithelial maintenance, particularly squamous differentiation and ciliation. However, these analyses revealed that SOX2 also regulates EGFR ligands and immune pathways, including downregulation of MHC class II subunits and the upregulation of extracellular serin protease inhibitors that counteract the tumour suppressive effect of neutrophil elastases. Whereas, the oxidative stress response downregulated interferon-α and -γ responses, together with the expected upregulation of redox- and xenobiotic detoxifying enzymes, amino acid transporters and pentose-phosphate pathway enzymes.

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TRANSLATIONAL ONCOGENOMICS

Group Leader
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Senior Scientific Officer
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Postdoctoral Fellows
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Graduate Students
Parsa Pirhady
Lucy Barton

Clinical Fellows
Martin Swinton
Diego Sanchez

Executive Assistant
Caroline Stone

[1]Joined in 2023
[2]Left in 2023

Figure 1. At CRUK MI we are uniquely placed to receive material from prostatectomy specimens from our clinical colleagues at the Christie Hospital. We have put in place protocols that allow us to establish immortalised prostate epithelial cell lines from any patient donor. As a major specialised cancer centre, the Christie NHS Foundation Trust manages the care of a large number of patients, meaning we can focus our sample collection from patients with relatively rare germline mutations driving predisposition to high-risk disease.

With an estimated 1.46 million new cases identified worldwide in 2022, prostate cancer ranks as the fourth most frequently diagnosed cancer across the globe. In the UK, there are approximately 52,000 new diagnoses and 12,000 prostate cancer deaths per year.

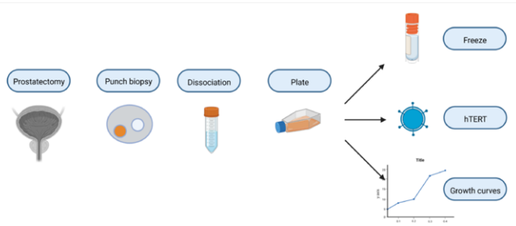
The natural course of this disease is difficult to predict and for clinicians the key challenge is to accurately assess the risk of progression to metastasis so that each patient can be managed appropriately. Whereas low to intermediate risk cancers are potentially curable with either surgery or radiotherapy approaches, higher risk localised cancers have an increased probability of producing incurable metastases, which may be undetectable at the point of diagnosis.

Here, localised therapy (radiotherapy/surgery) is combined with systemic treatments to target residual secondary disease. Crucially, although androgen deprivation therapy is initially effective, secondary tumours frequently progress becoming an incurable, metastatic castrate-resistant prostate cancer. Because metastatic tumours are recalcitrant to treatment, there is a 10-15 fold increased probability of prostate cancer specific mortality for high-risk prostate cancers. Clearly, there is a need to develop new biomarkers that give an insight into heterogeneity of outcomes in prostate cancer patients. In addition, new biomarkers that predict patient responses to the various treatment options will also be required. To this end, recent advances have shown that detailed understanding of the genomic/transcriptomic landscape of prostate cancer, together with knowledge of the degree of hypoxia within tumours, can provide important insights into the aggressiveness and likely prognosis of localised prostate cancer. By understanding the molecular landscape of high-risk disease, we hope to better understand what drives disease progression in some patients and to improve personalised treatment options for those men who need it most.

Familial predisposition to high-risk prostate cancer

Germline defects in DNA repair genes lead to heritable conditions which predispose carriers to a range of solid tumours. In prostate cancer the role of such mutations in driving high-risk, rapidly metastasising disease is becoming clearer. Accumulating evidence implicates a range of genes involved in DNA damage responses such as the ATM and CHK2 kinases, the mismatch repair machinery, and the tumour suppressor, TP53. However, the most frequently observed DNA repair defect is germline mutation of the Breast Cancer susceptibility-2 (BRCA2) gene, which confers an 8-9 fold increased risk of developing prostate cancer with subsequent failure during standard of care treatment. Overall, the cancer-specific survival is 5-8 years post diagnosis for 50% of BRCA2 carriers, compared with >90% 10-year survival for stage-matched non-carriers. The relatively poor outcome observed in germline BRCA2-driven prostate cancer reflects a rapid progression to metastasis, increasing the probability that diagnosis and subsequent treatment with localised therapies occur after the development of systemic disease. Arguably, this group of patients could benefit from a tailored approach to their care. For instance, inhibition of PARP, a strategy shown to be effective in the breast cancer setting, offers a new approach to systemic therapy and several clinical trials in prostate are underway. But conducting comprehensive trials in this relatively rare population is challenging and there is a clear need for pre-clinical models that can deliver useful information about likely efficacy of novel therapeutic approaches. Accordingly, we have made the development of novel prostate-specific, cellular models which recapitulate germline BRCA2 deficiency a key priority for our research.

Patient selection criteria:
- Known germline mutations in DNA damage response genes. *eg, BRCA2, TP53, ATM, CHEK2, MSH2, PMS2*
- Strong family history
- Early onset (diagnosis under 50 years of age)



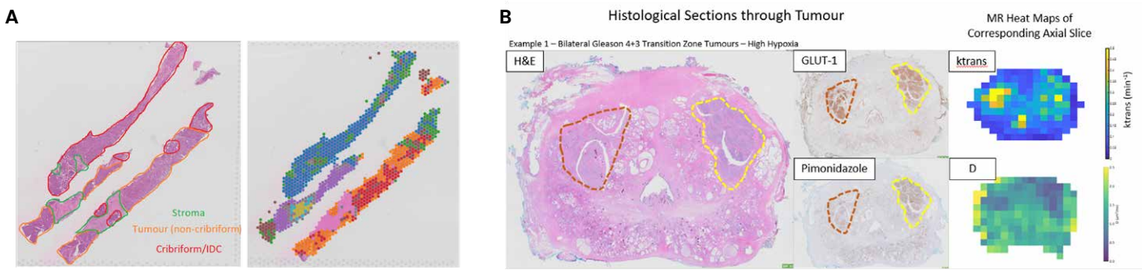


Figure 2. A Formalin-fixed, paraffin embedded needle-core biopsies sampled during HYPROGEN Arm A were processed for spatial transcriptomics using the Visium 10X platform. The left panel shows the result of mark-up by a pathologist – areas of tumour and stroma are indicated. The right panel shows the initial findings based on the transcriptome with distinct clusters mapping to specific regions of the tissue slice. We have a particular interest in characterising regions of Intra-ductal carcinoma with Cribriform Architecture (IDC/CA), a growth pattern associated with metastasis and known to correlate with the presence of hypoxia. Spatial transcriptomics will help us define the molecular landscape of these aggressive cancer sub-clones. **B** Analysis of a prostatectomy specimen performed as part of HYPROGEN Arm B. Two large primary tumours were marked during pathological review. Both tumours express the hypoxia-induced gene, GLUT1 to similar levels whereas the hypoxia tracer molecule, Pimonidazole reveals a lower oxygen tension in one of the tumours. Prior to surgery, the patient was enrolled onto a novel imaging study that aims to test whether a non-invasive imaging technique could be used to detect patients with tumours displaying high levels of hypoxia. Example images are shown on the right. The long-term goal of the study is to use hypoxia-based biomarkers to better predict which patients are likely to fail localised therapies.

We have developed robust protocols for establishing in vitro cultures of primary prostate epithelial cells (PrECs) using tissue samples taken from germline BRCA2 carrier patients undergoing radical prostatectomy at the Christie NHS Foundation Trust. Subsequently, we have succeeded in immortalising these cell cultures in a protocol that employs expression of the human TERT (telomerase) gene. By employing high depth whole-genome sequencing, we have confirmed these immortalised PrECs possess a largely unaltered genome – a major advance over the alternative prostate tumour cell lines currently available which harbour extensive chromosomal aberrations and nucleotide variants. In order to develop unique models of BRCA2 deficiency we have developed a gene-editing strategy to target a RAD51 binding domain in the final exon of the BRCA2 gene – thus creating a hypomorphic (partially functional) allele. When deployed to PrECs that already harbour a germline defective allele, our exon27 editing approach can be used to target the remaining wild-type allele and thus create a unique model of BRCA2 loss-of-heterozygosity (Figure 1). BRCA2 has essential roles during proliferation and to date relatively few cellular models of BRCA2 loss-of-function have been described. We are therefore excited to develop cellular systems that will provide important information about treatment combinations involving PARP inhibition, the nature of genome instability triggered by BRCA2 loss, and novel mechanistic insights into DNA repair. Going forward, we plan to build new experimental approaches into our PrECs models such as conditional expression of BRCA2. Furthermore, we will be increasing the repertoire of the genes we study to include other germline deficiencies such as ATM and TP53. By combining these approaches along with analysis of clinical samples we aim to provide much needed information about how best to care for patients with hereditary predisposition to prostate cancer.

Investigating the hypoxic tumour microenvironment

Many aspects of tumour biology are modified by exposure to hypoxia including resistance to radiotherapy, epithelial-to-mesenchymal transition and interactions with the immune system.

The HYPROGEN trial is designed to examine the potential of hypoxia to drive genome instability and metastasis, with patients currently being recruited to one of two arms, which aim to analyse hypoxic tumour biology in either the metastatic or localised setting (Figure 2). In Arm A, patients with oligo-metastatic disease are recruited to the study prior to receiving systemic treatment. Hypoxia is assessed by means of the tracer molecule, pimonidazole, which is administered 24 hours before biopsies are taken from both the primary tumour and selected bone metastases. In hypoxic tissues, a bioreductive reaction causes pimonidazole to be adducted to intracellular proteins and then detected by subsequent immunohistochemical analysis of tissue samples. Genome sequencing and in-depth spatial analyses is now underway so that the impact of a hypoxic TME on metastasis can be assessed in detail. In particular, we will ask whether genomic aberrations associated with hypoxia are also detected in metastatic lesions. This will provide insights into the potential of hypoxia to drive disease aggression. In Arm B, patients with fully localised disease and scheduled to undergo radical prostatectomy receive pimonidazole before surgery. Since we can access samples across the entire prostate, this setting permits the assessment of heterogeneous features such as hypoxia gradients or, where polyclonal disease arises, tumours with different levels of hypoxia. These data will inform new approaches to improving outcomes for patients with high-risk tumours such as drugs targeting or modifying hypoxia pathways.

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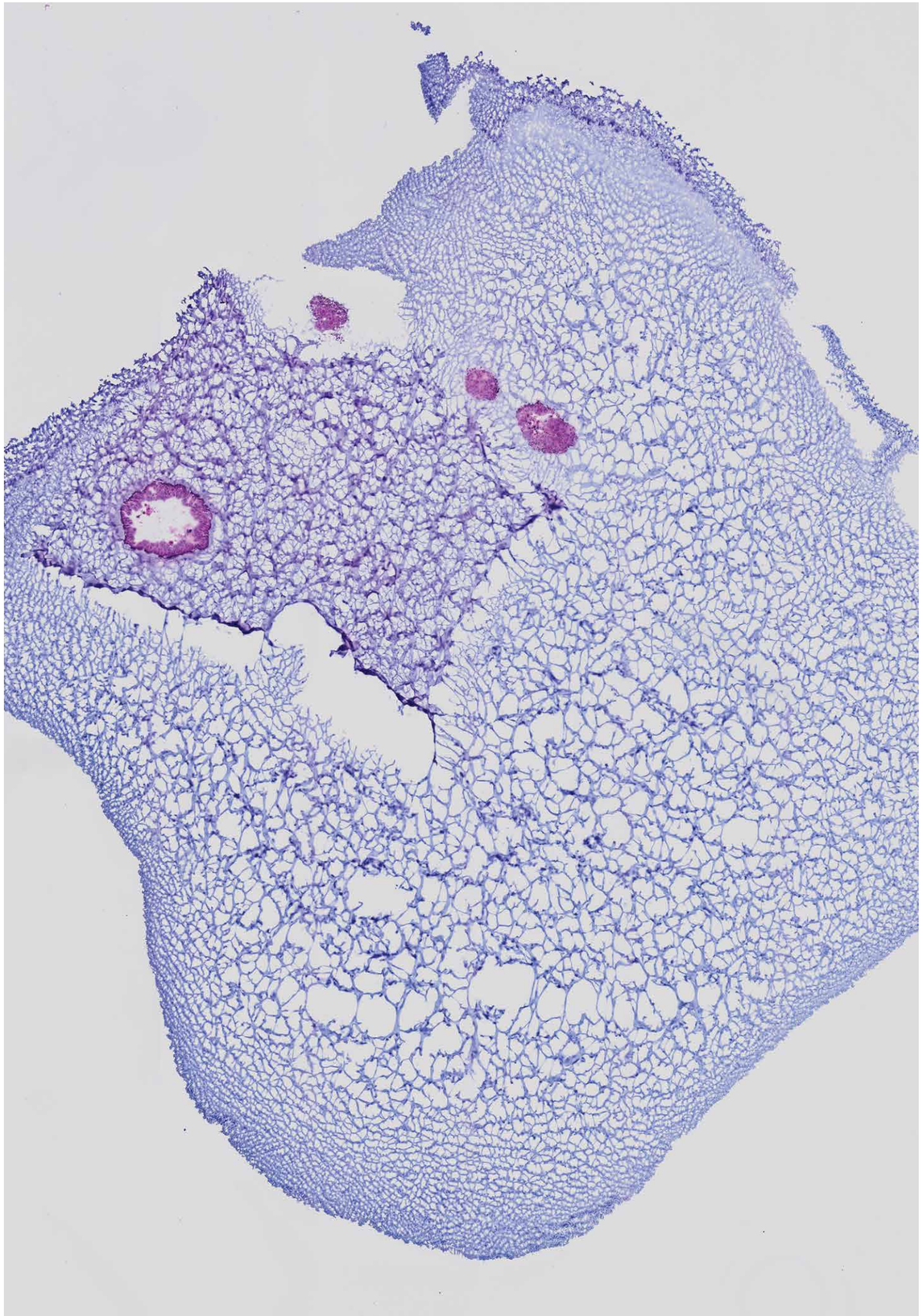


RESEARCH SERVICES

H&E image of a PEG hydrogel of 21 kPa stiffness with PDAC organoids grown as single cells embedded into a gel of 8 kPa. The thickened organoid appears similar to a pancreatic duct undergoing transformation into precursor PanIN lesions. The organoids that migrated to the softer gel have filled, similar to PDAC tissue.

Image supplied by Carmen Rodriguez-Cupello (Systems Oncology)

RESEARCH SERVICES

Chief Laboratory Officer
Stuart Pepper

During the first half of 2023, there was a lot of activity in preparation for the relocation, and then for the actual move. All the core managers worked hard to ensure that interruption to services was kept to a minimum and, as the following section shows, there have been plenty of developments during the year. By the second half of the year, all the services were up and running in the new building and the core facilities are now reaping the benefit of occupying laboratory spaces that have been designed specifically to provide a flexible working space for technology focussed laboratories.

Chief Laboratory Officer
Stuart Pepper

A key event this year was the relocation of the core facilities into the new Paterson Building. During the design phase, each team was able to guide the development of their own lab space so that it is optimised for the work they carry out. The building has also been designed so that the core facility labs are next to each other, and the teams all sit together facilitating cross team working, which is becoming increasingly important. As the sections below show there have been lots of developments but a key theme is how the core facility teams are more easily able to work together.

The relocation of *in vivo* units is inherently more complex than for equipment and it was amazing to see that we were able to move all three teams (Experimental, Breeding and GEMM) who carry out *in vivo* work in the new building and had everything up and running by September.

Across MBC, Histology and VIA there have been continual developments in spatial genomics as new platforms and applications have become available. In this technology area there is no one platform that is the best, it is always a case of fitting the best option to answer specific questions. The Institute now has a selection of options that research groups can access.

For the mass spectrometry facility the biggest development this year has been the introduction of data independent acquisition. This approach allows a step change in the depth of profiling that can be achieved on a sample, without having to purchase a new mass spectrometer. The key requirement for this application is access to appropriate analysis support, which is provided by the Computational Biology Support team (CBS).

CBS has a critical role supporting both the core facilities to develop and check new workflows, and then providing support for research groups analysing data. The now well-established team give a flexibility to rapidly adopt new approaches such as DIA in mass spec but are also opening options to move towards open source software, with potentially significant savings in costs.

This year saw the final integration of IT and Sci Com into one team, at the same time as relocating the entire IT and Sci Com technology platforms into our new data centre. This was another complex and challenging move, which was achieved with minimal impact on live services for the staff in the Institute.

Although the year was dominated by the relocation and then exploring the possibilities that our new lab layouts will facilitate now that we have settled in, that is not all that happened and the sections that follow detail some of the other achievements from the core facility teams.

Biological Mass Spectrometry

Duncan Smith, Yvonne Connolly, Robert Faulkner[1], Adam Flinders[1,2]

[1]Joined in 2023
[2]Based in Computational Biology Support

The remit of the Biological Mass Spectrometry core facility is to support our world class cancer researchers by providing access to innovative proteomics services to amplify the impact of cancer discoveries. We provide access to LCMS technology with an Oribtrap Lumos coupled to nano chromatography, embedded within an ecosystem of established and evolving workflows, enabled with numerous biochemical and software driven solutions to biological problems.

This year saw the retirement of our 12-year-old RSLCnano HPLC and replacement with the Vanquish Neo as the front-end solution of choice to the Lumos. The Neo system is a state-of-the-art ultra-high pressure (1500 bar) HPLC capable of supporting the latest column chemistry technologies. The flexibility of the Neo has allowed us to test and exploit the latest column chemistries from both PharmaFluidics (uPAC) and IonOpticks (Aurora Ultimate). The resolution and sensitivities of the latest nano LC columns make all our analyses more powerful, with distinct improvements in the limits of detection for low abundance peptides that makes entire proteome analyses with higher penetrance. We are now able to profile approximately 5000-6000 human proteins per hour, an increase of over 30% from the previous nLC setup.

The facility's premier complex label free proteomics workflow was transitioned to a Data Independent Acquisition (DIA) workflow during 2022. This year we have assessed the power of DIA workflows in the analysis of less complex mixtures, such as protein-protein interaction studies using immunoprecipitation. We have demonstrated the key advantages of DIA datasets in terms of reproducibility, sensitivity, and quantification accuracy in collaboration with the Stem Cell Biology group, Leukaemia Biology group and Computational Biology Support. The advantages of the workflow mean this transition is now all but inevitable despite the sparsity of downstream informatic workflows available currently. We will address this in collaboration with CBS in the coming year to fully support protein-protein interaction studies in the DIA domain.

We have also developed an intelligent staggered gas phase fractionation (sGPF) approach to offer next generation complex proteome type performance on the previous generation mass spectrometer. The approach shows much promise if the informatic hurdles to analysis can be overcome in the near future.

We plan to push DIA workflows beyond their current boundaries in the area of post translational modification analysis in the coming year. This area offers promise and challenge in equal measure.

Biological Resources Unit

Transgenic Breeding
Team Leader: **Jen Hughes**[1], **Natalia Moncaut**[2], Lauren Street[2], Irana Bakhtiari-Cunado, Daniel Bennett, Tim Bloor, Carl Conway, Wesley Moore, Victoria Preston[3], Rose Storey[3] , Martin Vincent

[1]Maternity leave June-December
[2]Maternity cover
[3]Left in 2023

The Transgenic Breeding Team specialises in breeding mice to support the research initiatives at the CRUK Manchester Institute. Currently, a dedicated team of six members ensures the day-to-day care of 92 distinct transgenic mouse lines housed in approximately 600 cages. Their responsibilities encompass comprehensive care, including husbandry, welfare maintenance, and breeding management, which involves coordinating timed matings as required.

Additionally, the team conducts regular genotyping sampling of the mice. To safeguard against the introduction of infections, the team employs non-sacrificial sampling methods for routine health checks. Maintaining a high health status is paramount, necessitating stringent protocols for introducing new transgenic lines from external sources. These lines are typically transferred into the facility as embryos or sperm by the GEMM team and undergo thorough health screening to ensure that resultant offspring are specific-pathogen-free.

Until July, the unit was situated on the main campus of The University of Manchester. However, in September, the majority of the mouse colonies were relocated to the new animal unit at the Paterson Building. This relocation facilitates enhanced integration of the Breeding Team with other teams within the Institute, notably providing opportunities for collaboration with the Experimental Team in a shared workspace. Having everyone under the same roof makes it much simpler for the team to attend seminars or workshops held in the Paterson Building, as well as to access training in new techniques. Furthermore, researchers enjoy direct access to the mouse stocks, allowing them to conduct tissue sampling and experiments without having to wait for an acclimatisation period.

Experimental Services

Team Leader: **Lisa Doar**
Lisa Dique, Jo Roberts, Laura Dean, Rachel Walker, Eirini Symeon, Tom Bosley, Emma Playle[1], Jacqui Clayton[1], Lewis Woolley, Diane Beeston, Lisa Flynn, Jessica Walker, Gary Cooke, Kimberley Kirkham, Oliver Lesser

[1]Left in 2023

The start to the year was busier than usual for the BRU Experimental Team, as research groups were keen to get studies in ahead of the Institute relocation back to the new Paterson Building in May. During the same period, we had commissioning work taking place at the new facility, where we had a few equipment integration issues to navigate, despite all the careful planning and attention to detail.

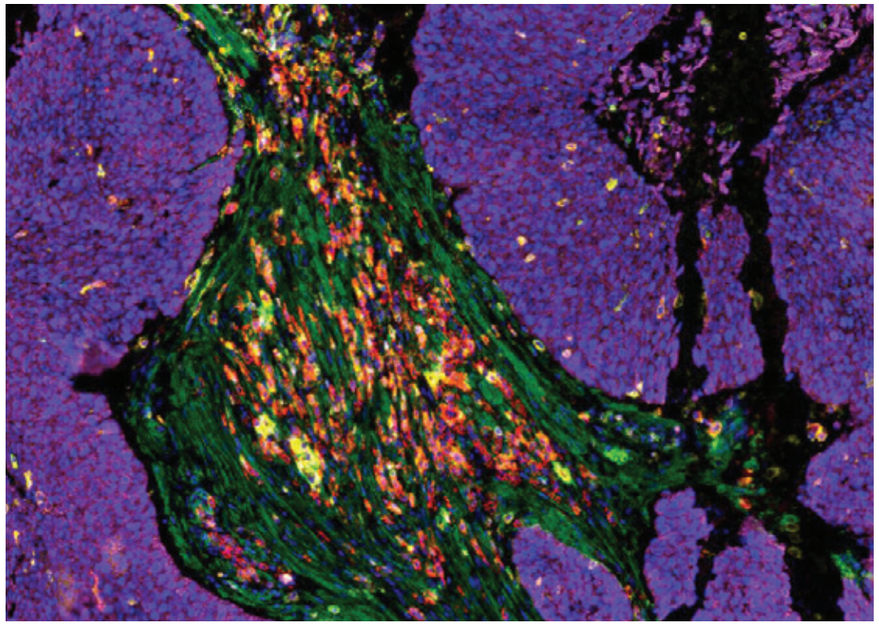


Image generated using multiplex immunohistochemistry assay. One tissue section stained with different cancer, stromal and immune cell-associated antibodies over multiple rounds of staining. Each image is overlaid using specialised software; different stains are shown in different colours.

Image supplied by Victoria Fife (Cancer Biomarker Centre)

However, the programme was successfully completed in the required timeframe.

When opening a new animal research facility, the Home Office must approve its addition onto our existing Establishment Licence. The process of approval requires the review of the design details of the facility, where it sets out how it meets the housing needs of the animals and the methods of working. We had to demonstrate to the Home Office Inspector that temperature and humidity in the animal holding rooms could be maintained within the required parameters for a full 14 days. Each step of the process went well, and the Inspector was impressed with the thought and innovation that had gone into the design.

The BRU move took place between the end of May and beginning of June and went as smoothly as we could have hoped for. Moving the equipment took several van loads over a few days, but the mouse move was completed in one day, and the animals seemed to settle into the new facility quickly and adapted to the change in the water supply (auto-water rather than water bottles).

Since the move we have dealt with the various expected teething problems before getting settled with working in the new facility. We carried out over 100 inductions in the first 2-3 months and were back up to pre-move levels of work by around September/October 2023. Although the move was the biggest achievement of the year for the BRU Experimental Team, we continued to work on various developments in the background. The most significant piece of work has been developing a jig for use with our cabinet X-ray irradiator, which allows us to use inhalation anaesthetic rather than the injectable anaesthetic currently used. We have been working on this for a couple of years, but progress had been slowed due to other priorities. During Q4 of this year we were finally able to test a working prototype. It worked well, and we are now planning a couple of minor tweaks to the design with the intention of switching fully to inhalation anaesthetic early in 2024. There are major welfare advantages over injectable anaesthesia, which is harder on the mice and we commonly see weight-loss. Some strains are particularly sensitive, and we have seen the occasional non-recovery incident. It also takes up to an hour for the mice to come around after the injection. However, with the inhalation anaesthetic there is much more adjustability in the dose given and recovery time is only a couple of minutes, so anaesthesia related complications will be minimised.

We have enjoyed meeting and discussing projects with some of the newer groups that have joined the Institute this year and who have recently obtained Project Licences to enable them to start their animal work. There will be some new models coming along for us to help get established, so we are looking forward to a more 'business as usual' year in 2024, where we will be back to helping develop and deliver the animal research.

Flow Cytometry

Antonia Banyard, Emily Scanlon, Andrew Heuze[1]

[1]Left in 2023

After the successful move of the entire Flow and Mass Cytometry facility to the Paterson Building in May 2023, our services continue to be central to Institute research groups, with them taking advantage of the ever-expanding capabilities.

The Cytof XT (mass cytometry) is being utilised continuously since we acquired the upgraded instrument with the capability to run overnight. There are now many different studies being completed for translational patient work, along with murine experimental studies.

Cell sorting has also been updated with the procurement of a spectral 7 laser system, The Bigfoot cell sorter. This has enabled our researchers to develop exponentially more complex labelling panels producing far superior resolution of different cell types, resulting in more accurate cell sorts and cleaner readouts for down-stream analysis, such as RNA sequencing. Not only does this bring exceptional improvement on phenotyping, but this instrument is also capable of sorting extremely quickly into 96, 384 and 1592 well plates, with up to 4 different cell populations within a matter of seconds, which has never been possible before. This enhanced capability will improve the cell viability and efficiency of reporter screens for CRISPR experiments.

With the more recent procurement of the flow analyser, the Novocyte Quanteon, researchers are now able to expand the number of cell types from each sample, increasing the cellular content information without having to increase the number of mice or samples used in their studies. This new platform set-up enables researchers to sample from a full 96 well plate and the instrument will run this without supervision, resulting in less time being spent using the instruments and giving more consistent results.

With the installation of new instruments, the team provide full training on the cytometers, and the use of the software Flowjo for post-acquisition data analysis, including high dimensional data from the Quanteon and Cytof XY. The facility is developing the training portfolio to include cell preparation protocols and practical knowledge and advice to maximise the use of all its platforms and continue to provide a comprehensive and knowledgeable, world class facility for all our researchers.

Genome Editing and Mouse Models

Natalia Moncaut, Athina Papaemmanouil, Lauren Street

The Genome Editing and Mouse Models (GEMM) core facility, operating at the forefront of technology, specialises in providing new genetically engineered mouse models for cancer research. Collaborating closely with researchers at CRUK MI, GEMM offers strategic guidance on generating cutting-edge mouse models tailored to investigate the mechanisms underlying tumour initiation, progression, and response to therapy. Utilising CRISPR-mediated gene editing, GEMM efficiently modifies the mouse genome to introduce precise genetic alterations, mimicking those observed in various human cancer types. This collaborative effort ensures the development of optimal cancer mouse models to advance our understanding of the disease.

This year marked a significant transition for us as we relocated our facility from the main UoM campus to the new animal unit in the Paterson Building. This move has brought about substantial advantages, granting us convenient access to our Molecular Biology and Cell Culture lab. Being situated alongside the entire Institute has fostered greater collaborations and interactions with researchers and with the *in vivo* team. Additionally, we have enriched our mouse repository by importing over 20 new mouse lines through the rederivation process, ensuring the maintenance of high health standards within the unit.

We continue our commitment to maintain a safely archived stock of all the mouse strains bred at CRUK MI. Routine sperm and embryo cryopreservations serve as a precautionary measure against potential strain loss resulting from various adversities, including breeding challenges, genetic variations, environmental factors, and disease outbreaks. With the majority of our mouse lines safely archived, we have been able to discontinue several strains that were not immediately required, streamlining our operations for enhanced efficiency.

Histology

Garry Ashton, Caron Abbey, Nicola Tonge, David Millard, Amy Lawrence, Peter Magee

Following the Institute's relocation earlier in the year, the facility is now housed together with other core facilities on the 4th floor of the new Paterson Building. The lab is subdivided into areas allowing the unit to offer a full range of both routine and advanced histological services for oncology research. The bespoke design of the area has allowed for range and complexity of the services offered to continue to grow and for a large number of both basic and translational research groups to adopt various tissue-based experimental approaches. Alongside this growth in services, the training and continued professional development of staff has ensured excellent staff retention whilst allowing the unit to always offer a comprehensive and flexible service.

Special stains have been used by various groups. These have included Masson Trichrome, PAS, Cresyl Violet, Oil Red O and reticulin stains. In addition, the unit has routinely prepared both FFPE and frozen human and mouse tissue in addition to organotypic assays, spheroids, agar plugs and cell pellets. These have all been used for both routine and advanced analysis with an array of techniques. Groups have also used vibratome tissue sections (50–250µm) for ex vivo cultures of tumours to evaluate and develop three dimensional studies.

The number of groups requiring access to human tissue continues to grow. All histology samples accessed through the biobank are prepared by the core facility. A dedicated scientific officer is responsible for ensuring the unit is compliant with current human tissue legislation working closely with biobank staff.

The high throughput routine immunohistochemistry service, troubleshooting and antibody validation services have once again proved popular. The unit incorporates both the Leica and Roche IHC platforms into its workflows ensuring consistency, reproducibility, and standardisation.

Sophisticated labelling techniques are now offered as part of our routine service. mRNA in situ hybridisation together with or without protein immunohistochemistry is in routine use. High plex protein detection incorporating directly conjugated barcoded primary antibodies and cyclic detection and removal of antibodies have both been extensively used. Over the last year, the use of spatial transcriptomics has seen expansion. Delivery of the Nanostring GeoMX DSP system has supported this expansion together with the roll out of 10X Visium slide technology. Laser capture microdissection, followed by the downstream extraction of both RNA and DNA, giving sufficient quantity and quality for NGS from relatively small amounts of material, continues to prove popular.

Several more tissue microarrays have been built, which together with existing arrays have been well used as they allow for high throughput sample analysis and reduces costs. Extensive evaluation of the 3DHISTECH TMA Grand Master enabling the precise selection of specific ROIs from the H&Es of donor blocks has been completed. The system will play a pivotal role in the spatial analysis of high numbers of samples.

With the Cancer Inflammation and Immunity group, the facility has helped develop an IHC panel to look at the effects of glucocorticoids on the tumour-infiltrate in an intradermal mouse model of melanoma. Further work looking at promising potential mechanistic markers is ongoing together with the analysis of human samples to validate the in vivo findings.

One area of interest for Systems Oncology is the expression of Endoglin/CD105, which can be highly expressed on endothelial cells and show low expression on fibroblasts. Automation utilising signal amplification has ensured reproducible single plex and multiplex IF staining.

The POBIG study, which delivers radiotherapy before surgery, is an example of how the Brain Tumour Biology Group - in the Division of Cancer Sciences at The University of Manchester - want to target the tumour at an earlier (preoperative) stage. An important translational aspect of POBIG is to elucidate the impact of radiotherapy and find novel combination regimens that can improve the treatment outcomes. Collected samples have been processed within the core facility for spatial transcriptomics using 10x Visium platform.

The Translational Oncogenomics group are interested in analysing the hypoxic tumour microenvironment. Our expertise has allowed the use of IHC-based markers of hypoxia in tissue specimens and help build novel datasets based on the latest spatial transcriptomic platforms. By combining immunostaining and transcriptomic data, the group can probe the relationship between hypoxia and genome instability in situ.

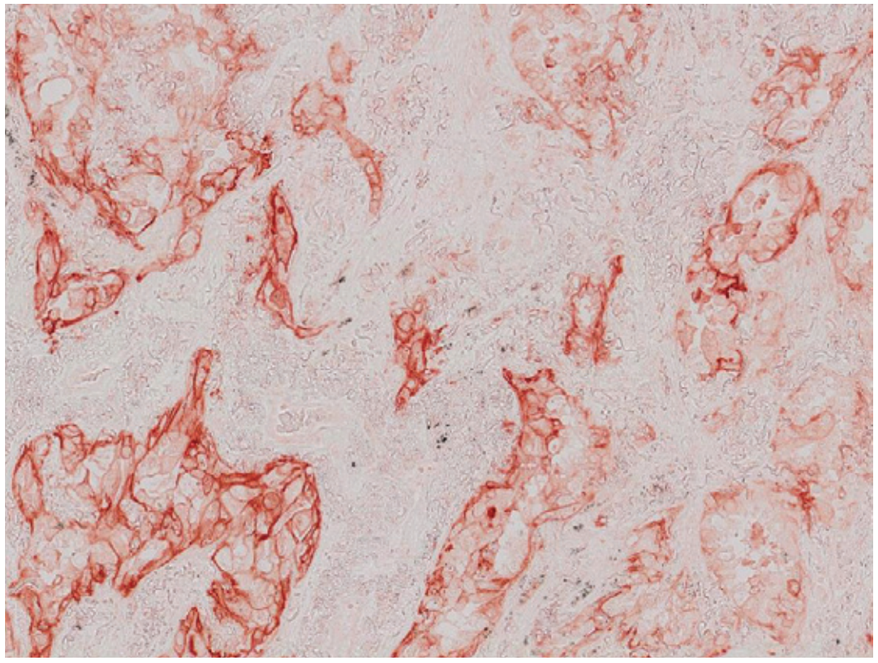
Molecular Biology Core

Wolfgang Breitwieser, Andzhela Abu Rashed, Lucy Goodman, Rachel Horner, Jason Rumley, John Weightman

Spatial Transcriptomics (ST) describes technologies that enable the interrogation of whole transcriptomes in the context of the native tissue environment. These methodologies offer huge opportunities in the analysis of cellular neighbourhoods or the interrogation of cellular interactions between tumour tissue and the immune system. Thus, since the introduction of ST services in the Institute they have been more than holding up their promise to be transformative for the understanding of biological activities, particularly for cancer prognostic and

Stage II lung adenocarcinoma cells showing PD-L1 staining (red). PD-L1 is a common immunotherapeutic target. These therapeutics aim to help the immune system recognise and fight the cancer.

Image supplied by Victoria Fife (Cancer Biomarker Centre)



diagnostic purposes. From the beginning it has been clear that the implementation of these technologies requires cross departmental efforts to achieve seamless workflows for sample processing and analysis, starting from tissue preparation and moving to tissue imaging, sequence library preparation and clonal sequencing, not ignoring the provision of storage facilities for the substantial and complex data sets as well as the computational pipelines for bioinformatic analysis.

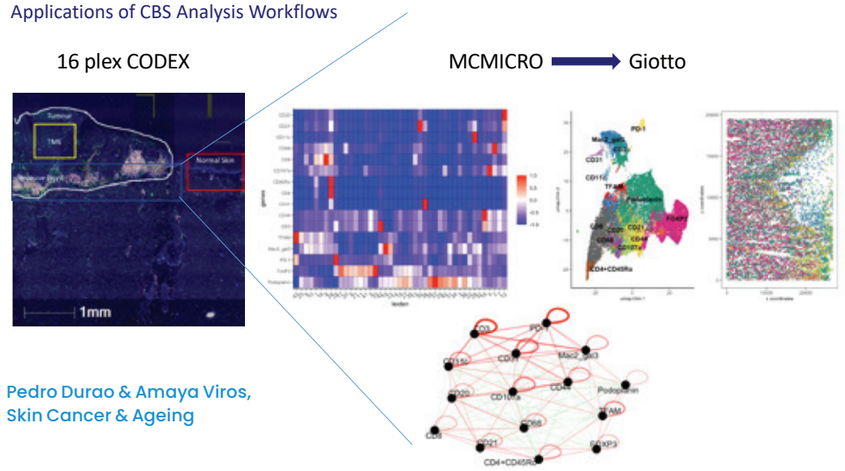
The first commercial ST technology, 10X Genomics Visium has been applied at the Institute for several years. Fresh/frozen or formalin fixed tissue sections are placed on technology compatible tissue slides that are coated with barcoded oligonucleotides to allow for the capture and spatial allocation of cellular transcripts. In its current iteration, Visium ST produces transcriptome data with a resolution of 5-10 individual cells, but computational methods allow for further segmentation and resolution to identify individual cell types. Furthermore, recently released High Density Visium reagents promise significant improvements in the segmentation of tissue up to single cell resolution. To enable these capabilities and to facilitate high throughput an automation platform, the Visium Cytassist, has been acquired this year. The Cytassist will therefore further help in improving transcriptome capture and future proofing the technology.

A second ST technology has been introduced with the recent purchase of the Nanostring GeoMx Digital Spatial Profiler (DSP) platform. GeoMx DSP technology enables the capture of cellular transcriptomes identified in specific regions of interest. A key feature in this technology is the additional immunofluorescent staining of the same tissue slide using morphology marker antibodies. This results in further tissue segmentation and thus allows for the separation of cell type specific transcriptomes. For example, in a typical GeoMx ST experiment, tumour biopsy tissues are processed for the separation and identification of transcriptional profiles between distinct tumour compartments or to distinguish between tumour and stroma tissue areas.

Following its recent introduction, a third ST methodology, STOmics StereoSeq, has been validated by the Institute's Core teams. Like Visium, this technology is based on the capture of cellular transcripts from tissue sections embedded on glass slides that are precoated with barcoded oligonucleotides. However, StereoSeq distinguishes itself from other technologies through an unmatched ultra-high resolution in the barcoding, thereby allowing for cellular to subcellular segmentation of the target tissue. Currently this technology is used for analysis of fresh/frozen tissue types, including embryonic as well as tumour tissues. Overall, with the capabilities to offer a diverse range of ST technologies, the Institute Core teams are now in a unique position to offer tailored solutions to individual scientific challenges in this field.

In separate NGS technology development, MBC has made great use of the recently acquired Oxford Nanopore PromethION P2 instrument. This long read sequencing platform is used for a variety of applications and proves to be a

An example of the application of our workflow for the analysis of a multiplex-stained melanoma skin sample. Shown in the figure is the expression of immune markers for different cell populations (for example, population 24 can be annotated as memory CD4 cells), cellular neighbourhood pattern and proximity analysis of invasive font (blue) region.



Pedro Durao & Amaya Viros, Skin Cancer & Ageing

valuable addition and emerges as a strong alternative to short read sequencing technologies. Nanopore technology identifies bases and derives sequences as nucleotide strands pass through biological pores embedded in a membrane as the main component of a sequencing flow cell. The emerging sequencing reads are independent of molecule sizes and can result in uninterrupted contigs of many thousands of bases. In bioinformatic analysis, long reads overcome some of the challenges posed by short read technologies, for example in the stitching together of sequence reads to produce comprehensive information of genome architecture. PromethION P2 can produce whole genome information of multiplexed samples in single runs, and therefore overcomes the limitations in terms of throughput seen with the previously applied MinION instrument. Consequently, we have seen significant uptake of this technology for whole genome sequencing applications, for example for the examination of aberrant genome structure in cancerous tissue. Additionally, a unique feature of native DNA sequencing using the Nanopore platform is its ability to recognise biologically occurring nucleotide modifications, such as methylation or hydroxy methylation. This feature in base calling is now widely used to identify modified, e. g. hypermethylated, genomic domains without the need for chemical or enzymatic treatment of the analytes. In addition, base calling algorithms can be trained to recognise experimental modification of DNA, e. g. through the treatment with the nucleotide analogue BrdU. Therefore, the technology lends itself for the analysis of cell cycle or cell replication.

Computational Biology Support

Sudhakar Sahoo, Dave Lee[1], Robert Sellers, Richard Reeves[2], Adam Flinders[2]

[1]Left in 2023
[2]Joined in 2023

The Computational Biology Support (CBS) core facility is engaged in providing high quality computational biology and bioinformatics analysis solutions for the researchers across the CRUK Manchester Institute. The primary aim of the CBS is to develop computational analysis infrastructure that enables the application of state-of-the-art bioinformatics and statistical analysis to support the forefront scientific research within the Institute.

Most of our work focuses on developing and applying computational approaches to high throughput sequencing and multiplex imaging data sets locally generated by advanced technological platforms. We develop workflows and pipelines combining custom built tools and open source software that run on the high performance computing facility provided by the Scientific Computing core facility at the Institute.

The CBS team has the broad expertise to analyse and interrogate genomic, epigenomic, transcriptomic and proteomic datasets at bulk, from single cell to spatial resolutions. Over the year, CBS has produced workflows and pipelines to perform a variety of high throughput omics, multi-omics and multimodal data analysis and integration. Of particular interest in cancer research is uncovering biological complexities using single cell omics/multi-omics and spatial omics integrated methods. To tackle this, we have developed and successfully applied workflows and pipelines in single cell and spatial omics (proteomics and transcriptomics) projects. As such, some spatial technologies require tissue imaging, and it is important to analyse those high-plex tissue images with greater detail to integrate with the sequencing data.

Over the year, CBS has developed several workflows and pipelines for genome analysis that have helped researchers at the Institute to publish high quality publications. A recent highlight is the high throughput image analysis workflow which is available Institute wide. This automated high throughput image analysis pipeline has been developed in collaboration with Systems Oncology and Scientific Computing (SciCom) and can analyse a variety of tissue images generated by ultra-plex imaging platforms such as CODEX, mIF, mIHC, MIBI, cyCIF and Hyperon. The workflow has also been extended by implementing a downstream spatial analysis software suite for cell clustering, annotation, neighbourhood construction and cell-cell interactions, which may help to study tumour microenvironment in data sets generated by spatial-transcriptomics and proteomics platforms (see the figure as an example using data from the Skin Cancer and Ageing group).

As usual, the CBS team have also provided continued support to our core facilities. The adoption of innovative technologies within the Institute requires planning and knowledge at all levels; our involvement here is within the validation of the data produced before being used in research studies and the streamlining of its analysis. Explicit examples of our team's involvement can be seen in the adoption of nanopore sequencing (P2 solo), spatial transcriptomics (GeoMx and Visium [+Cytassist]) and the spatial proteomics high-plex imaging CODEX platform. As technologies develop, so will the need for analysis workflows and we are striving to keep our workflow and pipelines up to date.

The CBS team have been at the forefront of the Institute this year. We have continued to play our role as both analysts and consultants for prospective research projects. The expansion of our role has come with the introduction of our well-received Institute wide NGS data analysis training programme, which we aim to deliver at regular intervals. This data analysis educational programme is designed to include emerging technologies and communicating our advances in knowledge across the Institute, ensuring that our research methodologies are well-informed, and researchers follow best practice guidelines.

IT and Scientific Computing

Marek Dynowski, Brian Poole, Christopher McCauley[1], John Campion, Kevin Doyle, Krar Haider, Matthew Young, Stephen Kitcatt, Stephen Royle, Zhicheng Wang

[1]Moved from Operations to IT and SciCom in 2023

In 2023, we started merging the IT and Scientific Computing teams to increase cooperation and exploit synergies in the technical and administrative areas. The Cancer Research UK Manchester Institute (CRUK MI) IT Team is a small group of experienced IT professionals providing a wide range of IT support services to over 300 research and support staff across two sites. Combining the team with the highly specialised Scientific Computing team will facilitate designing and implementing coordinated IT, computing, and data storage services across the Institute in the future.The recently completed CRUK MI High Performance Computing data centre, designed and built in the last two years, has already proven its value. We successfully addressed several operational challenges that arose during the process. It now boasts fully redundant (N+1) uninterruptible power supplies (UPS) and cooling systems, and sufficient space and capacity to accommodate additional hardware in a highly secure environment.

In cooperation with our system integrator OCF, IT and Scientific Computing successfully moved an entire data centre from the Institute's temporary location at Alderley Park to the brand-new data centre in the new Paterson Building. In eight days, we relocated 46 servers, 25 storage enclosures, 19 switches, one large tape library, a 400TB enterprise-class file storage system, and hundreds of cables and tapes to the new Manchester Institute data centre. After that, IT and SciCom rebuilt the infrastructure and worked effectively with scientific workgroups and core facilities to connect all the instruments to the CRUK MI network and storage system. Before the move, IT and Scientific Computing planned and successfully implemented contingency measures to minimise downtime and continue providing essential services, such as user authentication, during the relocation.

At the same time, the team installed the Institute's new High Performance Computer (HPC) system, Griffin, in the new data centre. We designed the system with a focus on performance and high availability. It is five times faster than its predecessor, Phoenix, and all essential components are designed to be redundant. The design allows users to use the system to analyse large, time-critical, and highly sensitive data.

Griffin is a heterogeneous InfiniBand Linux cluster designed to analyse a large variety of different data types using different methods and algorithms. It consists of 100 x standard compute nodes[1], 2 x high memory nodes[2], and an NVIDIA Redstone GPU (graphic processing unit) system[3] and an FPGA. We decided to replace the former MOAB/Torque-based batch system with the modern and widely used SLURM for more effective job management. HPC nodes and storage are connected via a high-speed InfiniBand 100Gb/s connection, allowing high-speed data transfer between the components. Accessing the system is also much faster than before, as the network bandwidth has been upgraded from 10GbE to 25GbE.

With its massive computing power, Griffin is a crucial component of SciCom's High Throughput Data Analysis platform. It is tightly integrated with various storage systems, the High-Performance virtualisation platform, bare metal servers and cloud services to provide an integrated platform for analysing research and clinical data. The platform covers the entire data analysis lifecycle, spanning from data generation, processing, downstream analysis, and visualisation, publication, and archiving, while following FAIR principles.

The focus of the IT and Scientific Computing team is now on making the resources more accessible. Tools like our recently upgraded RStudio Web server, the Galaxy web-based platform for data-intensive biomedical research and technologies like Remote Desktop Access via XRDP are important to simplify the usage of High Performance Clusters like Griffin. However, easy access does not mean such systems should be used as a "black box". Therefore, the IT and Scientific Computing team is working closely with the Computational Biology Support team and the Bioinformatics and Biostatistics team from the Cancer Biomarker Centre on training programmes to ensure users have the essential knowledge needed to use these powerful resources.

While the cyber security incident at The University of Manchester did not directly impact CRUK MI due to the Institute having its own recently updated perimeter firewall, it did prompt us to conduct a thorough review of our infrastructure and security protocols. As a precautionary measure, we strengthened access controls to the Institute's services, including the implementation of two-factor authentication.

We also implemented new secure methods for external collaborators to access our Institute's services during this process. This is crucial for maximising the Paterson Building's potential, where scientists from multiple organisations collaborate.

[1] 2 x Intel CPUs, 24 x cores per CPU, 256GB RAM per node
[2] 4 x Intel CPUs, 24 x cores per CPU, 4096GB RAM per node
[3] 4 x A100 GPUs, 2 x AMD CPUs, 24 x cores per CPU, 512GB RAM per node

Visualisation, Irradiation & Analysis

Steve Bagley, Alex Baker, Jianhua Tang, Henry Banks[1]

[1]Joined in 2023

The facility's remit is to support the researchers with imaging and irradiation instrumentation, quality control of the equipment and their outputs, support for image processing analysis, and the development of new techniques and instrumentation. The facility is not isolated in its outlook; it values working alongside other facilities and researchers in developing novel workflows.

In addition to relocating the facility and being operational in two weeks, the team has performed 148 training sessions, and the instruments have been utilised for a total of 20,800 hours of microscopy and time-lapse imaging, 2,900 hours of high-content screening, and produced 14,300 histology slides. The facility supports both commercial and open-source software for image processing and 2D, 3D, 4D image analysis, and consequently a total of 7,500 hours of image analysis was undertaken.

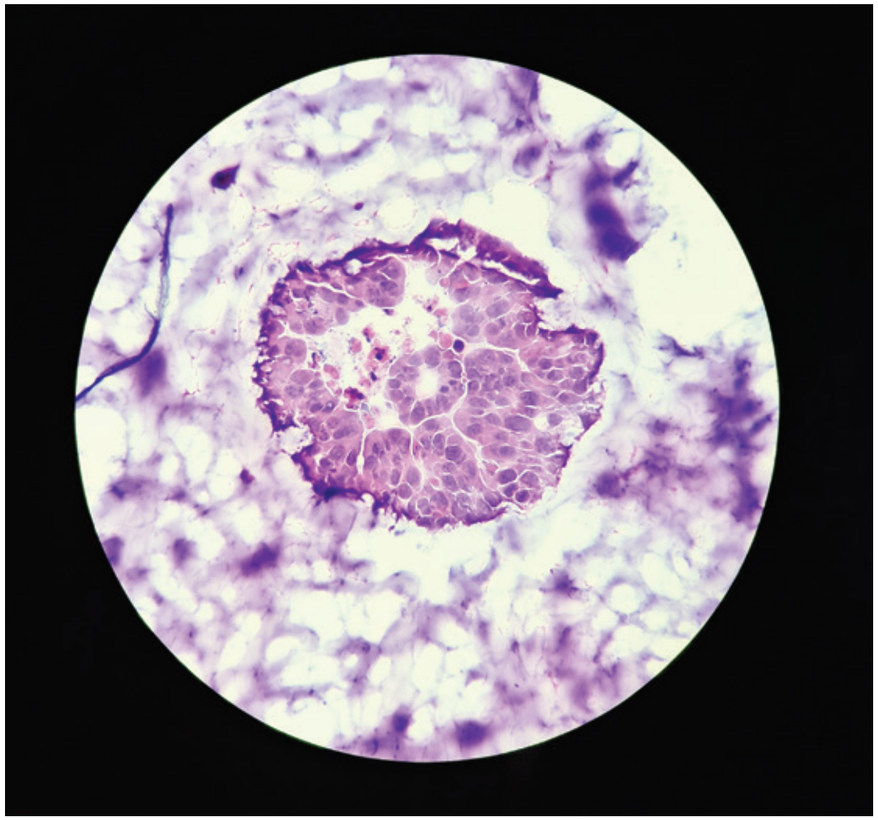
Over the year the facility completed the development of several workflows, including:

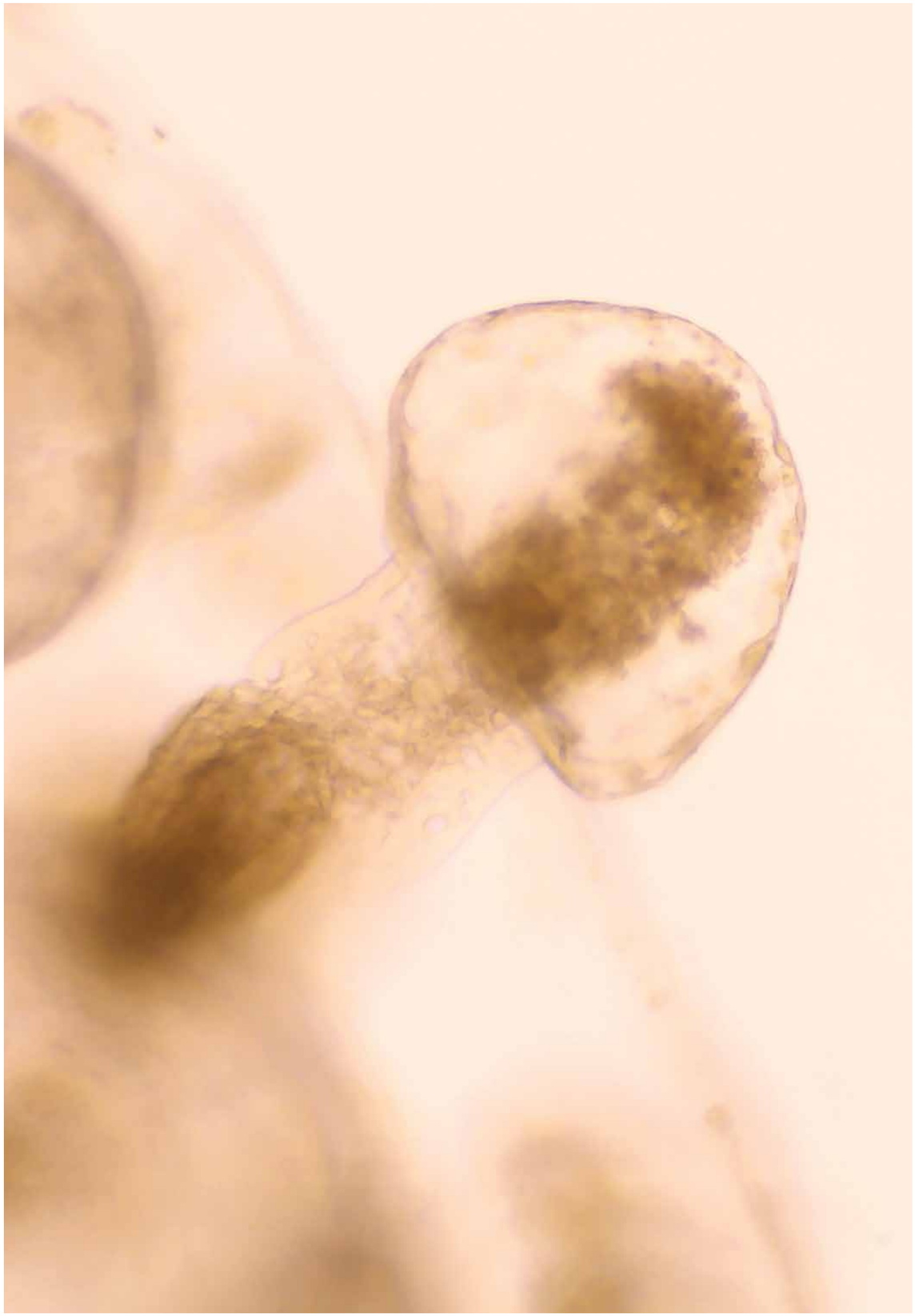
- Low light time-lapse imaging using regenerative AI/ML techniques to extend the imaging window.
- QC of instruments allowing instrument stability and fully characterised outputs over the lifetime of a study.
- Polarised light imaging automation to operate alongside transmitted light and fluorescence imaging of whole slide histology sections.
- Working alongside the in vivo unit to develop an irradiation shielding system with gaseous anesthesia.
- Seven colour fluorescence imaging for whole slide and mega slide imaging to enable complex hi-plex investigations at low cost to the research groups.
- Integrating a laser system for DNA ablation to a spinning disk confocal system for rapid time-lapse.
- Working with Iain Hagan's Cell Division laboratory to develop a new workflow for image analysis of microscopy data.

Challenges have been based on the amount of data the facility generates for the researchers; single datasets of 1TB are becoming more common and handling that volume of data in image analysis space is demanding. The facility is reviewing how we can cost-effectively handle and process this data. Over the coming year, the facility has several planned developments based on imaging instrumentation so to enable more complex forms of phenotypic analysis.

Polyethylene glycol (PEG) hydrogel of 21 kPa stiffness containing PDAC organoids seeded as fragments was embedded into a new gel of 8 kPa stiffness. Prolonged growth within the stiffened gel has allowed these traditionally hollow organoids to fill similarly to precursor PanIN lesions in tissues.

Image supplied by Carmen Rodriguez-Cupello (Systems Oncology)





PUBLICATIONS AND OPERATIONS

PEG hydrogel of 18 kPa stiffness was seeded with PDAC organoid fragments with later embedding into a gel of 8 kPa stiffness. This organoid, 4 days after embedding into a new stiffness, has created a tunnel within the hydrogel to the border between stiffnesses in order to eject cells outwards into the softer gel, eventually creating another organoid.

Image supplied by Carmen Rodriguez-Cupello (Systems Oncology)

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Cancer Biomarker Centre

(page 14)

Caroline Dive

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Cancer Inflammation and Immunity (page 22)

Santiago Zelenay

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Cell Division (page 24)

Iain Hagan

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Cell Signalling (page 26)

Angeliki Malliri

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Leukaemia Biology (page 28)

Tim Somervaille

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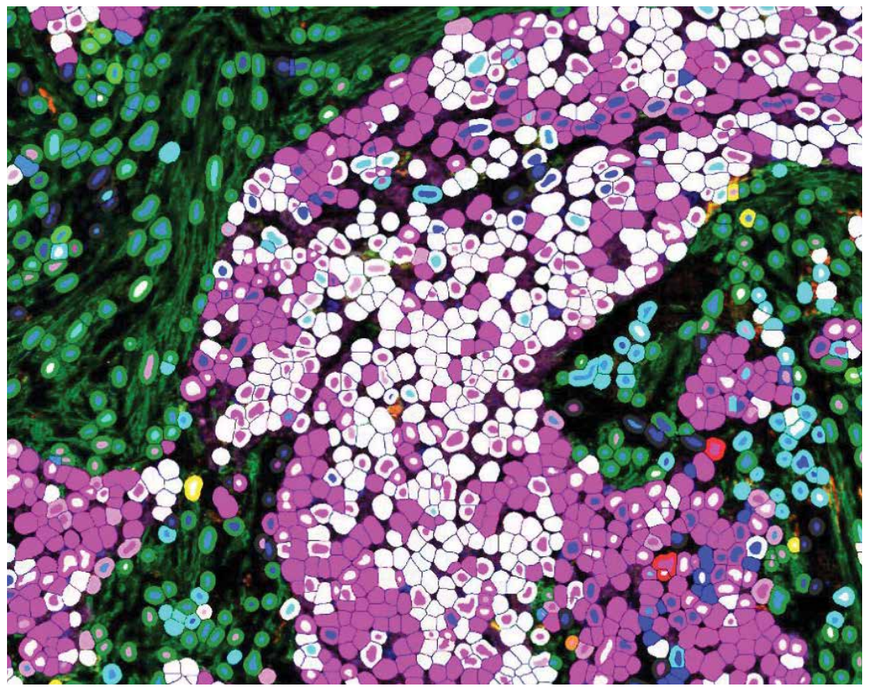
A neighbourhood analysis mask overlayed the stained tissue - lung squamous cell carcinoma showing tumour infiltrating immune cells (red and yellow) in stromal (green) and cancerous (purple) cells. Neighbourhood analysis enables investigation of tissue areas; the characteristics of each cell shown as different colours. This analysis aids our understanding of the composition and heterogeneity of tumours, and why certain therapies work in some areas of the tumour but not in others.

Image supplied by Victoria Fife (Cancer Biomarker Centre)



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Skin Cancer and Ageing (page 32)

Amaya Virós

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Stem Cell Biology (page 34)

Georges Lacaud

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Systems Oncology (page 36)

Claus Jørgensen

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Translational Lung Cancer Biology (page 38)

Carlos Lopez-Garcia

Refereed research publications

Roberts M, Ogden J, Hossain ASM, Chaturvedi A, Kerr ARW, Dive C, Beane JE, Lopez-Garcia C. (2023)
Interrogating the precancerous evolution of pathway dysfunction in lung squamous cell carcinoma using XTABLE.
Elife 12:e77507.

Translational Oncogenomics (page 40)

Rob Bristow

Refereed research publications

Dubec MJ, Buckley DL, Berks M, Clough A, Gaffney J, Datta A, McHugh DJ, Porta N, Little RA, Cheung S, Hague C, Eccles CL, Hoskin PJ, Bristow RG, Matthews JC, van Herk M, Choudhury A, Parker GJM, McPartlin A, O'Connor JPB. (2023)
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Cell Death Differ. 30(6):1533-1549.

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EXTERNAL SEMINAR SPEAKERS 2023

The seminar series that we run is vital for the Institute, connecting world-class researchers across the broad spectrum of cancer research. Following the move back to the Paterson Building and our original site next to the Christie NHS Foundation Trust, we were able to reconnect with colleagues across the Manchester Cancer Research Centre and build on those in person scientific interactions to invite an excellent set of internationally renowned speakers. The postdoctoral researchers and technical staff at the Institute continue to give weekly seminars, which are important in bringing our scientists together and helping to integrate the entire cancer research efforts of the Institute.

Ketan Patel
University of Oxford

Payam Gammage
CRUK Beatson Institute

Donald E Ingber
Wyss Institute at Harvard

Andrew Sharrocks
University of Manchester

Greg Hannon
Cancer Research UK Cambridge Institute

David Crosby
Cancer Research UK

Paul Nurse
The Francis Crick Institute

Julie George
University of Cologne

Jason Carroll
Cancer Research UK Cambridge Institute

Anindita Roy
University of Oxford

Andrew Jackson
University of Edinburgh

Owen Sansom
CRUK Beatson Institute

Neta Erez
Tel Aviv University

Cigall Kadoch
Dana–Farber Cancer Institute

Jesus Gil
MRC Laboratory of Medical Sciences

Paco Real
CNIO

Carmit Levy
Tel Aviv University

Sebastien Jaillon
Humanitas University

Inigo Martincorena
Wellcome Sanger Institute

Axel Behrens
Institute of Cancer Research

Michelle Garrett
University of Kent

Chris J. Halbrook
University of California Irvine

Andrew Wood
University of Edinburgh

Sridhar Hannenhalli & Vishaka Gopalan
NCI Center for Cancer Research

Thomas Milne
University of Oxford

Sam Au
Imperial College London

Thijn Brummelkamp
Netherlands Cancer Institute

Gerard Evan
University of Cambridge

Sankari Nagarajan
Manchester Breast Centre

Martin McMahon
University of Utah

Nicholas McGranahan
UCL

Inigo Martincorena
Wellcome Sanger Institute

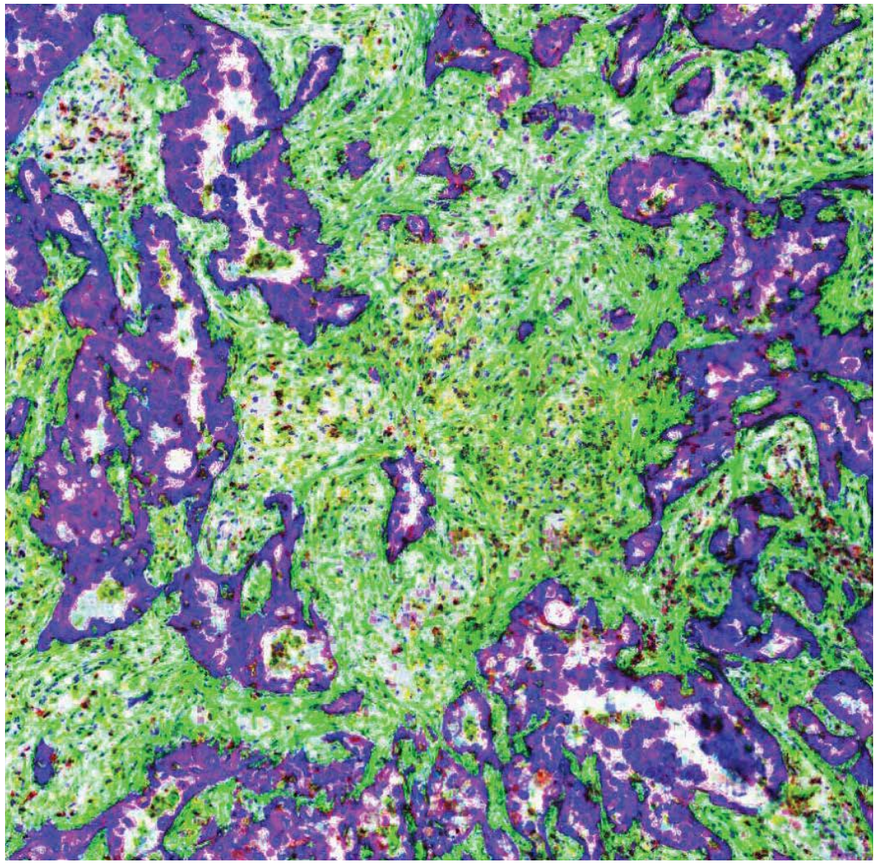
Christine Harrison
Newcastle University

Elaine Mardis
Nationwide Children's Hospital

Ilaria Malanchi
The Francis Crick Institute

Lung squamous cell carcinoma displaying cell nuclei in blue. Different antibodies were used to identify subsets of immune cells (red and yellow), identify cancer cells (purple) and cancer-associated stromal cells (green).

Image supplied by Victoria Fife (Cancer Biomarker Centre)



OPERATIONS

Chief Operating Officer
Caroline Wilkinson

Chief Laboratory Officer
Stuart Pepper

Chief Finance Officer
Mike Berne

Chief Human Resources Officer
Rachel Powell

The past year marked the end of a prolonged period of disruption as the Institute moved back to our original location on the site of the Christie NHS Foundation Trust in Withington. Many within the Operations team were heavily involved in organising and co-ordinating the relocation of the Institute, while others worked hard to set up operations in the new building. Prior to the move, the team worked closely with our new Landlords, the Christie NHS Foundation Trust, to set up new operational arrangements in the facility and have established excellent working relationships with both Christie and the Facilities Maintenance Team from Dalkia.

Chief Operating Officer
Caroline Wilkinson

We worked hard to ensure that all necessary licences were in place, including the Establishment Licence for work conducted under the Animals (Scientific Procedures) Act and a permit from the Environment Agency to conduct experiments using radio-chemicals.

At the start of the relocation, some groups who had remained at the Oglesby Cancer Research Building in Withington moved to the new Paterson Building or moved to a new location in OCRB, ably assisted by our Logistics team, followed by most teams relocating from Alderley Park where we have been based since 2017/2018 following the fire in the original Paterson Building. The move took place over several weeks and started just three weeks after practical completion of the build, which had been delayed by three months from its original target. A consequence of this was that commissioning and finalising operational arrangements happened concomitantly with the relocation. Despite the challenges that this posed, the relocation went very smoothly and was completed on time and under the target budget. As part of the move, we have reviewed all our emergency response and business continuity plans and updated the Institute's risk register.

Since settling into the building, the operations team has supported numerous visits of major donors, the media and other regular Institute events, which we have adapted to our new surroundings, such as the PhD student recruitment process.

In parallel with working on the relocation, the Health and Safety team conducted a comprehensive audit of our health and safety management processes (HASMAP) achieving high compliance in each category of the audit.

Our Human Resources team continued their efforts to roll out our Equality, Diversity and Inclusions strategy and have launched a wellbeing strategy, which has included wellbeing champions being recruited across the Institute.

During the year the Cancer Biomarker Centre spun out from the Institute to become the CRUK National Biomarker Centre. They remain aligned with the Institute and with ongoing assistance from our Operations team, and we look forward to supporting them.

In terms of team changes, we said goodbye to Helen Jones and Emma Lloyd and look forward to welcoming Minerva Chang as our new Administrative Services Co-ordinator in 2024. We welcomed Ruth Cox back from maternity leave. The IT team merged with the Scientific Computing Team with this integrated endeavour now overseen by Marek Dynowski. As described elsewhere in this report they worked incredibly hard to relocate their activities and establish IT/SciCom infrastructure into the new building. The Institute's Web Developer Chris McCauley moved from Scientific Administration team to join the newly merged team.

The Operations team is looking forward to 2024 which, amongst other events, will feature the official opening event for the Paterson Building.

Administration Team
Ruth Cox, Naomi Samuels, Karen Lee, Helen Jones[1]

[1]Left in 2023

The Institute Administration Team plays a crucial role in supporting the Senior Management Team and Faculty, ensuring the smooth operation of the Institute. Ruth Cox is Executive Assistant to Caroline Dive in her role as Interim Institute Director and Claus Jorgensen, Deputy Director. Ruth's role was covered by Naomi Samuels for the first few months of 2023 during maternity leave, following which Naomi continued to support Caroline Dive in her role as Director of the CRUK Cancer Biomarker Centre (CBC). Karen Lee is Executive Assistant to Caroline Wilkinson, Chief Operating Officer and Stuart Pepper, Chief Laboratory Officer. Helen Jones held the position of Administration Services Coordinator until Summer 2023.

This year, the Administration Team's central focus was facilitating the Institute's relocation to the Paterson and Oglesby Buildings, ensuring a smooth transition of people and processes into their new home. We have been pleased to restore in-person events and meetings, with the Student Showcase and CBC Showcase both providing excellent platforms to share our research and it was a joy for the Institute and collaborators to interact on our new site. We have been involved with hosting many tours of the new Paterson Building for groups including architects, fundraisers, donors, and colleagues from the University and CRUK.

We hosted 35 external seminar speakers in 2023 and have organised a busy programme of internal seminars, student talks and PhD viva talks alongside a range of education and engagement events for staff and students throughout the year. Our gratitude extends to all the invited speakers who generously shared their insights. Details can be found at cruk.manchester.ac.uk/seminars.

We were delighted to host our Welcome Party in Autumn at the local Red Lion pub, during which we celebrated the new students and staff who have joined the Institute recently. The event also felt like a 'Welcome back home' to Withington and The Christie site. We ended the year with a fantastic Christmas party, held at The Woodstock Arms in Didsbury.

Finance and Purchasing
Mike Berne, Denise Owen, David Jenkins, Muhammad Raja, Vikki Rosheski

This year we continued to see growth in the charitable funding availability following the pandemic and the move into the new Paterson Building has provided a springboard for new collaborative opportunities. The increasing cost of inflation continues to be of concern, particularly on running costs and consumables, but our continued hard work to ensure good relations with our suppliers means we can remain competitive with our research applications despite budgetary constraints.

The Finance team continues to support the Institute Director and the management of the reduced £17m budget to account for the spinout of the CRUK National Biomarker Centre, the finances of which are also managed by the Finance team. Finance also assists in providing costs and advice for new research proposals and contracts for all our groups. Despite global financial pressures, we continue to be successful in winning a number of new awards, with several million pounds flowing to the Institute in relation to research applications and contractual agreements.

The Institute's move to the Paterson Building went exceptionally well. It has been an exciting time for everyone, and the Finance team are looking forward to the new challenges and opportunities it brings.

Human Resources
Rachel Powell, Andrew Haines, Julie Jarratt, Laura Jones, Emma Lloyd[1], David Stanier[2]

[1]Left in 2023
[2]Joint with Scientific Operations

Over the past year, the HR Department has continued to deliver a high-quality proactive service to the Institute. The department provides advice and guidance to managers and staff on all employment-related matters such as recruitment, onboarding, policy guidance, employment legislation and best practice.

During 2023, we were successful in appointing and onboarding 43 new individuals into the Institute and eight individuals through internal transfers, which is in keeping with the Institute's commitment to the development and progression of its employees. We look forward to continuing to support the growth of the Institute over the next couple of years.

We are committed to developing our staff and ensuring that Personal Development Reviews (Contribution and Development Reviews) are undertaken. This year, we reviewed the Contribution and Development Review process and increased the performance ratings from

three to five. This has created a more meaningful and motivational process which has been well received by staff. As part of the roll out of the revised process we delivered refresher training to managers. The department also facilitated the successful promotion of nine individuals this year.

Over the year, there has been a drive on wellbeing and supporting mental health within the Institute. The Institute launched its Wellbeing Strategy, and we are delighted to have recruited three Wellbeing Champions with another three due to undertake the training shortly.

The department has strongly supported the Institute's Equality, Diversity and Inclusion (EDI) vision, which is to create a diverse and inclusive culture that develops, attracts, and maintains a positive environment for staff and students whilst achieving its aim to deliver world class cancer research. Implementing the Institute's EDI action plan has resulted in positive promotion of EDI at the Institute, including its incorporation into the Institute's induction session as well as being promoted at a variety of Institute events.

We continue to collect and analyse appropriate data to help inform our approach to EDI ensuring that any inequality is highlighted, and initiatives are put in place to work towards addressing this.

In addition, we have continued our commitment to joint partnership working with the union, which has resulted in the revision of several HR policies and procedures throughout the year. One such policy was the the Institute's Respect at Work Policy; this confirms the requirement for equality, respect, and dignity at the Institute.

We have also reviewed and updated our family friendly policies, this resulted in an increase in paid leave for staff during maternity, adoption and shared parental leave. In addition, we reduced the Institute's weekly contracted working hours (from 37 to 35), which supports staff with EDI-related requirements and enables staff to have greater flexibility at work.

In addition, we visited the CRUK Scotland Institute to continue with our CRUK cross-institutes working practices. We are in the process of reviewing the scientific pay and grading framework, in partnership with other CRUK Institutes, to ensure that we are strategically reactive to the market trends for researchers within cancer research.

Next year, the department will continue its focus on Wellbeing, Equality, Diversity and Inclusion and supporting the Institute in working towards the Athena Swan accreditation. In addition, we plan to launch the revised scientific pay and grading framework.

Safety and Facilities Management

Colin Gleeson

Health and Safety

Colin Gleeson, Chris Bamber, Jeff Barry

The Health and Safety Team played a significant role in the relocation of the Institute to the new Paterson Building, on The Christie NHS Foundation Trust site. This involved finalising the arrangements for relocation of our biological material and chemical agents, ensuring that the moves were legally compliant and suitable contingency plans were in place in case of difficulties, and moving a range laboratory research and office equipment. At the same time, we also made significant contributions to operational readiness plans for the new building, ensuring that critical systems were in place, commissioned and operable prior to our occupation. This included life critical systems such as the fire detection and alarm system, low oxygen, and other gas monitoring systems, which included critical operational systems such as autoclave, glass washing and RO water systems and fume cupboard and microbiological safety cabinet commissioning. It also included the provision of adequate first aid and fire evacuation marshal cover at our new location as well as an induction programme. Following the implementation of our move plans, it was pleasing to see the move go so smoothly, and we occupied the new building without any significant issues.

Once we had occupied the building the next phase of our work begun. We prioritised and delivered a risk controls assurance programme, which tested and provided evidence that the risk control measures we employ in our work were effective in our new environment. We also ensured that information for all our equipment requiring statutory examination and testing was gathered and provided to the appropriate competent authorities.

As research groups increased their throughput of work, our more routine work picked up in parallel. We reviewed and updated our risk register, updated numerous risk assessments to reflect our new location, delivered a laboratory self-inspection programme, completed a major University of Manchester led Health and Safety audit achieving high compliance in all fields, and reviewed and improved our arrangements for occupational health surveillance of staff working in our Biological Resources Unit (BRU). We also applied for an Environmental Permitting Regulations permit from the Environment Agency for radioactive work with radio-chemicals. This was granted in July 2023, and we set up our radioactive laboratory, appointed Radiation Protection Advisors and Radioactive Waste Advisors, and developed local arrangements and risk assessments for the work, which subsequently began in December 2023.

All the above has been overseen by our Health, Safety and Wellbeing and Biosafety Committees. Importantly, we have developed good working relationships with our Landlord and their Facilities Management Team. This has proved beneficial in many respects, for example, in the development of comprehensive emergency response plans via our Emergency Response and Business Continuity Committee.

Electronics

Yunis Al-hassan

The electronics engineer provided substantial support in the relocation programme to the new building. This included disconnection and reconnection of numerous pieces of laboratory equipment, including those which were hard-wired. He also played a significant role in re-assembling numerous items of laboratory equipment. Additionally, he continued to provide the Institute with an equipment repair, maintenance, and PAT testing service, which resulted in less equipment down time and significant economic benefit to the Institute with minimisation of repair costs and avoidance of the unnecessary cost of equipment replacement. The Institute's electronics engineer also tracks equipment which is under warranty, service contract or in-house repair. This service also provides a considerable economic benefit to the Institute.

Laboratory Services

Mark Craven, Christine Williams, Corinne Hand, Tony Dawson, Busola Atuegbe, Alex Fletcher[1]

[1]Left in 2023

During 2023, Lab Services has continued to supply the various sites with sterile fluids, glassware, plastics, and microbiological media. These sites include Alderley Park (until we moved back to the Paterson Building in spring 2023), the Oglesby Cancer Research Building (OCRB), Proton Beam Centre, MCRC Biobank, and University of Manchester Incubator building.

The department continued to operate at the OCRB throughout the relocation programme, and supplying the Alderley Park site with sterile tips and microbiological media. We also opened the new Paterson based Lab Services in spring, ahead of the research groups move, using all the newly purchased equipment to quickly ramp up the delivery of a full package of sterile products.

We continue to oversee the delivery of clean lab coats across the site, and we continue to operate a monthly pipette clinic for the researchers to use.

We manage the supply and maintenance of some portable safety related items, such as lab and office based First Aid, handheld anemometers, and portable gas monitoring devices. By having access to autoclaves, we can sterilise bespoke items required by labs and in coordination with our Media Coordinator can, by prior agreement, adapt our current microbiological media and make any new alternatives requested by researchers.

As the service matures on site, we are also looking to incorporate new recycling strategies to help with Cancer Research UK and The University of Manchester sustainability targets.

Logistics

Andrew Lloyd, Michael Alcock, Edward Fitzroy, Nigel Fletcher, Sedia Fofana, Wayne Howarth Jonathan Lloyd, Robin Sherratt

During the past year, the Logistics team has had an instrumental role in the relocation of the Institute back to the Paterson Building. Before the relocation began, the team helped with the disposal of aged or redundant equipment. The team also provided additional support to some of the Core Facilities to help with their preparation in setting up in the new building. The team were taking receipt of and storing equipment, and then later delivering it back to the Paterson Building. The team also supported the assembly of the new animal cages in the BRU and the deliveries of items of hardware to help set up the data centre.

As part of the early setup, we had to ensure the building was operationally ready for the newly arriving groups. New accounts had to be set up for the provision of bottled gas, liquid nitrogen deliveries and new waste procedures established. The goods in and stores were also set up and made functionally ready for the first groups to arrive.

The team based over at the OCRB supported the internal re-organisation of groups, which involved moving groups from the first floor to the ground floor, and conversely from the ground floor to the first. They were tasked with moving all the heavy equipment, such as ultra-low temperature freezers.

During the relocation period the team worked tirelessly operating business as usual over the three sites. The Logistics team has now settled in and continues to deliver an efficient and reactive service, providing the back of house support for the research activity carried out in the Paterson Building and OCRB.

Scientific Administration

Caroline Wilkinson, Gillian Campbell, Julie Edwards, Andrew Porter, David Stanier[1]

[1]Joint with HR

The Scientific Administration team provides a variety of services to aid the smooth running of the Institute. This year many of the team have also been involved in the relocation of the Institute to the new Paterson Building and in setting up operations in our new location in addition to their normal duties. One of the many advantages of the move is that the team are now all based together, which has facilitated the many interactions and collaborative working that takes place within the team as well as with other colleagues from across the Operations team. There was a welcome return to student talks and pre-viva seminars at the Oglesby Cancer Research Building lecture theatre with our Postgraduate Education Programme continuing to be overseen by Postgraduate Education Manager Julie Edwards (see the Education section of this report).

Gill Campbell is our Grants Advisor and Scientific Operations Officer who assists our scientists in sourcing external funding opportunities and in preparing applications. She also provides support to the Grants Committee, chaired by Iain Hagan, who peer review applications and help applicants prepare for grant/fellowship interviews. This year there was funding success through applications to CRUK ACED, CRUK, Royal Society, Blood Cancer UK, AMMF, and Wellcome (through The University of Manchester Translational Partnership Award).

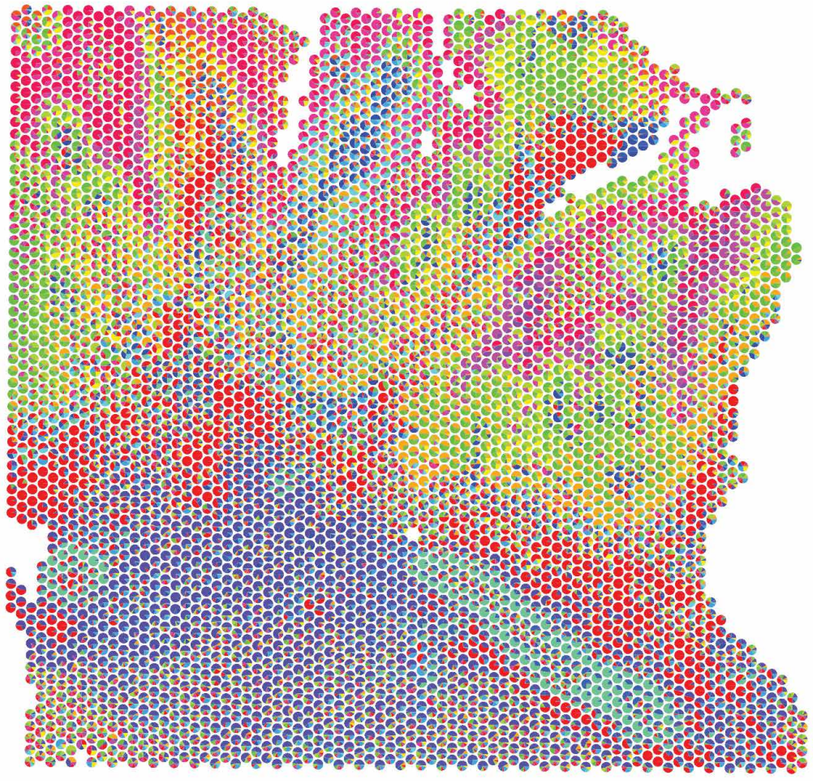
Gill also plays a vital part in the Institute's communications activities and compiles and edits this report as well as the Institute's newsletter and keeping the external website up to date. This year Gill has led a project involving other members of the Scientific Administration/ Administration team to refresh our external website with a launch date due in 2024. Although we did not hold an in-person colloquium this year, Gill organised a highly successful one-day student showcase in October where our second-year students gave presentations and we heard from some of our alumni as well as a plenary talk from Gerard Evan.

Andrew Porter is our Research Integrity and Training Adviser who supports the Institute's scientific community through a manuscript review process and training sessions for our early career researchers covering topics such as statistical analysis, meta data recording and a research integrity induction for new students and staff. This year, he has created a seminar and online training content on generative AI to help our researchers navigate its complexities and apply institutional and funder policy requirements. A major undertaking this year has been to investigate electronic lab notebook solutions together with the Scientific Computing team with a view to introducing a platform to aid with our data management strategy in 2024. He continues to expand his research integrity network and gave a presentation at the UKRIO Annual Conference 2023, as well as writing well-received blogs on various RI topics for CRUK.

Andrew is also a key part of the Institute's communications team overseeing our social media channels and has played a major role in the communications around the new Paterson Building as well as facilitating many tours and filming opportunities.

David Stanier is the Institute's Information Governance Coordinator and Administrative Officer supporting the Institute's Information Governance Guardian, Caroline Wilkinson, with the management of information security, data protection and record management to ensure information governance disciplines are embedded within working practice across the Institute. To facilitate this, David regularly liaises with the University's Data Protection Officer and Information Governance Office over best practice and produces reports for the IGO capturing metrics relating to the Institute's Information Governance activity. David was a key part of the Operations relocation team that planned our relocation, adapting many of our services and processes to the new Paterson Building. He leads the meetings of our Institute Information Governance (IG) Committee, which amongst other activities, seeks to review any institutional IG requirements, and oversees the continued deployment of Teams across the Institute. This year David joined the Institute's Emergency Response and Business Continuity Committee (ERBCC), which is chaired by Caroline Wilkinson.

The ERBCC reviewed its suite of emergency response plans ahead of the relocation during which time the Institute was split between Alderley Park, the Oglesby Cancer Research Building and the Incubator Building at The University of Manchester. Following the relocation, the ERBCC reviewed the plans again to take account of the new location and our interactions with our landlords and the facilities maintenance contractor for the Paterson Building. In parallel, the Institute's risk register has been reviewed and updated. Caroline Wilkinson and Stuart Pepper worked closely with our landlords to co-ordinate the operational readiness of the Paterson Building, producing over 30 SOPs ranging from fire responses to reception services. They continued to refine operations as we all settled into the building.



Colourful image from the spatial transcriptomic analysis of skin cancer representing various cell type signatures (each different colour) and showing the cell type layers in the skin.

Image supplied by Tim Buddon (Skin Cancer and Ageing)

Animal Welfare

Simon Poucher, Regulatory Liaison and Training Officer, Sally Robinson, Animal Welfare & Ethical Review Body (AWERB) Chair, Stuart Pepper, Chief Laboratory Officer, Caroline Wilkinson, Establishment Licence Holder.

The Institute upholds high standards of welfare for the laboratory mice used in our research. All animal research activities in the UK are conducted under the Animals (Scientific Procedures) Act 1986 (A(SP)A). The Institute's Animal Welfare and Ethical Review Body (AWERB) has oversight of research involving animals at the Institute and is required to conduct several tasks under A(SP)A. The AWERB supports all staff involved with animal research by promoting a Culture of Care, reviewing processes, staff training, and facilities for the care and use of mice, and encourages implementation of the replacement, reduction and refinement (3Rs) of the use of mice in our research. AWERB also reviews proposed collaborations and grant applications involving animal research. During 2023 we moved to the new Paterson Building in Withington, having been at either The University of Manchester or more latterly Alderley Park since the Paterson Building fire in in April 2017. Hence there was a period from May to July where fewer studies were performed with mice while the experimental facility for the whole Institute re-located.

The number of mice used in the production and supply to CRUK MI researchers was 6817 (a decrease of 21.5% compared with 2022). Overall, there were 6293 mice used in regulated procedures, including production and supply, under the Act in 2023 (a reduction of 34.4% compared with 2022).

The Institute continues to uphold high standards of regulatory compliance, promptly reporting any unexpected findings or events to the Animals in Science Regulation Unit (ASRU), all of which have been resolved. AWERB reviewed five new project licence applications and one amendment prior to their submission to ASRU for approval. The reviews by AWERB are

a required task and ensure that projects will be conducted causing least harm, using the fewest mice only when non-mouse alternatives are not appropriate or available.

We built on the Culture of Care Recognition Award that was introduced at the end of 2022 to encourage activities that further enhance the Culture of Care. In 2023, there were nine awards given for a wide range of activities ranging from supporting the animal husbandry team in an emergency, supporting a particular challenging experiment, and refining clinical endpoints for a study. The award process helps re-enforce good practices across the Licensees. In promoting the "People and Culture" aspects of our Culture of Care we have held the first workshop, with more planned, for Licensees to discuss the ethical aspects of two case studies (taken from the NCR3s website) in small groups. This generated lots of discussion around the topic areas, sharing different points of view and working through the scenarios.

We regularly share our best practices with other establishments. For example, our Named Animal Welfare and Care Officers have regular meetings with those from other CRUK Institutes. This year we relaunched our annual 3Rs' award. This was run as a poster event with two winning entries. The winner in the science category was an in vitro method developed to study a specific type of dendritic cell found in tumours. These cells could play a crucial role in the immune response against cancer but studying them in live animals is challenging. The new approach not only reduces the need for mice by about 90%, but also eliminates the need for animals to undergo experimental procedures. The winner in the technical category described the new cabinet X-ray system, which posed challenges as there is now a need to irradiate the mice top down when carrying out tumour targeted irradiations, which means the mice require an anaesthetic to keep them still during the procedure. A new irradiation jig was developed and refined (using 3D printing prototypes) to allow for accurate targeting of irradiation. The team worked to develop an inhalation anaesthesia system rather than using an injectable one, meaning the mice can recover in a few minutes (rather than in up to an hour using injectable anaesthetic).

In addition, a poster describing the collaborative efforts to improve the welfare of SKH1 Mice was recognised externally and won the Institute of Animal Technology's Andrew Blake Tribute Award for the technical refinements made to improve the mouse welfare.

Our scientists have taken part, by invitation, in online forums and conferences, and contributed to expert groups arranged by national bodies: for example, the National Centre for Replacement, Refinement and Reduction of Animals in Research (NC3Rs) webinar series on 'creating the right environment for animal care'; the Royal Society for the Prevention of Cruelty to Animals (RSPCA) meeting on running an effective AWERB; and the Laboratory Animals Science Associations (LASA) annual meeting (e.g. animal sciences transgenics section programme) to further the sharing of knowledge and advice on laboratory animal use.

- Individuals at CRUK MI continue to be part of an expert working group that are collaborating to update the 2010 guidelines on the welfare of animals used in cancer research. The guidelines are used by researchers nationally and internationally to support good practice and high standards in animal care. The update to the 2010 guidelines is being facilitated by the UK NC3Rs and National Cancer Research Institute.

- Members of CRUK MI have also contributed to meetings on sharing good practice for AWERBs such as the AWERB Hub workshops.

- Caroline Wilkinson and Sally Robinson are members of the Establishment Licence Holders Forum, Sally is Secretary General to the Council of the Laboratory Animal Science Association and a member of the NC3Rs Board, Simon Poucher is a member of the Home Office Liaison, Training and Information Forum.

Commercialisation and Innovation Support

Nathalie Dhomen, Martyn Bottomley

The Cancer Research UK commercial partnerships team is a specialist oncology-focused development and commercialisation team which is part of Cancer Research Horizons (CRH), Cancer Research UK's innovation engine. By arrangement with The University of Manchester, CRUK owns and is responsible for the development and commercialisation of intellectual property arising from CRUK funded research at the University. The commercial partnerships team aims to bridge the gap between cutting edge academic research and industrial development of cancer therapeutics, medical technologies, and diagnostics.

Our Cancer Commercialisation and Innovation Lead, Nathalie Dhomen, links into the commercialisation support infrastructure of both Cancer Research Horizons and The University of Manchester Innovation Factory – the University's technology transfer office. She is the first point-of-contact for all the research groups and clinicians working within the Manchester Cancer Research Centre partner organisations, including the CRUK Manchester Institute. She supports researchers and clinicians in identifying new high-value IP and innovations arising from their research and can facilitate access to oncology-focused expertise in technology evaluation, patent applications and management, funding for development, commercialisation, preclinical and clinical development, drug discovery, market intelligence and spin-out formation.

It has been a busy year with nearly a dozen disclosures from researchers across the MCRC, and the filing of three priority patents by researchers at the CRUK Manchester Institute. We have seen strong trends towards the development of software-based clinical decision-making tools and the advent of AI and machine learning in the clinical space, as well as a wide array of biomarker discovery and development. To address the need for guidance in software commercialisation, The University of Manchester Innovation Factory has been working with Imago Software, The University of Manchester's student software company, to translate their expertise in developing commercial software offerings into training materials and workshops for researchers seeking to bring their own software tools to market.

In the space of biomarker innovation and commercialisation, CRH and the MCRC have organised a Biomarkers Commercialisation Summit to support Manchester researchers seeking to introduce novel biomarkers to the clinic. Bringing together experts from industry, academia, intellectual property management, and diagnostics companies, as well as case studies of successful biomarker offerings and investors, the day highlighted the variety of avenues and expertise available to support the journey of a biomarker from discovery to patient benefit. The day resulted in new connections and discussions of potential collaborations, which we hope will have a positive impact on our biomarker commercialisation goals.

CRH and the MCRC have also sought to support early-stage translational projects with a strong potential for patient impact through a pump priming programme, the Springboard Award. Working with the commercialisation teams at both The University of Manchester Innovation Factory and Cancer Research Horizons, this funding aims to launch exciting ideas and overcome the financial obstacles that academics typically face when translating an idea to a product or service. Four projects, including one project developed at the CRUK MI addressing the diagnostic challenges of biliary tract cancer, have been funded through the scheme. All will have access to an infrastructure of industry connections, commercial advice, and business-planning workshops. We are looking forward to seeing these projects develop and supporting their translation journey to patient benefit.

Another exciting development in 2023 was IDEAYA announcing its first patient dosed in its phase 1 clinical trial for IDE161, a potential first-in-class PARG inhibitor targeting HRD solid tumours, that was developed right here at the CRUK Manchester Institute. We are excited to see how these trials progress and look forward to seeing this new drug making an impact on patient outcomes.

We actively manage a broad portfolio of development programmes and exciting licensing opportunities originating from the Cancer Research UK Manchester Institute that continue to attract commercial partners. These projects include novel pan-cancer treatment response biomarkers from the Cancer Inflammation and Immunity group, assets from the Cancer Biomarker Centre, novel blood production technologies from the Stem Cell Biology group, as well as unique cancer research tools, both laboratory-generated and digital. We are seeing an increasingly diverse range of research innovations being developed by CRUK funded researchers in Manchester, and we look forward to advancing their discoveries to beat cancer in the years ahead.

https://www.cruk.manchester.ac.uk/Education/Education-Home

POSTGRADUATE EDUCATION

Postgraduate Education Manager
Julie Edwards

Postgraduate Tutor
Santiago Zelenay

Postgraduate Director and Chair of the Education Committee
Tim Somervaille

The Cancer Research UK Manchester Institute offers postgraduate degrees (PhD) for students interested in a career in cancer research. The Institute considers education of both research and clinician scientists to be a major investment in the future of cancer research and has an excellent track record of launching careers in basic, translational and clinical research. As part of this commitment, we have an active postgraduate programme that provides students and clinical research fellows with the potential and opportunity to study for a cancer-related PhD degree.

This is achieved through a structured training programme that aims to improve effectiveness in research, provide professional and management skills and enhance career development. Our PhD students have exceptional employment prospects following graduation, with the vast majority continuing in academia, industry, or healthcare, and securing positions in destinations across the UK, Europe, and the USA. In 2023, 100% of our graduates found positions following PhD completion: academic (27%), scientific industry (64%) and return to clinical training (9%).

In 2023, we welcomed twelve new graduate students and two clinical research fellows to our PhD programme, working in a variety of fields, including cancer immunology, leukaemia biology, cancer biomarkers, small cell lung cancer biology, and translational oncology.

It was also particularly gratifying to see that over the past twelve months, some of our PhD students and clinical research fellows had published first author papers in a variety of journals including *Proc Natl Acad Sci U S A*, *Cancer Cell*, *Journal of Thoracic Oncology*, and *iScience*. Other students contributed to several publications as middle authors, including *Nature Communications*.

The Cancer Research UK Manchester Graduate Programme
We aim for each student to receive high quality training in scientific research through an intellectually demanding but achievable research programme. Each project is peer-reviewed in advance of commencement and monitored with formal student assessments at key stages throughout the duration of the programme. Modes of assessment include annual written reports, oral presentations and progress meetings, which are designed not only to provide formal points at which progress (of both the student and the project) can be monitored but are beneficial in the development of presentation skills fundamental to most academic careers in science and beyond.

Graduate training and student welfare is monitored by the Institute's Education Committee, with members including Institute group leaders and fellows, operational managers, and student representatives (see below) providing a diverse range of experience and expertise. A main supervisor and a second or co-supervisor are nominated for each student, providing advice and support on both academic and non-academic matters. Students are assigned an advisor whose role is to provide impartial support and advice in a pastoral capacity.

The CRUK Manchester Institute has an established internal and external seminar series featuring talks from leading scientists in cancer research, and all our students benefit from these events. Speakers are internationally renowned scientists, and we consider it essential that our students are exposed to outstanding research from leaders in different disciplines, providing a broad understanding of many aspects of cancer research and basic biology. In addition, we hold an internal series of weekly postdoctoral and technical research seminars that also plays an integral part in the student learning pathway. While students themselves are asked to give talks at key points during their PhD, they also have opportunities to present their work at lab meetings, student forums and external conferences. Conferences and seminars play an essential role in connecting and networking with colleagues across the Institute, nationally and internationally.

Staying connected with peers and colleagues has remained a key component for students over the last three years, not only in terms of research progress, but also mental health and wellbeing. A programme of in-house training events, external and internal seminars are all held on site and in person, providing an invaluable opportunity in encouraging students from the CRUK Manchester Institute and Division of Cancer Sciences at The University of Manchester to engage and connect with the wider scientific community.

The CRUK Manchester Institute relocated from Alderley Park, Cheshire back to its original site at the new Paterson Building, Manchester in June 2023. The move went according to plan with most PhD students experiencing little or minimal disruption to their studies. Student research and activities continue to thrive in the new building with access to advanced state-of-the-art equipment and excellent core facilities alongside the Oglesby Cancer Research Building and the Christie NHS Foundation Trust.

STAy (Science TakeAway) is a committee group run by junior scientists and students in the CRUK Manchester Institute with the aim of providing a forum for discussions and training related to research, communication of scientific engagement and development of social and networking opportunities. STAy is keen to encourage networking, career progression and personal growth of early-career researchers encouraging students, scientific staff and post-docs to engage ensuring the research community remains well connected.

Due to the relocation of the Institute, the 2023 annual colloquium was postponed, and a one-day Student Showcase was held in its place in October in the Oglesby Cancer Research Building. The event provided a platform for our 2nd year students to present highlights of their latest research and progress to-date. All current students from years 1, 3 and 4 were invited to submit a poster. An alumni session and a seminar from external guest lecturer Gerard Evan, closed the day followed by a welcome evening social event.

We congratulated two PhD students, Maria Koufaki (4th year) from Cancer Inflammation & Immunity and Yitao Chen (4th year) from the Cancer Biomarker Centre, who were jointly awarded the Lizzy Hitchman prize for the best poster: 'Phenotypic and functional characterization of conventional dendritic cells in cancer' by Maria and 'Multi-omics analysis of CDX models to identify potential biomarkers that predict drug response in small cell lung cancer' by Yitao.

Cancer Research UK contributes towards PhD student attendance at the annual International PhD Student Cancer Conference (IPSCC) allowing high calibre students (typically 2nd - 4th years) from the top cancer research institutes across Europe to organise and present at their own scientific conference. The conference is organised by students for students from core participating Institutes: the Francis Crick Institute, CRUK Manchester Institute, Cambridge Institute (CI), CRUK Scotland Institute (formerly the Beatson), Netherlands Cancer Institute (NKI), European School of Molecular Medicine, Milan (SEMM, IFOM & IFEO), Max Delbruck Center (MDC), Berlin and the German Cancer Research Centre (DKFZ).

The 16th annual International PhD Student Cancer Conference brought 120 students together in June 2023, hosted by students from the CRUK Cambridge Institute. The two-and-a-half-day programme featured high profile keynote speakers, student talks, poster sessions, career workshops and opportunities for networking and interacting with plenary speakers.

POSTGRADUATE EDUCATION (CONTINUED)

CRUK Manchester Institute was represented by 15 students from years 1-4. There were 30 talks in total over the 2.5 days, and two excellent talks were given by our students:

Oliver Bartley, Cancer Biomarker Centre. "Circulating Tumour Cell-Derived Explant (CDX) Models to investigate SCLC's Intrinsic Immunobiology".

Lobsang Dolma, formerly of the Tumour Suppressor group. "Engulfment of cells and vesicles by GOF-mutp53 and its implications in cancer".

At the end of the conference, students voted for the best oral presentation and the top three posters from 88 entries. We are delighted that two CRUK Manchester Institute students were awarded the 1st and 2nd poster prizes:

1st Prize - Bradley Revell, 4th year student from Leukaemia Biology. He showcased his work on "The biology of transcription factors in Acute Myeloid Leukaemia".

2nd Prize - Parsa Pirhady, 4th year student from Translational Oncogenomics. He showcased his work on "Multiomics Approaches to Investigate the Role of MSH2 in Localised Prostate Cancer".

We are looking forward to joining the students at the Max Delbruck Center, Berlin at the 17th IPSCC in June 2024.

PhD studentship recruitment
PhD recruitment to our core funded studentships is highly competitive, with between 300-500 applicants competing for between four and eight places each year. CRUK core funded studentships are full time for 4 years with an approved research project to be undertaken in one of our core funded research groups. Some students are allocated joint supervisors in different groups, fostering important collaborations, and providing exposure to different disciplines. Interviews are typically conducted annually over a two-day period in January/February, however, additional PhD studentships afforded by alternative funding routes, may be advertised at various times throughout the year.

PhD studentships and clinical fellowships in 2023 were awarded to the CRUK Manchester Institute core funded groups via CRUK core funding to the Institute, Cancer Research UK Manchester Centre Clinical Training Fellowships, Manchester Biomedical Research Centre (BRC) and the Manchester Cancer Research Centre MB-PhD Scheme.

Education Committee 2023
The Education Committee acts for postgraduate students and consists of group leaders, the Chief Operating Officer, the Postgraduate Tutor, and the Postgraduate Education Manager from the CRUK Manchester Institute.

Our goal is for every student to have a project that is both achievable and intellectually stimulating and demanding. Projects and students are monitored by the Education Committee ensuring that the proposed plan of research is achievable, and that progress is made consistently throughout the course of the studentship. Various assessments at key stages throughout a CRUK Manchester Institute PhD studentship are a vital component in ensuring successful PhD completion and graduation of our students. Such assessments are not only crucial in the development of students throughout their postgraduate programme, but importantly enhance future employability and academic careers.

Education Committee Members
Tim Somervaille, Postgraduate Director & Chair
Caroline Dive, Ex-Officio Member
Julie Edwards, Postgraduate Manager
Santiago Zelenay, Postgraduate Tutor

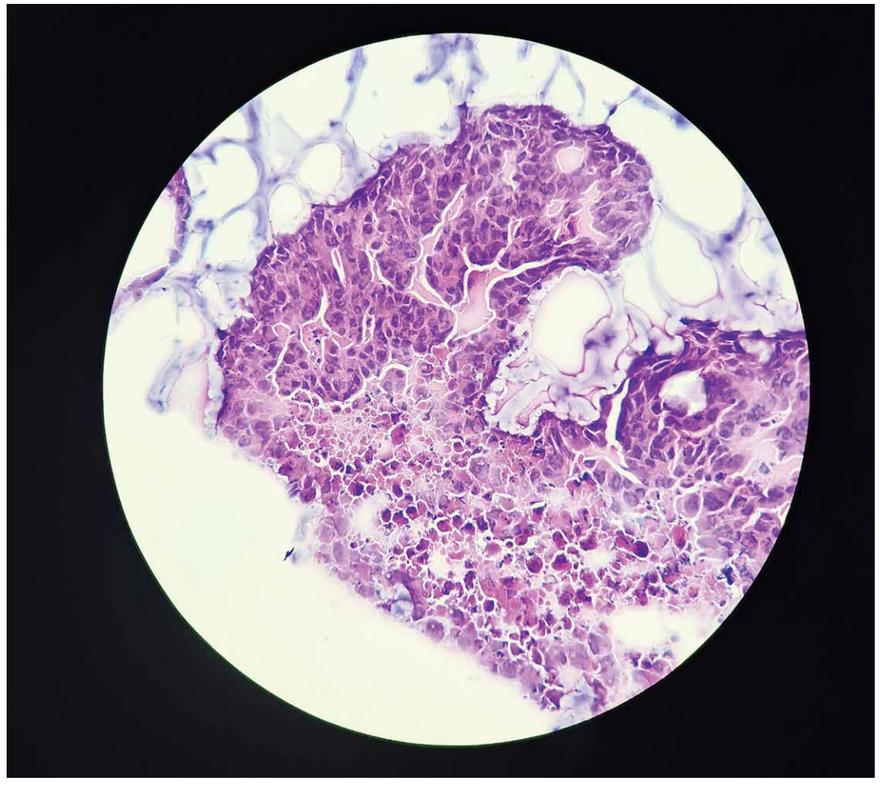
Amaya Virós
Carlos Lopez Garcia
Caroline Wilkinson
Claus Jørgensen
Dominic Rothwell[1]
Georges Lacaud
Mark Williams

Student Representatives
Seung Lee[2]
Victoria Fife[2]
Florentia Moussoulou[1]
Sophie Richardson[1]

[1]Joined in 2023
[2]Left in 2023

PEG hydrogel of 21 kPa stiffness was embedded into a new gel of 8 kPa stiffness where PDAC organoids had been growing from single cells. These have exited the original stiffness into the softer area and created ductal like structures reminiscent of tissue.

Image supplied by Carmen Rodriguez-Cupello (Systems Oncology)



THESES

Hasse Bossenbroek
Epigenetics of Haemopoiesis Lab

A multiomic study of epigenetic dysregulation in chronic myelomonocytic leukaemia

Alexandru Suvac
Translational Oncogenomics

Studies in genetic instability under hypoxia in prostate cancer

James Stratford
Drug Discovery

Design and synthesis of targeting molecular imaging probes to identify lysyl oxidase and a suicide gene therapy transgene in vivo

Mihaela Ficu
Drug Discovery

Discovery of novel Proteolysis-Targeting Chimeras for cancer-associated proteins

Melissa Frizziero
Cancer Biomarker Centre

Dissecting the neuroendocrine landscape of extra-pulmonary neuroendocrine carcinoma to discover biomarkers with clinical utility

Lucy Ginn
Cell Signalling

The Evaluation of RAC Signalling as a Therapeutic Target in Non-Small Cell Lung Cancer

Bradley Revell
Leukaemia Biology

The Biology of Transcription Factors in Acute Myeloid Leukaemia

Naseer Basma
Leukaemia Biology

Mesenchymal stromal cell reprogramming and aberrant inflammatory signalling in myelofibrosis: insights from single cell RNA sequencing

CANCER RESEARCH UK MANCHESTER INSTITUTE'S RESEARCH ENGAGEMENT

Research engagement forms an integral part of the culture at the Institute, with our early career researchers being the driving force behind the organisation of many activities. In this section, we celebrate their passion and enthusiasm for communicating science and reaching out to engage with local communities. This year they made the most of being back in Withington and reconnecting with our neighbours.

Engagement activities start ramping up from World Cancer Day, held every year on 4 February. The aim is to raise worldwide awareness, improve education and catalyse action by working together to save millions of preventable cancer deaths and make access to life-saving cancer treatment and care equitable for all. It is an opportunity for our researchers to highlight the international impact of the important work they do, via social media channels.

Helping to inspire the next generation of cancer researchers is a key part of engaging with young people. National events can facilitate this interaction and our researchers are always keen to get involved. British Science Week is an annual celebration of science, technology, engineering and maths that supports thousands of activities across the UK.

The Research Engagement Team at the Cancer Research UK Manchester Institute created a new and stimulating outreach experience called 'The Biomarker Lab'. This activity replicates an ELISA experiment and allows students to screen simulated patient samples for cancer by detecting a fictional biomarker called FLAVO. It was designed in such a way that school children could easily understand.

The Biomarker Lab

The Biomarker Lab debuted at several locations, including the 1st Didsbury Guides, Queensgate Primary School, and Manchester Museum during British Science Week 2023. Everyone got a comprehensive overview of how ELISAs work and their importance in cancer screening and treatment monitoring, all while getting hands-on with pipettes, samples and plates.

The Lab was seen as an enormous success and gave students an excellent insight into work carried out at the Institute. The activity was brought to life thanks to the fantastic team effort of Bradley Revell, Joanna Kelly, Andrew Porter, Molly Glenister-Doyle, Sophie Richardson, Catherine Felton, Mollie Anne Halford, Erminia Romano and Ana Vitlic.

Guide group in Didsbury take part in the Biomarker Lab and learn all about ELISAs with Sophie.

Catherine and Tim showcase the Biomarker Lab at the Bruntwood Schools' day at Alderley Park.

Institute scientists take the Biomarker Lab to more than 1000 students at the Inspiring Futures event at the Manchester Museum.

This year David's Movember fundraising events raised an enormous £1,922. Well done! Movember fundraising kicked off with 'David's Great British Bake-Off Halloween Special' on 31 October, held in the foyer of the new Paterson Building. Everyone was pleased that David baked a fine selection of his favourite and most popular treats, including delicious cupcakes, loaf cakes, rocky road and much more!

During British Science Week, the activity was carried out at several locations by members of the team, captivating the interests of young people from across Greater Manchester. Sophie took the Biomarker Lab to her guide group in Didsbury, whilst Kathryn Simpson visited a local school, carrying out the activity with around 30 children.

At the Bruntwood Schools' day at Alderley Park, Catherine, Joanna, Sophie and Tim Budden showcased the Biomarker Lab. The event was aimed at young people who were exploring careers in science and saw year 10 students from across local schools in Cheshire take a hands-on approach with cancer research. The students loved the activity, with a Bruntwood representative commenting, "the cancer research activity came up several times as a highlight of the day!"

Finally, a team of nine scientists from the Institute – Andrew Porter, Deepti Wilks, Duncan Smith, Erminia Romano, Hannah Sheedy, Maria Peiris Pages, Mollie Anne Halford, Molly Glenister-Doyle and Mukkarram Hossain – attended the Inspiring Futures event at the recently reopened Manchester Museum. This event aimed to promote future study in healthcare and showcased diverse careers within the NHS. Cancer Research UK staff teamed up with Stephanie Seville from the Museum of Medicine and Health, and Ellie Chambers from the Manchester Cancer Research Centre to provide a collection of stalls focusing on cancer research and treatment. With a total of 1137 students attending across two days, the Biomarker Lab got great exposure, with students enjoying the interactive experiments and talking to the researchers.

The hope is that this activity can easily be recreated and adapted for use at future events and by other Cancer Research UK Institutes. The team plan to continue engaging and building relationships with local communities through participating in similar future events, especially now we have moved to the new Paterson Building and the team are back together at the heart of the cancer campus in Manchester.

Fundraising for Movember 2023

Institute Finance Purchase Officer David Jenkins has been fundraising for men's health for over ten years. The Movember Foundation is the only global charity focused solely on men's health and this Movember David worked exceptionally hard to raise funds to tackle some of the biggest health issues faced by men: prostate cancer, testicular cancer, and poor mental health.

After a few years' break due to the pandemic, David resumed his long-awaited Movember Fundraising events this November, arranging four sell-out events.

The Movember Raffle alone raised a staggering £749.00, together with David's Great British Bake Sale Halloween Special, David's Lucky Dip, the Movember Café Talk, and other donations made this David's most successful Movember yet, raising a jaw-dropping grand total of £1,922.00. We would like to thank David for his continued hard work with his fundraising campaigns and to everyone at the Institute for their generosity.

CANCER RESEARCH UK MANCHESTER INSTITUTE'S RESEARCH ENGAGEMENT (CONTINUED)

Images left to right: Stuart Pepper gives a presentation to the supporters in the Oglesby Lecture Theatre; A demonstration in the lab is given to some of the supporters; University of Manchester President, Dame Nancy Rothwell addresses major donors during a tour of the Paterson Building.

Paterson Building tours with donors and sponsors
Following the move into the Paterson Building, between September and December 2023, the Institute held 13 separate tours and events for the many sponsors and donors that made the build possible. We also hosted the architects, fundraisers and colleagues from the University and CRUK.

Guests included the Taylor Family Foundation, Northwest and CRUK Supporters, PepsiCo, as well as individual donors such as Yakub Patel, Ada Haywood, Mike Jackson, Richard Bevan and Peter George, plus many others. Further tours are also planned for early next year.

The many visitors were delighted to observe the impact of their donations first hand. They were impressed with the state-of-the-art laboratories, appreciating how fully interconnected lab spaces across all floors remove traditional barriers to researchers' movement and facilitate the sharing of equipment and ideas.

The Northwest Supporters tour was a great success and included talks in the lecture theatre followed by a tour of the laboratories. Here are just some of the positive quotes from the supporters who attended:

"Very uplifting and very, very impressed with what is happening in these buildings."

"The explanation of the scientific discoveries during the morning is inspirational – it makes it very clear how important fundraising is and the impact the research has."

"A very inspiring day, a privilege to be shown round a centre of such excellent research. Proud to have seen where world class work on research to beat cancer takes place in such a magnificent building with such dedicated scientists."

"The tour was a fantastic experience. Understanding more about how treatments are advancing was very powerful."

The Institute is immensely grateful to all the donors and sponsors. To express our gratitude, next year plaques commemorating the funders will be erected throughout the building, with the naming of floors after organisations giving large donations. Floor 5 for example will be named the Wolfson Foundation Floor.

ACKNOWLEDGEMENT FOR FUNDING OF THE CANCER RESEARCH UK MANCHESTER INSTITUTE

The total funding of the CRUK Manchester Institute and the CRUK National Biomarker Centre for 2023 was £22.1m. The major source of this funding was awarded by Cancer Research UK (CRUK) via a core grant of £10.8m, a CRUK National Biomarker Grant of £2.5m plus additional strategic funding of £1.8m. This funding enables the various scientific groups and service units within the Institute to carry out and support impactful research.

The infrastructure of the CRUK Manchester Institute is funded by Research England generated income at a cost of £2.1m.

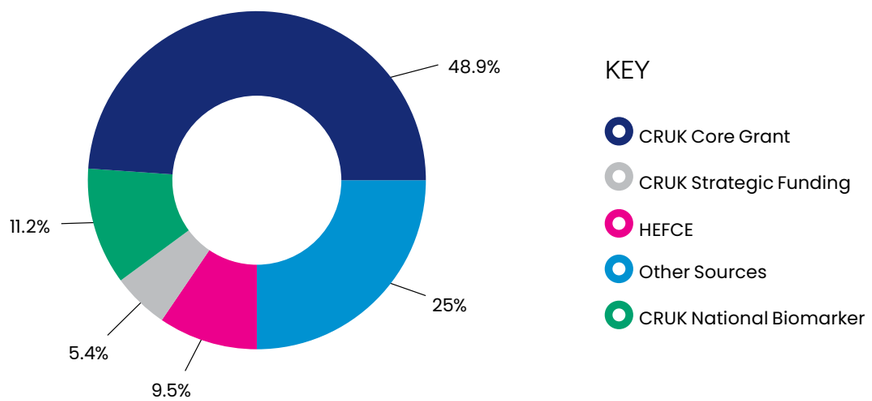
The balance of the Institute's funding is received from a number of additional sources. The research carried out through these additional projects enhances and supports the research undertaken by the core funding.

These sources are as follows:

- Amgen
- Angle Inc
- Astex Pharmaceuticals
- Astra Zeneca
- Bioven
- Blood Cancer UK
- Boehringer Ingelheim
- Carrick Therapeutics
- CellCentric
- Christie Hospital NHS Foundation Trust
- European Commission
- European Research Council
- Fondation ARC pour la Recherche sur le Cancer
- GlaxoSmithKline
- Harry J Lloyd Charitable Trust
- Institut de Cancerlogie Gustave Roussy
- Imago Bioscience
- Kay Kendall Leukaemia Fund
- Lady Tata Memorial Fund
- Lustgarten Foundation
- Medical Research Council
- Medimmune LLC
- Melanoma Research Alliance
- Menarini Biomarkers Singapore
- Merck
- Moulton Charitable Trust
- My-T Bio Ltd
- National Institute of Health Research
- NC3Rs
- Neuroendocrine Cancer UK
- Ono Pharmaceuticals
- Pancreatic Cancer Research Fund
- Perfusion Biotech
- Pickering Leukaemia Research
- Prostate Cancer UK
- Rosetrees Trust
- Sosei Heptares
- Taiho Oncology Inc
- The US Department of Health and Human Services
- UKINETS
- Wellcome
- Worldwide Cancer Research

We are immensely grateful to all our sponsors.

CRUK MANCHESTER INSTITUTE FUNDING 2023



A NEW CANCER RESEARCH ECOSYSTEM IN MANCHESTER

"Having the Institute all together on one site - alongside our colleagues at the Christie and in the Division of Cancer Sciences - in the Paterson Building that is designed to maximise collaborations, makes driving innovative cancer research easier and paves the way for breakthroughs in our discovery science and translational research."

Caroline Dive, Interim Director, Cancer Research UK Manchester Institute

After a devastating fire CRUK Manchester Institute returns to a brand-new cancer research facility

Six years after the catastrophic fire that resulted in the relocation of the Cancer Research UK Manchester Institute to temporary premises at Alderley Park, we returned to a new building on our original site at The Christie NHS Foundation Trust, having turned disaster into a unique opportunity to create a state-of-the-art facility that heralds a new era of cancer research in Manchester.

This new facility – the Paterson Building – will lead world-class transformational cancer research. By supporting the integration of our discovery and translational research, we can transform patient outcomes through advances in the prevention, early detection and treatment of cancer.

Collaboration and Innovation

More than twice the size of the original building and covering over 25,000 square metres, the ambitious vision for the new Paterson Building was delivered through the collaborative efforts of numerous stakeholders, including the Christie NHS Foundation Trust, The University of Manchester, and Cancer Research UK – who formed the Paterson Redevelopment Project – along with generous donations to the 'Re-Write Cancer' campaign, a £20 million joint fundraising appeal that helped meet the £150 million cost of the building.

Thanks to the resilience and hard work of many people across the Manchester Institute over the past six years, we finally relocated to the new comprehensive cancer facility in spring this year. The ten-storey building is now occupied by one of the largest collection of scientists, clinicians, allied healthcare professionals and administrative support staff in Europe.

The design

Starting afresh made it possible to rethink how we work. Fully interconnecting lab spaces across all floors allows for seamless movement of researchers, streamlining workflows and facilitating the sharing of equipment and ideas.

Key to the integration of discovery and translational research is our proximity to the Christie Hospital – we are directly linked by a bridge. This connection accelerates the collection and processing of patient samples and access to clinical trials, further driving progress in personalised medicine. Tissue and blood samples from patients can now be transferred from research labs to the clinic in a matter of minutes.

Detailed planning of our animal facility incorporated many features designed to support the highest standards of both animal and staff welfare. Locating together teams from Experimental Services and Breeding, along with the Genome Editing and Mouse Models facility, has enhanced integration and collaboration and helped streamline processes.

Breakout spaces on each floor encourage informal and spontaneous gatherings, while bespoke meeting rooms incorporate the latest technology for hybrid meetings with research colleagues from across the world. Private meeting pods dotted on each floor complement open space working.

Research clusters

The new building provided the exciting opportunity to cluster research activities and maximise collaboration and synergistic interactions across the cancer campus here in Withington. The assemblage of research teams has been carefully thought out, bringing groups that were previously quite distant into neighbouring research areas, creating a comprehensive team science approach.

Haematological cancer research

For example, the breadth and depth of clinical and non-clinical research in haematological cancers has significantly increased in strength over the last few years and for the first time these groups can be colocated, which will undoubtedly promote even greater collaboration and free flow of ideas.

On Floor 5 of the Paterson Building we have grouped together Leukaemia Biology, led by clinician scientist Tim Somervaille, with Leukaemia Immunology and Transplantation, headed by Institute Fellow Mark Williams, and Stem Cell Biology, run by Senior Group Leader Georges Lacaud. Also on this floor are Kiran Batta and Dan Wiseman from the Division of Cancer Sciences, who both focus on haematological cancer research. Working alongside each other in this shared space facilitates discussion around their connected interest in blood cancers.

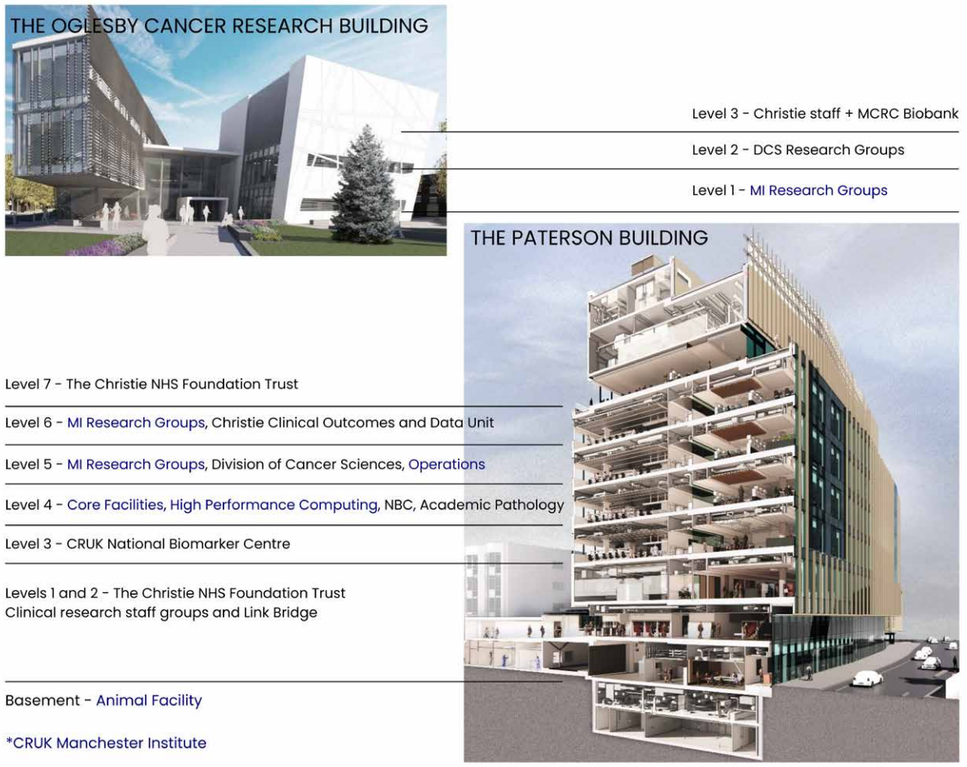
"Having different groups all around us - who tackle different parts of immune-oncology and different aspects of the biology of blood cancer - really is essential for our work."

Mark Williams, Institute Fellow, Leukaemia Immunology and Transplantation

Amplifying this alliance is the Christie being one of 13 Leukaemia Care Trials Accelerator Programmes Centres in the UK and a Myeloma UK phase I centre, and other haematological research groups within the Division of Cancer Sciences – based in the Oglesby Caner Research Building across the road – covering basic and clinical research that cuts across several research themes.

We anticipate this colocation will hugely benefit the translation and reverse translation of discoveries and insights between the laboratory and clinic.

A NEW CANCER RESEARCH ECOSYSTEM IN MANCHESTER (CONTINUED)



The Withington Cancer Campus co-locates scientists, clinicians and support staff to drive transformative breakthroughs in cancer research and treatment. Architectural drawings of the Paterson Building (credit BDP) and OCRB (credit Gillespies) shows floor allocation and how flexible spaces create a truly collaborative environment.

Supporting the Institute is the Operations department, also on Floor 5, comprising Scientific Administration, Finance and HR teams, working side-by-side in open plan office space. This open clustering allows greater interaction between the different teams to help coordinate and facilitate Institute operations while supporting the wellbeing of all staff.

Elsewhere in the building, Floor 6 houses research groups Cancer Inflammation and Immunity, headed by Santiago Zelenay – bringing their expertise in cancer immunity; the Systems Oncology group led by Claus Jorgensen, who study the complex ecosystems of solid tumours – which include immune cells, fibroblasts and endothelial cells; and the newest Institute group leader Evangelos Giampazolias and his Cancer Immunosurveillance group, who study the interaction between tumours and the host immune system. Angeliki Malliri and her Cell Signalling group are temporarily based on this floor prior to their move into the Division of Cancer Sciences in early 2024, where they will be located in the Oglesby Cancer Research Building.

The Paterson Building is also home to the Cancer Research UK National Biomarker Centre, on Floor 3 and part of Floor 4, sandwiched between the research communities it constantly interacts with – the NHS research clinicians on Floors 1 and 2, and our discovery research teams on the upper floors. Floor 4 contains our core facilities and Data Centre, juxtaposed to Christie Academic Pathology, providing the ideal opportunity for synergy and sharing of expertise and technologies.

The Oglesby Cancer Research Building, across the road from the Paterson Building, is home to three other CRUK MI groups. Rob Bristow's Translational Oncogenomics group moved from the first floor down to the ground floor to join Skin Cancer and Ageing group – led by Amaya Virós – and the Cell Division group – led by Iain Hagan – who both relocated from Alderley Park.

"This is the dawn of an exciting new era for cancer research in the North West. Out of the tragedy of the fire, this globally significant facility has emerged and it's something that a great many people across Manchester and beyond can be proud of helping to achieve.

"Critically, the new building will allow clinicians and scientists to work alongside one another on a daily basis, facilitating the kind of interactions and collaborations that spark innovative ideas and lead to advances that bring hope to people with cancer and their families."

Caroline Wilkinson, Chief Operating Officer, Cancer Research UK Manchester Institute.

Power of Colocation

The Oglesby Cancer Research Building, together with the Paterson and the Christie facilities form the impressive 'Cancer Campus' in Withington. These three buildings, with their complementary spaces and resources, bring together on one site a critical mass of clinicians, researchers, technicians, and clinical trials staff all focused on solving the complex problems in cancer.

Colocation of such a large cancer community on the Christie site, the largest single-site cancer centre in Europe, will foster more powerful collaboration between these specialists, accelerating progress for cancer patients not only in Manchester but across the globe. With this unique collaboration, we are poised to be one of the top cancer research centres in the world.

"Six years after the fire, we're finally back together on site and in our magnificent new building. It's been a long journey filled with many and varied challenges as we all know, but thanks to everyone's resilience and the strength of our partnerships, we are already transforming how we work to deliver our research goals.

"As the latest piece of the cancer campus jigsaw, along with the Christie and the Oglesby Cancer Research Building, we are together shaping the future of cancer research and our goal to improve the lives of patients with cancer in Manchester and beyond."

Caroline Dive, Interim Director, Cancer Research UK Manchester Institute

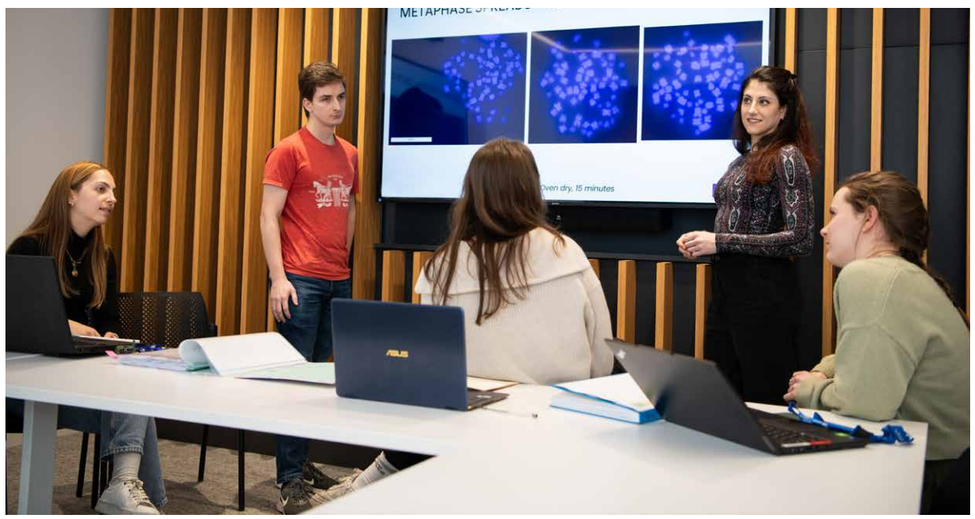
Looking to the future

Since our relocation earlier this year, our research groups are settling well into their new surroundings, and the Paterson Building has become a hive of activity, stimulating collaboration and innovation. With our cutting-edge laboratories and equipment, advanced computing infrastructure, and dedicated collaboration spaces, the facility is primed to accelerate discoveries in cancer biology and treatment.

Every aspect of the build, from its innovative and open design to its highly interconnected laboratory spaces and proximity to the clinic, has been conceived to advance cancer research and treatment.

Representing the future of cancer research in Manchester, the Paterson Building is designed to accommodate cancer research activities for years to come as well as attracting new talent and building strength in key priority areas where we can really excel and make a difference.

We look forward to realising our ambitions of improving outcomes for cancer patients across the globe.



CAREER OPPORTUNITIES AT THE CANCER RESEARCH UK MANCHESTER INSTITUTE

The Cancer Research UK Manchester Institute has a strong programme of basic and translational research. There are close links with clinical and translational research groups throughout the Christie Hospital site.

The Institute offers excellent laboratory facilities and outstanding core facilities, including molecular biology services, next generation sequencing, real-time PCR, mass spectrometry, flow cytometry, histology, advanced imaging, and a biological resources unit. Details of all groups and facilities are given in this report and can guide interested parties to the appropriate contacts.

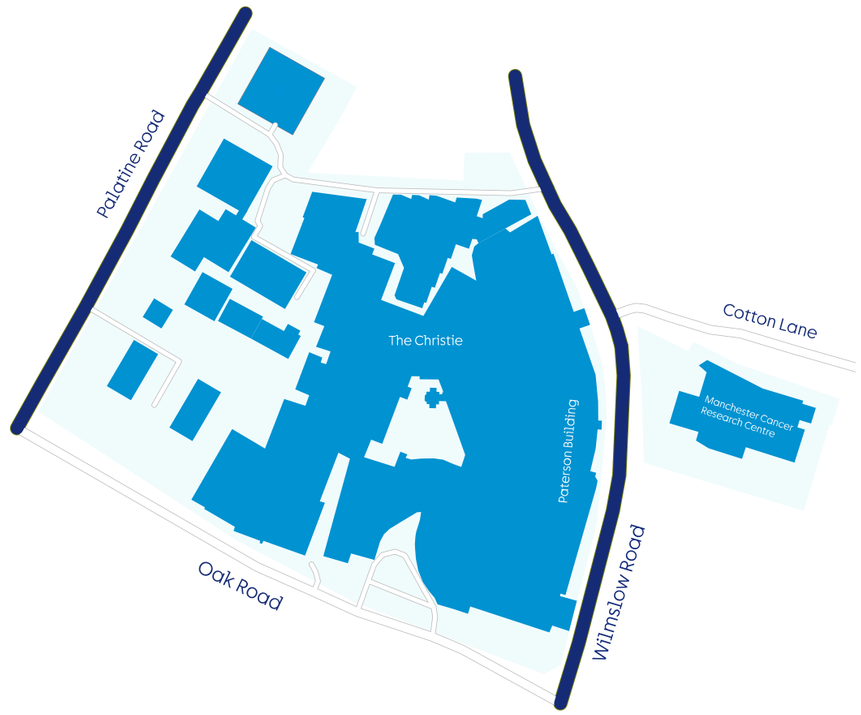
Opportunities exist at a number of levels in the Institute. We have a well-established programme of degrees by research which is described in the section on Postgraduate Education. We encourage applications from suitably qualified graduates to apply to join either the PhD or MD programmes. Graduates with a first or 2.1 honours degree in a biological science can apply each year to train for a four-year PhD in one of our research laboratories. The University of Manchester offers a wide range of training for new and existing students which provides opportunities to acquire skills that will complement the research programme and help achieve personal and career development goals. At the Institute, we also ensure that postgraduate students are provided with high quality, relevant and appropriate training alongside development opportunities. The Institute also has a well-developed process for ensuring excellent pastoral care and mentoring for all students.

Postdoctoral applicants of high calibre are regularly sought. Although Postdoctoral Fellows will be encouraged to apply for their own fellowships, funded positions are available for outstanding candidates. Interested applicants should contact the Group Leaders directly, with details of their research interests and recent experience.

In addition to postgraduate and postdoctoral opportunities, the Institute is seeking to recruit outstanding candidates to the positions of Junior and Senior Group Leaders. The packages provided are extremely attractive and commensurate with the experience of the applicant, with significant funding for personnel, recurrent expenditure and equipment. Junior Group Leaders are appointed for an initial six-year period with a review between five and six years for consideration of promotion to Senior Group Leader, with Senior Group Leaders appointed to non-time limited positions.

Specific vacancies can be found on our web pages (https://www.cruk.manchester.ac.uk/recruitment/candidate/searchvacancies), but suitably qualified and enthusiastic individuals should contact the Institute at any time to enquire about career possibilities.

CONTACT DETAILS



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Electronic version of this report can be found at: www.cruk.manchester.ac.uk/About/

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