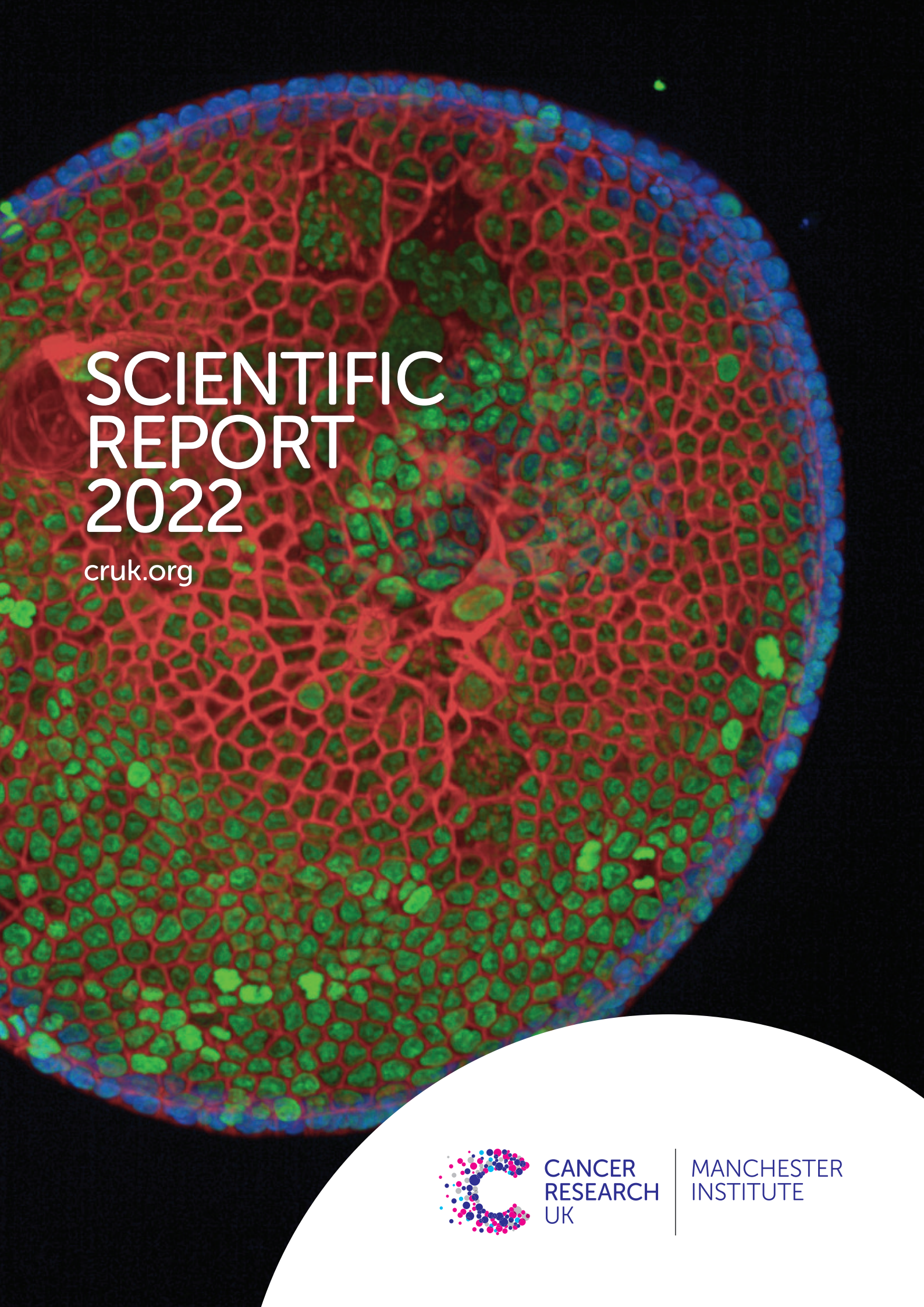

SCIENTIFIC REPORT 2022

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COVER IMAGE

3D structural cell organisation of murine pancreatic organoids cultured in Matrigel. Nuclei (cyan), actin cytoskeleton (red), and PI3K signalling activation (green).

Image supplied by Celia Cintas (Systems Oncology)

SCIENTIFIC REPORT 2022

MANCHESTER INSTITUTE

MANCHESTER 1824
The University of Manchester

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CONTENTS

The Cancer Research UK Manchester Institute is temporarily located at Alderley Park in Cheshire until we return to our original site in Withington. Some research groups and staff remain 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

During 2022, the Institute made the long-anticipated return to meeting in person after the restrictions and challenges posed by the SARS-CoV-2 pandemic were finally eased. This marked change set the positive tone for the year, allowing us to reconnect with each other again and with the wider scientific community.

It has been a productive and exciting year for our staff, and it is my great pleasure to highlight their accomplishments here.

Notably, Santiago Zelenay was promoted to Senior Group Leader following an excellent performance during his tenure review. He has been a Junior Group Leader at the Institute since 2015 and made important discoveries that have advanced the field of cancer inflammation and immunology, including taking his fundamental science through to biomarker-informed clinical trials with his clinical collaborators. Santiago is a great asset to the Institute, and we look forward to seeing his research continue to flourish in the coming years.

Former Team Lead of the Nucleic Acids Biomarker team, Dominic Rothwell was appointed as the new Deputy Director of the CRUK Manchester Institute Cancer Biomarker Centre (CBC). This is a pivotal appointment for CBC, the Institute, the CRUK Manchester Centre and the wider cancer research community. I look forward to working with Dominic in his new role as the CBC expands with the upcoming move into the third floor of the new Paterson Building next year.

I am delighted that Evangelos Giampazolias from the Francis Crick Institute accepted our offer of a Junior Group Leader position, and we look forward to welcoming Evangelos to CRUK MI in January 2023 to lead the Cancer Immunosurveillance group.

The Institute's Drug Discovery Unit closed at the end of 2022 after 13 successful years. I would like to wholeheartedly congratulate Caroline Springer and Richard Marais on setting up the CRUK MI's first spinout company, Oncodrug that will remain at Alderley Park and continue to work with Iain Hagan's group at CRUK MI. We wish Oncodrug every success and look forward to maintaining a close association with Caroline and Richard.

External funding augments the breadth of our research and helps support the development of our early career researchers. With congratulations we welcome Mark Williams to the CRUK MI Faculty following his success in winning a competitive Clinician Scientist Fellowship from the Medical Research Council. With this funding, Mark set up his independent research group as an Institute Fellow. His interest is in understanding the mechanisms of acute myeloid leukaemia (AML) relapse following allogenic stem cell transplantation, with the overall goal of preventing acute myeloid leukaemia relapse after stem cell transplant.

Institute Fellow Amaya Virós was awarded a grant from the Harry J. Lloyd Charitable Trust. One of four recipients in the final funding round of this US-based foundation, it is noteworthy that she was the only candidate outside the US to be selected. Tim Budden, a postdoc in Amaya's group, won this year's BACR Chris Marshall Prize for Cell Signalling. This is a prestigious prize and highlights the great work being carried out in the Skin Cancer and Ageing group. Congratulations to Amaya's team.

I would also like to congratulate postdoctoral researcher Nasir Haider from Claus Jorgensen's Systems Oncology group, who was awarded a Pancreatic Cancer UK Career Foundation Fellowship to target KRAS partners in pancreatic cancer.

We continue to publish an impressive collection of scientific discoveries, some of which are presented in our research highlights section. Among those featured is *Nature Communications* publication by Santiago Zelenay's team, which demonstrates the role of COX-2 enzyme in cancer and the development of chemotherapy resistance. They show the potential of combining common nonsteroidal anti-inflammatory drugs (NSAID) with chemo- and immunotherapy for hard-to-treat cancer models, such as triple negative breast cancer.

The CBC Nucleic Acids Biomarkers team developed a sensitive blood test to monitor patients with small cell lung cancer (*Nature Cancer* 2022, 3(10):1260-1270). Analysis of the methylation pattern of circulating cell-free DNA (cfDNA) from patients' blood can also identify the predominant molecular subtype of SCLC, potentially supporting future personalised clinical trials of novel therapies for this aggressive form of lung cancer.

Tim Somervaille and his group provided novel insight into protein interactions on chromatin and how this causes the cell differentiation block seen in acute myeloid leukaemia (*Oncogene* 2022, 41(44):4841-4854). They revealed a new genome-wide interaction between the HMG-box protein with LSD1 and GFI1, explaining the underpinning mechanisms behind AML and significance of LSD1 inhibitors in the studies of AML and their application in clinical trials.

Conferences are critical to the advancement of science, and this year has seen many scientific meetings return as in person events. The Institute's Colloquium, held virtually for the two previous years, was held at the Alderley Park Conference Centre this year. There was a real buzz throughout the event, and we greatly enjoyed getting together to share our latest research.

As Co-Director of the CRUK Lung Cancer Centre of Excellence, it was a pleasure to open the CRUK Lung Cancer Conference in Manchester and bring the lung cancer community together again. Several members of the CBC gave talks or presented posters, with two young Institute scientists, Victoria Fife and Rebecca Carroll, winning poster prizes.

Manchester welcomed the Greater Manchester Cancer Meeting in October and the first ever Greater Manchester Cancer Awards were launched this year. I am so proud that the digital Experimental Cancer Medicine Team won the Commitment to Equality Award for encouraging inclusivity in technology clinical trials. Congratulations to the team.

In my final days as President of the EACR, I chaired the EACR annual congress in Seville where Claus Jorgensen and Dominic Rothwell gave excellent oral presentations. Melissa Frizziero and Julia Ogden from the Cancer Biomarker Centre, alongside Ryan Guilbert from the Cell Signalling group, were all awarded competitive travel bursaries to attend the meeting which was a great success.

In early October, we welcomed the CRUK Trustees to Manchester to hear about our latest progress and future plans. They enjoyed a tour of the new Paterson Building that is due to be completed early in 2023 and learned about the opportunities the new building will provide, and how our Institute will sit at the heart of the wider Manchester research ecosystem.

And finally, as construction of our new building nears completion, we have all been working exceptionally hard planning for the move in spring of 2023. I would like to thank everyone for their enormous efforts this past year, particularly the Core Facilities, the wider Operations team and Chief Laboratory Officer Stuart Pepper and Chief Operating Officer Caroline Wilkinson, and the team of scientific officers led by Iain Hagan, who collectively and on behalf of the Institute, have led on the design of the building, coordination of the move and liaised with external partners on operational readiness and ongoing operating arrangements.

It will be tremendous to have everyone back together and I am excited about how this new facility will help to support and integrate our discovery and translational research to transform patient outcomes through advances in the prevention, early detection and treatment of cancer.

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

RESEARCH HIGHLIGHTS

In this section we highlight some research publications from 2022 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.

Maiques-Diaz A, Nicosia L, Basma NJ, Romero-Camarero I, Camera F, Spencer GJ, Amaral FMR, Simeoni F, Wingelhofer B, Williamson AJK, Pierce A, Whetton AD, Somervaille TCP. (2022)
HMG20B stabilizes association of LSD1 with GFI1 on chromatin to confer transcription repression and leukemia cell differentiation block.
Oncogene 41(44):4841-4854.

LSD1 is an enzyme that demethylates histone tails and has been the focus of much recent interest in view of encouraging pre-clinical and early phase clinical studies using candidate inhibitors. The Leukaemia Biology group previously demonstrated that pharmacologic inhibition of LSD1 induces molecular and morphologic differentiation of blast cells in acute myeloid leukemia (AML) patients harbouring *MLL* gene translocations. In addition to its demethylase activity, LSD1 has a critical scaffolding function at genomic sites occupied by the SNAG domain transcription repressor GFI1. Importantly, inhibitors block both enzymatic and scaffolding activities; in the latter case by disrupting the protein:protein interaction of GFI1 with LSD1. GFI1 is important in normal and leukaemic haematopoiesis, and its function as a transcription repressor depends upon its recruitment to chromatin of LSD1 and other proteins that associate with LSD1 in the CoREST complex.

Despite important prior insights, the role of many of the proteins found in the LSD1/CoREST complex and how they interact at sites of GFI1 binding to confer transcriptional repression remain elusive. The group explored the wider consequences of LSD1 inhibition on the LSD1 protein complex and discovered that the interaction of the HMG-box protein HMG20B with LSD1 was disrupted by LSD1 inhibition. Downstream investigations revealed that HMG20B is co-located on chromatin with GFI1 and LSD1 genome-wide; the strongest HMG20B binding co-locates with the strongest GFI1 and LSD1 binding. Functional assays demonstrated that HMG20B depletion induces leukaemia cell differentiation and further revealed that HMG20B is required for the transcription repressor activity of GFI1 through stabilising LSD1 on chromatin at GFI1 binding sites. Thus, HMG20B is a critical component of the GFI1:LSD1 transcription repressor complex which contributes to leukaemia cell differentiation block. Their study adds to emerging data that further clarify the mechanisms of action of LSD1 inhibitors currently under evaluation in diverse malignancies including AML, myelofibrosis and essential thrombocythaemia.

Cannistraci A, Hascoet P, Ali A, Mundra P, Clarke NW, Pavet V, Marais R. (2022)
MiR-378a inhibits glucose metabolism by suppressing GLUT1 in prostate cancer.
Oncogene 41(10):1445-1455.

Prostate cancer (PCa) is the second most common cancer in men, and the fifth leading cause of cancer related deaths worldwide. Advances in early detection enable therapeutic intervention before metastasis but can also lead to overtreatment and unnecessary morbidities. Thus, a major challenge is to distinguish primary tumours that will remain indolent from those that will metastasise. To address this challenge, efforts are ongoing to develop biomarkers for risk stratification. Amongst these the microRNAs (miRs) are of particular interest because of their high stability in body fluids and fixed tissues, and because they regulate messenger RNAs (mRNAs) and thereby cancer progression. Members of the Molecular Oncology group examined the functional and prognostic role of miRs in PCa and identified a 7-miR signature with prognostic value independent of Gleason score, pathological T (Tumour) state, N (Nodal) stage, surgical margin status and age. Within this 7-miRs signature, they found that miR-378a-3p levels progressively decrease from benign prostate tissue to lower grade tumours and decreased further in advanced grade primary PCa. The group identified glucose transporter 1 (GLUT1) mRNA as a direct target of miR-378a-3p and showed that GLUT1 inhibition hampers glycolysis leading to cell death in PCa models. Together, this data advances our understanding of PCa biology, particularly regarding the energy-related drivers of the metastatic phenotype as they show that miR-378a-3p regulates PCa glucose metabolism by targeting GLUT1. Thus, their data additionally identifies GLUT1 as a therapeutic target in early stage glycolytic PCa. In summary, the group define a 7-miR signature that following further validation in independent cohorts could be used alongside histopathological assessment to balance the uncertainty of disease progression against the potential of overtreatment. This signature could have particular utility in patients with intermediate-risk PCa for whom risk stratification and therapeutic options are uncertain.

Valpione S, Campana LG, Weightman J, Salih Z, Galvani E, Mundra PA, De Rosa F, Gupta A, Serra-Bellver P, Lorigan P, Germetaki T, Dynowski M, Kitcatt S, Sahoo S, Lee D, Dhomen N, Lord G, Marais R. (2022)
Tumour infiltrating B cells discriminate checkpoint blockade-induced responses.
European Journal of Cancer 177:164-174.

B cells are immune cells that are highly specialised to recognise non-self antigens and produce antigen-specific antibodies. So far, their role in immune oncology has mainly been limited to antibody production against cancer neoantigens, but there is evidence that B cells can also express both HLA class I and class II of the major histocompatibility complex. In this project, a diverse team of researchers from Molecular Oncology, Core Facilities, the Christie Hospital and the School of Biological Sciences at The University of Manchester, performed a single cell study of the transcriptome and surface proteome of sequential melanoma biopsy and peripheral blood samples. The differential expression analysis of the tumour immune infiltrate before starting treatment and after the first cycle of immunotherapy (week 3) with checkpoint inhibitors showed that the melanomas that then went on to respond were characterised by an upregulation of B cell genes at week 3 (immunoglobulins, including post isotype switch antibody genes, and genes involved in immunoglobulin production). The team also found that memory B cells had the highest expression of HLA class I and class II among the tumour infiltrating immune cells, and that at week 3 major histocompatibility complex pathways were among the most differentially expressed in memory B cells of patients who benefitted from treatment compared to the ones of patients who progressed. Intriguingly, the B cell surface expression of the checkpoint molecules targeted by the checkpoint inhibitors was negligible, suggesting that the B cell role in anticancer immunity might be downstream from the checkpoint regulation.

Selkirk E, Patel R, Hoyle A, Lie-A-Ling M, Smith D, Swift J, Lacaud G. (2022)
SGOL1-AS1 enhances cell survival in acute myeloid leukemia by maintaining pro-inflammatory signaling.
Heliyon 8(11):e11362.

The paper by the Stem Cell Biology group, along with colleagues from the Biological Mass Spectrometry facility and the Division of Cell Matrix Biology and Regenerative Medicine at The University of Manchester, investigates the role of a long non-coding RNA (lncRNA) SGOL1-AS1 in acute myeloid leukaemia (AML), a highly aggressive and difficult-to-treat haematologic malignancy.

To identify lncRNAs important in the proliferation of leukaemic cells, the team employed a CRISPR interference (CRISPRi) screening approach to induce sequence-specific repression of lncRNA expression. They selected the THP-1 cell line, a human AML cell line with an MLL-AF9 translocation, to express dCas9-KRAB (dead Cas9 fused to Krüppel associated box (KRAB) domain), which represses transcription at targeted loci. The authors screened 3882 lncRNAs, identifying 19 as significantly influencing cell proliferation. Within these 19 hits, they identified MIR17HG, a lncRNA that also encodes for the miR17-92a-1 cluster. The microRNAs within this cluster are essential factors in MLL-rearranged leukaemia, validating the screen's performance in identifying lncRNAs important in leukaemic maintenance.

Along with the other candidates, the researchers focussed their study on SGOL1-AS1. They found that SGOL1-AS1 is significantly upregulated in AML cells compared to normal bone marrow cells. Knockdown of SGOL1-AS1 resulted in decreased survival and proliferation of AML cells. Reduced proliferation occurred via the induction of apoptosis. This suggests that SGOL1-AS1 plays a crucial role in AML expansion. Further analysis revealed that SGOL1-AS1 maintains pro-inflammatory signalling in AML cells, which is essential for their survival.

The prognosis for AML patients remains poor, with a five-year survival rate of less than 30%. Therefore, there is a critical need for new therapies that can improve patient outcomes. The study highlights the potential of lncRNAs as therapeutic targets. LncRNAs are non-coding RNAs that regulate gene expression and play critical roles in cancer development and progression. The study demonstrates that SGOL1-AS1 is a crucial regulator of pro-inflammatory signalling in AML cells, suggesting that other lncRNAs may also play important roles in cancer pathogenesis.

Overall, this study provides new insights into the mechanisms of AML maintenance and identifies potential therapeutic targets for this devastating disease. Further research is needed to validate these findings and develop novel therapies based on the study's results.

Zhou C, O'Connor J, Backen A, Valle JW, Bridgewater J, Dive C, Jayson GC. (2022)
Plasma Tie2 trajectories identify vascular response criteria for VEGF inhibitors across advanced biliary tract, colorectal and ovarian cancers.
ESMO Open 7(2):100417.

VEGF inhibitors (VEGFi) are targeted anti-angiogenic agents that deliver promising treatment benefit in many cancer types. However, their use is compromised by lack of validated biomarkers. Previously, the team from the Cancer Biomarker Centre and Christie NHS Foundation Trust showed that reduction in the concentration of plasma Tie2 (pTie2) reflects a tumour vascular response to bevacizumab, an anti-VEGF antibody. In this study, they investigated the potential of pTie2 as a generic biomarker to predict treatment response and disease progression across 3 cancer types and 2 classes of VEGFi where data from 3 clinical trials were aggregated. In Travastin (n=70, colorectal cancer) and ICON7 (ovarian cancer, n=92), patients received bevacizumab or placebo; in ABC03, patients (n=124) with advanced biliary tract cancer (ABC) received cediranib (a small molecular VEGFi) or placebo.

Cediranib-treated ABC patients were deconvoluted into distinct groups by pTie2 trajectories: vascular complete response (vCR), vascular non-response (vNR) or vascular partial response (vPR). Despite limited improvement on PFS/OS from cediranib overall, vCR patients showed significantly longer PFS and OS compared to placebo and the 6.7-month OS improvement demonstrated the clinical value of identifying patients who benefit from VEGFi.

Integrating data across ovarian, colorectal and ABC, the criteria for vCR are consistent in all tumour types and both classes of VEGFi, although the percentage of patients classified as attaining vCR vary considerably. vCR patients have improved PFS/OS while vNR have not. Moreover, subsequent elevation of pTie2 in responding patients predicts resistance to VEGFi sufficiently in advance of radiological disease progression that could enable treatment switch before clinical deterioration occurs.

In summary, the team has shown in 3 different cancers using two different classes of VEGFi that a pTie2-defined vascular response is associated with a significant clinical benefit. This is the first biomarker that has shown broad utility for VEGFi use. As a result, CRUK has supported the VALTIVE (Validation of Tie2 for VEGF inhibitors) programme that aims to progress pTie2 towards NHS use for clinical decision making regarding VEGFi treatment.

Frizziero M, Kilgour E, Simpson KL, Rothwell DG, Moore DA, Frese KK, Galvin M, Lamarca A, Hubner RA, Valle JW, McNamara MG, Dive C. (2022)
Expanding therapeutic opportunities for extrapulmonary neuroendocrine carcinoma.
Clinical Cancer Research 28(10):1999-2019.

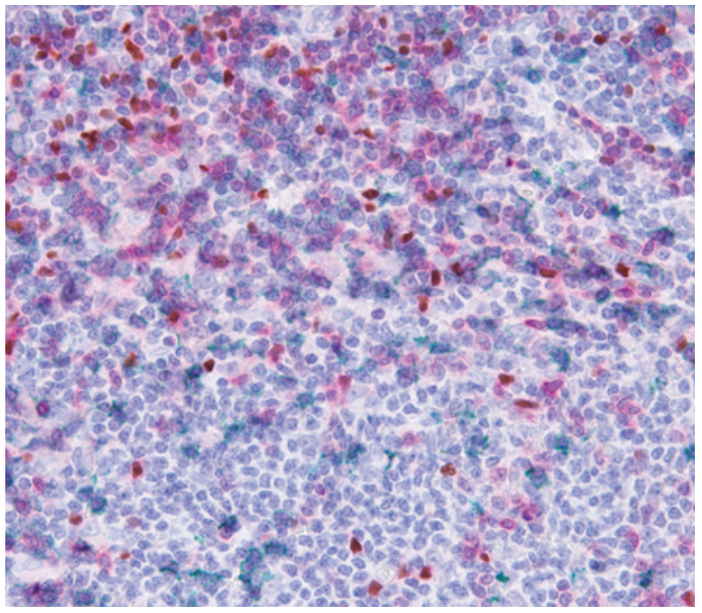
NeuroEndocrine Carcinomas (NEC) are rare yet aggressive cancers that are garnering interest within the scientific and clinical community as they become more commonly encountered in the clinic. This is due to improved diagnostic methods and the increasingly observed phenomenon of "NeuroEndocrine (NE) lineage plasticity," whereby non-NeuroEndocrine (non-NE) epithelial cancers transition to aggressive NE phenotypes after targeted treatment.

The most common and best characterised NEC is small cell lung cancer (SCLC) that derives from the NE body cells of the lung. However, a minority of NEC originate from anatomical sites outside of the lung and are currently classified and clinically managed as a single entity; so-called Extra-Pulmonary (EP)-NEC. Whilst advances towards precision-medicine are being made in SCLC research, EP-NEC remain understudied.

Identifying effective treatments for patients with EP-NEC is challenging for several reasons that include lack of recurrent molecular targets, paucity of patient-relevant preclinical models to study biology and test novel therapeutics, and lack of validated biomarkers to guide clinical management.

Histological image showing lung cancer cells.

Image supplied by Cancer Biomarker Centre.



This review by the Cancer Biomarker Centre, with colleagues from the Christie NHS Foundation Trust, provides a thorough portrait of the current knowledge of the EP-NEC molecular and immune landscape, drawing parallels with SCLC and non-NE cancers from the same sites of origin, with a view to informing new avenues for research and drug development. The team highlighted the heterogeneity of molecular vulnerabilities (SCLC-like, same-organ non-NE cancer-like and tumour-type-agnostic) within the EP-NEC family, focusing on those with potential for therapeutic exploitation. Emerging evidence towards an 'immune-altered' phenotype for EP-NEC where PD-L1 expression and immune cell infiltration are commonly confined at the tumour edge or tumour/stroma interface are presented; a scenario where immunotherapy may find a role combined with strategies to elicit tumour immunogenicity. They finally discussed hypotheses surrounding the origin of EP-NEC and how "NE lineage plasticity" can be leveraged for therapeutic purposes.

Chemi F, Pearce SP, Clipson A, Hill SM, Conway AM, Richardson SA, Kamieniecka K, Caeser R, White DJ, Mohan S, Foy V, Simpson KL, Galvin M, Frese KK, Priest L, Egger J, Kerr A, Massion PP, Poirier JT, Brady G, Blackhall F, Rothwell DG, Rudin CM, Dive C. (2022)
cfDNA methylome profiling for detection and subtyping of small cell lung cancers.
Nature Cancer 3(10):1260-1270.

Small cell lung cancer (SCLC) is a high-grade neuroendocrine carcinoma characterised by high proliferation and early metastatic spread. SCLC is treated as a homogenous disease, but recent studies describe distinct molecular subtypes based on transcription factor expression. These subtypes have been shown preclinically to exhibit differential therapeutic vulnerabilities, suggesting potential for patient-specific therapy. Molecular characterisation of patient tumours remains challenging due to scant or non-existent biopsies and serial biopsies to chart subtype evolution rarely occurs.

In this study, researchers from the Cancer Biomarker Centre established a novel method (T7-MBD-seq) for detecting genome-wide DNA methylation. This method was applied to DNA from patient derived models of SCLC as well as circulating cell-free DNA (cfDNA) extracted from plasma from 78 patients with SCLC and 79 non-cancer controls (NCCs).

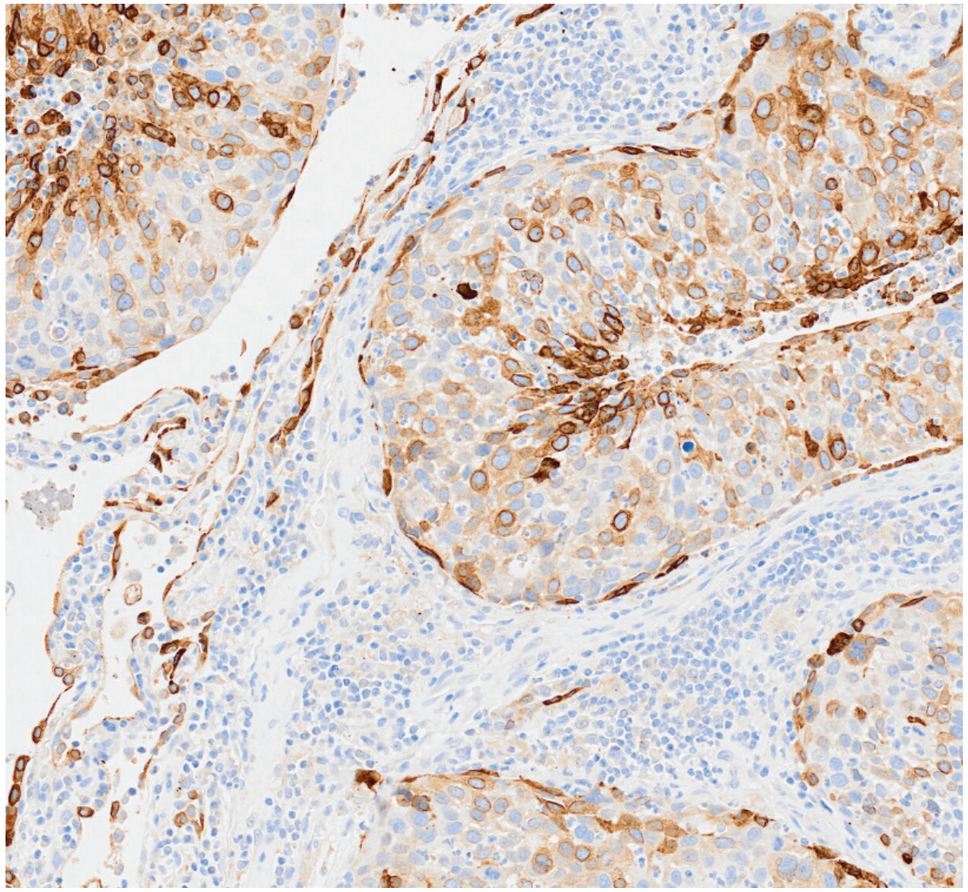
Firstly, a classifier was generated to discriminate patient cfDNA samples from NCCs. They used

in silico spike-in samples consisting of reads from NCCs mixed with reads from SCLC tumour DNA to mimic the low tumour fractions (0.5-5%) often observed in cfDNA. This achieved an area under the receiver operator curve (AUROC) of 0.986 and 1 for limited (including 6/6 stage I patients) and extensive stage SCLC respectively and correctly classified samples starting from 0.22% tumour content in a synthetic spike-in.

Secondly, a SCLC subtype classifier was built using an innovative method to convert publicly available methylation array data to generate data comparable with T7-MBD-seq data. This classifier correctly assigned all 33 preclinical models and 10/11 cfDNA samples with known subtypes. Distribution of predicted subtypes across all cfDNA samples concurs with literature-expected proportions. Together, this work shows that cfDNA methylation has potential as a sensitive liquid biopsy to detect SCLC and to determine molecular subtype.

Bell CR, Pelly VS, Moeini A, Chiang SC, Flanagan E, Bromley CP, Clark C, Earnshaw CH, Koufaki MA, Bonavita E, Zelenay S. (2022) Chemotherapy-induced COX-2 upregulation by cancer cells defines their inflammatory properties and limits the efficacy of chemoimmunotherapy combinations. ***Nat Communications*** 13(1):2063.

Cytotoxic therapies, such as chemotherapy and radiotherapy, constitute mainstays of cancer treatment for both early-stage cancer and unresectable advanced disease. In addition to inducing tumour shrinkage through direct cytotoxic effects on proliferating cancer cells, these therapies can also stimulate the immune system to help control tumour outgrowth. However, chemoresistance and tumour relapse continue to pose significant challenges, indicating that anti-cancer immunity following cytotoxic therapy may be weak, short-lived, or overridden by immune evasive mechanisms. Previous studies led by the Cancer Immunity and Inflammation group have implicated prostaglandin E2 (PGE2) release from dying cancer cells in stimulating cancer cell proliferation and tumour repopulation post-cytotoxic therapy. In the group's recent study, they revealed that cancer cell activation of the cyclooxygenase (COX)-2/PGE2 pathway after chemotherapy is a widespread phenomenon that alters the inflammatory properties of the treated cancer cells. By screening multiple mouse and human cancer lines, the group showed that enhanced PGE2 synthesis post-cytotoxic treatment is an active process that occurs only when the cancer cells have pre-existing COX-2 expression and activity. Using a library of >1200 market-approved compounds added to COX-2-transcription reporter cancer cells combined with quantitative real-time live-cell imaging, they found that all classes of anti-neoplastic drugs rapidly upregulated COX-2. This phenomenon coincided with the arrest of cancer cell proliferation induced by the various neoplastic agents independently of their mechanism of action. These data indicate that the upregulation of COX-2/PGE2 by cancer cells undergoing cell death might be a significant obstacle to the development of tumour immunity resulting from cytotoxic therapy. Accordingly, tumour shrinkage and inhibition of metastatic spread using a poorly immunogenic breast cancer model was exclusively observed following the triple combination of PD-1 blockade, chemotherapy and a selective COX-2 inhibitor. Thus, their study highlights a potential approach for enhancing the effectiveness of combinations of immunotherapy and chemotherapy.



Lung squamous carcinoma, 20x whole tissue. Brown indicates specific immunostaining of COX-2 and light blue indicates nuclear haematoxylin staining.

Image supplied by Victoria Fife (Cancer Biomarker Centre)

CANCER RESEARCH UK MANCHESTER INSTITUTE

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It has been another busy year for the CBC. Our multiple ongoing projects progressed, we developed new liquid and tissue biopsies, launched new biotech/Pharma partnerships, tested novel therapies in our patient derived models and deployed digital approaches in clinical trials.

We were sad to see the departure of Cells and Proteins Team lead Dr Elaine Kilgour and digital Experimental Cancer Medicine Team (dECMT) lead Dr Donal Landers; we thank them both for their great work with us. We welcomed Dr David Millrine to lead the new Translational Immunology Team (TI) and recruitment for a new dECMT lead is underway. Our circulating tumour cell specialists transitioned to the Nucleic Acids Biomarkers Team, which will be led by Dr Florent Mouliere, joining us in June 2023. We congratulate Dr Dominic Rothwell who took up his new role as Deputy Director of CBC in November 2022. We also welcomed Naomi Samuels as Executive Assistant to the CBC Director.

The Cancer Biomarker Centre will become independent from but firmly aligned to the CRUK Manchester Institute during the summer of 2023, as we move to our new, bespoke design CRUK core funded facility in the new building at the Christie NHS Foundation site. Our Small Cell Lung Cancer (SCLC) Biology team will remain in CRUK MI studying vasculogenic mimicry (the formation of blood vessels by SCLC cells) and mechanisms of drug resistance and metastasis. We thank the past CRUK MI Directors Profs Nic Jones and Richard Marais for the wonderful opportunity and their continued support of the evolving CBC within CRUK MI since 2004.

The Preclinical Pharmacology Team

Patient derived preclinical models reveal novel biology of SCLC

The PP team continue to characterise SCLC molecular subtypes using our biobank of patient-derived circulating tumour cell explant models (CDX, >65 models) that reflect the genetic heterogeneity and phenotypic plasticity of SCLC. Having discovered the ATOH1 subtype, we showed that ATOH1 expression is prevalent in 41% of 197 clinical tumour samples (Figure 1), expressed alone or with other subtype transcription factors ASCL1 or NEUROD1. Our *in vitro* and *in vivo* studies using ATOH1 subtype CDX models showed that ATOH1 promotes cell survival and supports CTC dissemination to the liver, a frequent site of SCLC metastasis in patients.

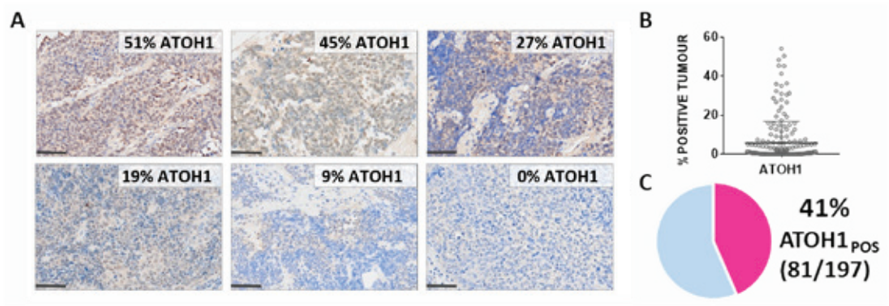

Figure 1. Representative IHC for ATOH1 expression in selected SCLC patient samples from a large clinical cohort (n=197) and HALO analysis data. Scale bar represents 100 µm (A). Quantified using HALO (B). Proportion of CDX models expressing ATOH1 (C).

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Naomi Samuels[1]

[1]Joined in 2022
[2]Left in 2022
[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)

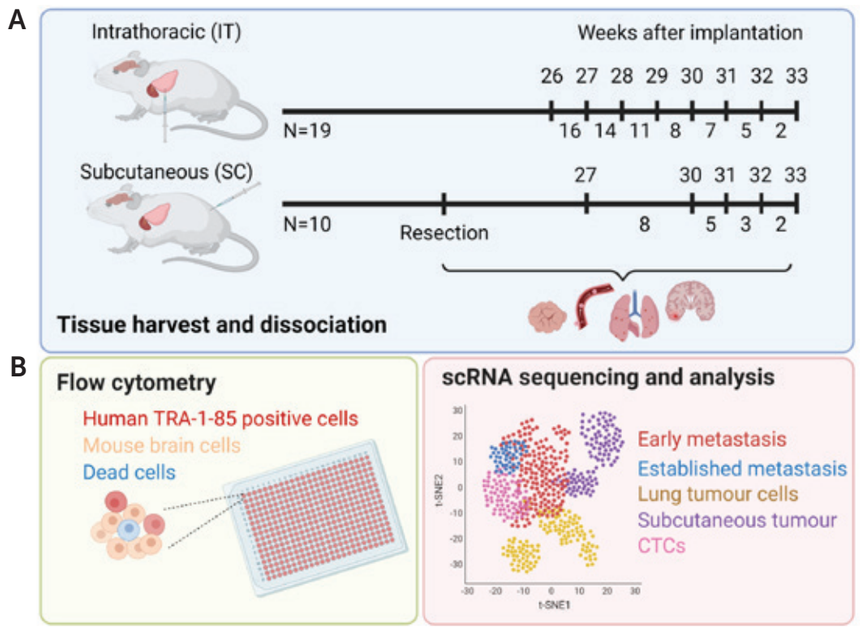


Figure 2. Study of SCLC brain metastasis using CDX models. A) Workflow to isolate brain founder tumour cells and established macro-metastases from CDX. B) FACS-mediated isolation of tumour cells and scRNAseq analysis.

Paucity of brain biopsies and consequent lack of robust patient-derived models hampers studies of SCLC brain metastasis, a clinical occurrence that increases to ~80% of patient cases during disease progression. We developed the first documented tumour resection protocol enabling study of SCLC brain metastasis *in vivo* (Figure 2). Single cell RNA sequencing of CDX CTCs and brain metastatic founder cells showed that the first cells to seed into the brain display distinct transcriptomic programmes with altered metabolic pathways compared to CTCs or resected tumour cells. We continue to explore modulation of altered pathways to suppress brain tropism to improve the quality of life for SCLC patients.

The Translational Immunology Team

Searching for predictive biomarkers to stratify immunotherapy for SCLC

Despite its notoriety as an 'immune cold' tumour type, the introduction of immunotherapy for SCLC was a rare significant improvement in treatment. However, as only a minority of patients respond, we seek a liquid biopsy to predict immunotherapy responses. With Fiona Blackhall (Christie NHS Foundation Trust, CFT), we are collecting serial blood samples from patients treated with chemotherapy with or without immunotherapy and have optimised a high dimensional multi-parameter CyTOF antibody panel to characterise immune responses in peripheral blood mononuclear cells (PBMC), aiming to identify 'immune signatures' predictive of immunotherapy response. Efforts are also ongoing to detect and monitor expansion of peripheral blood T cells responding to immunotherapy using T cell receptor sequencing in partnership with ThermoFisher.

Characterising the immunogenicity of SCLC

We are exploiting our SCLC CDX models to indirectly assess immunogenicity, focusing on expression of MHC I molecules that play a crucial role in determining susceptibility of SCLC cells to cytotoxic killing by Natural Killer cells (Figure 3 overleaf). Our emerging data revealed that within a CDX tumour, Neuroendocrine (NE) cells express lower levels of MHC I compared to NonNE cells. Early data suggest that NE cell MHC-I expression can be enhanced following treatment with an EZH2 inhibitor acting on the main enzymatic component of the polycomb repressive complex, commonly overexpressed in SCLC and known to regulate gene expression via histone methylation (H3K27). Using our newly developed *ex vivo* co-culture platform of CDX cells with autologous PBMCs, we are assessing whether EZH2 inhibition increases CDX immunogenicity and promotes expansion of SCLC cell reactive T cells present. We hope to exploit this platform to assess new immunotherapies.

Immune cells within melanoma microenvironments

With Dr Rebecca Lee (UoM/CRICK Institute) and Prof Sarah-Jane Dawson (Peter MacCallum Cancer Centre, Melbourne, Australia) and the CBC Tissue Biomarkers Team, we are

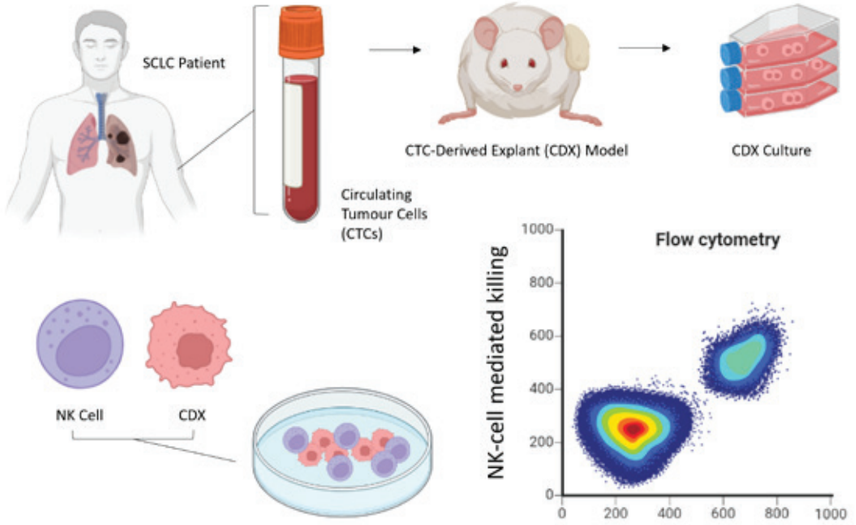


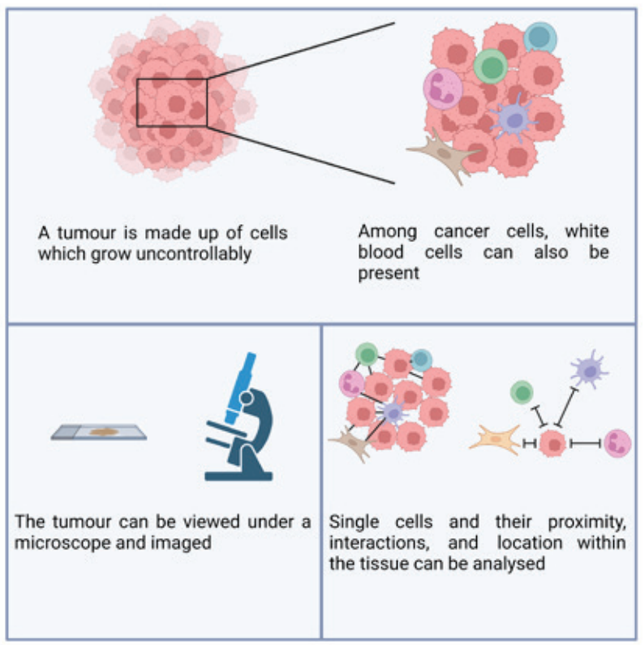
Figure 3. CTCs are enriched from the blood of SCLC patients and implanted into immunodeficient mice to generate CDX tumours. Disaggregated tumour cells are incubated with natural killer cells and subject to FACS-based profiling to ascertain cell killing capability.

Figure 4. Development of PhenoCycler panel to define immune cell neighbourhoods in Stage II melanoma. The infiltration of immune cells into tumours is an important determinant of response to therapy. The PhenoCycler antibody panel combined with fluorescence-based imaging identifies specific cell populations and their interactions.

developing a PhenoCycler (formerly CODEX) antibody panel to enable high dimensional multiparameter immunofluorescent imaging of immune cells within melanoma microenvironments in FFPE tumour sections (Figure 4 overleaf). This addition to the CBC immune biomarker 'toolkit' enables not only identification of the activation status of immune cells but also spatial phenotyping of cellular neighbourhoods. By measuring distances between cell types to identify cell-cell interactions and networks, we aim to garner additional prognostic information to other measured clinical features to predict risk of relapse following surgical resection of stage IIA melanoma.

Prediction of relapse after surgery with curative intent in NSCLC

In collaboration with Dr Santiago Zelanay (CRUK MI, page 16) and Prof Phil Crosbie (UoM, Manchester University NHS Foundation Trust, MFT), we are exploring the prognostic significance of the pro-tumourigenic inflammation signature (PTI) discovered by Zelanay in early-stage NSCLC. Early data using a Nanostring PTI, validated for clinical use, is encouraging and warrants further evaluation before we apply it to our community-based, low dose-CT screening cohort. We posit that understanding the immune/inflamed landscape of these stage I resected tumours will aid identification of the 20% of patients whose tumours will recur.



The Nucleic Acids Biomarker Team (NAB)

Whole genome methylation profiling of cfDNA

With the BBS team (see overleaf), we have continued to develop and utilise our in-house T7-MBD-Seq cfDNA methylation workflow across a number of exciting liquid biopsy projects, examining the cfDNA methylome in SCLC, NSCLC, and Cancer of Unknown Primary (CUP).

SCLC: After exciting early data using donor-matched plasma and SCLC CDX models in which the molecular subtype (SCLC-A, -N or -P) was known, we refined our SCLC molecular subtype classifier and published our findings (Chemi et al, *Nature Cancer*, 2022). Our data has been presented at several international conferences and has generated considerable interest. Our SCLC molecular subtype liquid biopsy is now undergoing validation in three independent cohorts, including two from large clinical trials. Since molecular subtypes are associated with specific vulnerabilities, we aim to prospectively deploy our subtyping liquid biopsy in future stratified clinical trials in collaboration with Prof Fiona Blackhall and our Pharma partners.

NSCLC: The NAB and BBS teams with Prof Philip Crosbie (MFT, UoM) were awarded a CRUK Early Detection Programme grant this year allowing us to analyse our precious blood sample collection from the community-based low dose-CT screening programme for cfDNA methylation, ctDNA mutations (with Prof Max Diehn, Stanford) and rare circulating cells (using the HDSCA platform with Prof Peter Kuhn, USC). We are developing a multi-modal liquid biopsy to augment LD-CT screening, also integrating radiomics features with Prof Marcel van Herk (UoM). A study of 200 stage I/II screened cancers and 200 cancer negative risk matched controls is underway.

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) to support treatment decisions in the challenging context of CUP. Our TOO classifier was developed across 29 cancer types and incorporated into a universal cfDNA workflow that provides mutation, CNA and methylation profiling from a single blood draw (Figure 5).

Application of the classifier to 143 cfDNA samples from patients with 13 different tumour types showed 98% specificity and 87% sensitivity. Sample collection to expand the CUP cohort and further validate the TOO classifier is underway. A patent for our CUP TOO liquid biopsy (CUPiD) is being filed.

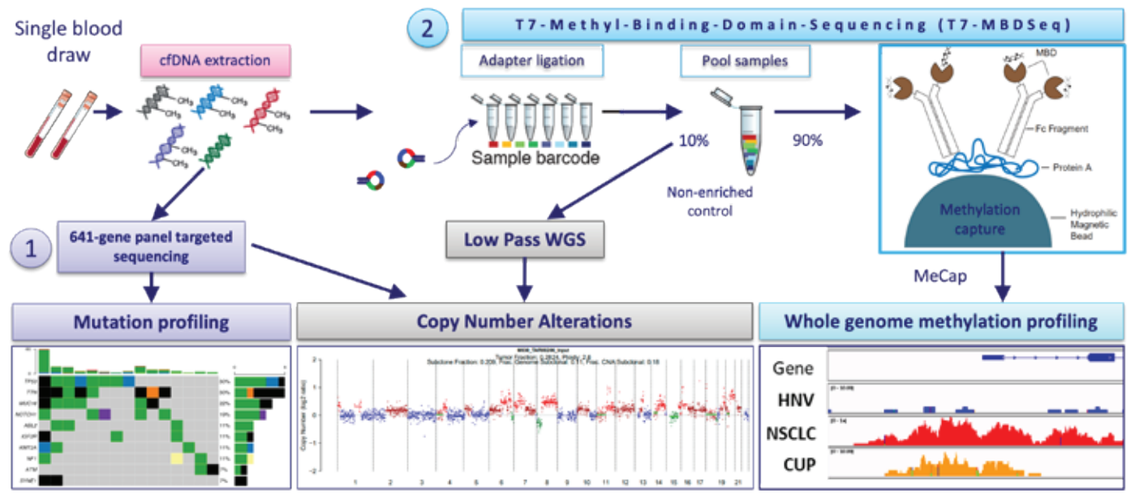
ctDNA mutation profiling

We have optimised our ctDNA profiling assays using NGS-based large comprehensive gene panels (+400 cancer associated genes) and studies using smaller, focussed DNA damage response and disease-specific panels and deployed these in clinical studies with pharmaceutical and biotech collaborators. We also developed highly sensitive unique molecular indexed (UMI) based assays which can detect somatic mutations at low tumour fractions (<1% VAF).

ddPCR-based primary assay to direct therapy decisions in melanoma

With the continued 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). This included analysing blood samples from the first patients in the DETECTION trial, which involves using ddPCR to detect early relapse with a panel of BRAF and NRAS specific assays. We also completed

Figure 5. cfDNA workflow enabling comprehensive genomic profiling of CUP patient samples.



validation of cfDNA ddPCR assays to monitor tumour activity and burden (TAB) levels for the upcoming DYNAMIC trial, which uses BRAF V600 ddPCR assays to monitor TAB and inform adaptive BRAF-MEK inhibitor therapy in Stage III unresectable/IV cutaneous melanoma.

The Bioinformatics and Biostatistics Team (BBS)

The BBS team develops and integrates bioinformatics and statistical methods and advises on experimental design across multiple CBC projects and clinical trials.

cfDNA methylation pipelines and packages
Working with NAB (see earlier section), we developed analytical pipelines to analyse DNA methylation sequencing data, including a bioinformatics package to be made publicly available as a research tool. We developed the SCLC molecular subtype and CUP TOO classifiers, which are being refined in larger ongoing studies. As part of our recently awarded CRUK Early Cancer Detection Programme, BBS and Dr André Freitas (digital ECMT, see below) are partnering with bioinformatics teams in Stanford and University of Southern California in the multi-omics integration of circulating DNA and rare cell outputs for our multi-modal liquid biopsy for the early detection of NSCLC.

Supporting Phase I trials - the TARGET protocol
A key focus of BBS is to enable robust, reproducible workflows to analyse different types of next-generation sequencing data. TARGET, a molecular profiling programme to match patients with a broad range of advanced cancers to early phase clinical trials (Clinical Lead, Dr Matthew Krebs a CBC alumnus), is now completed. This involved a thorough analysis of both somatic mutations and copy number alterations across a 641 cancer-associated gene panel assessed in ctDNA. Data from the first 100 enrolled patients on TARGET were published in *Nature Medicine* (Rothwell et al 2019) and our approach has been adopted across the CRUK Experimental Cancer Medicine Centre Network ECMCs as TARGET National. We have now analysed NGS data from the complete TARGET cohort (520 patients) using updated analysis tools, implementing the latest versions of variant callers and added a functionality to detect variants from cfDNA NGS data without a germ line control. These pipelines will be used across CBC projects involving liquid biopsies to guide effective treatment strategies.

The REACTION SCLC trial
With Prof Benjamin Besse (Paris-Saclay University, Orsay, France), Dr Jessica Menis (EORTC), Dr Pernalle Lavaud (EMBO visiting fellow) and Prof Fiona Blackhall and our TI team, we analysed CTCs in extensive disease SCLC patients who underwent treatment with immunotherapy and/or chemotherapy on the REACTION trial (EORTC 1417). Survival analysis revealed CTC number was prognostic on treatment, suggesting that CTCs are a potential biomarker for patient stratification in future clinical trials of immunotherapies.

The NOTION trial
We worked with the TI team to develop a bespoke QC metric for ELISA assays of cytokines in home-based acquisition of blood samples sent to CBC as dried blood spots on paper from patients in the NOTION trial led by the digital ECMT (see below). The QC metric uses tolerance intervals to determine whether assays are performing consistently through time.

The VALTIVE1b trial
Following the launch of the VALTIVE1a trial (Clinical Leads, Prof. Gordon Jayson (CFT, UoM), Prof. Richard Adams (University of Cardiff)), we are advising on the design of the subsequent VALTIVE1b trial that seeks to optimise use of VEGF inhibitors in advanced ovarian cancer patients using the CBC developed vascular response biomarker plasma Tie2.

The digital Experimental Cancer Medicine Team (digital ECMT)

The digital ECMT seek digital solutions to support treatment decisions for cancer patients and to digitally empower patients and healthcare professionals to innovate and design new cancer care pathways. The team provide next generation patient cancer care through comprehensive data-driven evidence, enabling transformation of clinical decision-making, evolving the patient's role and improving patient outcomes. They achieve this by listening to patients and healthcare professionals, understanding their needs and working proactively with them to develop ethical algorithms (AI) to build digital solutions and evaluate technologies under clinical trial conditions (technology clinical trials).

The NOTION study (with TI and BBS teams and Immuno-oncology Group at CFT) utilises Mitra® collection devices to assess whether patients can collect blood microsamples at home. These blood samples are analysed in CBC to assess whether changes in patients' cytokines correlate to development of immune-related adverse events (cytokine storms). This approach may help detect the onset of immune-related toxicities sooner and help manage them better for patients.

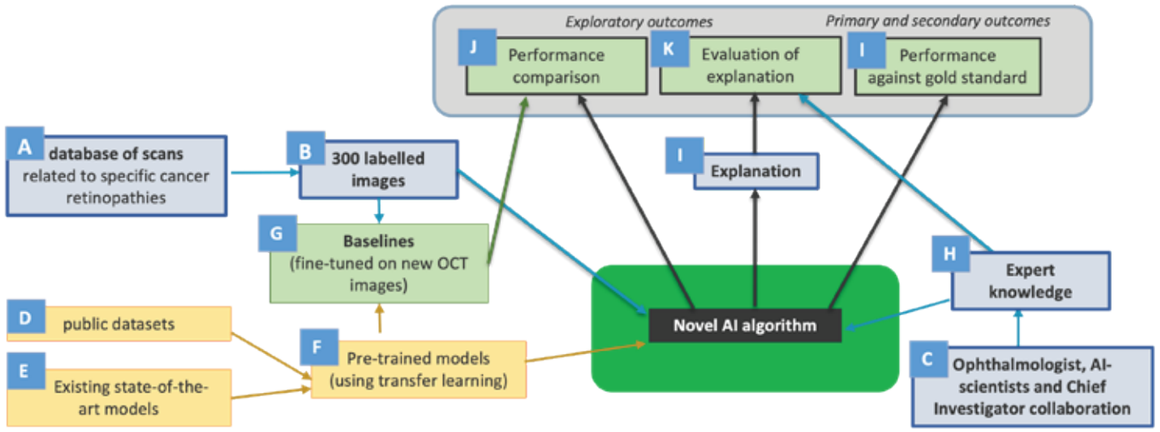


Figure 6. AI algorithm development to detect adverse retinal abnormalities caused by cancer treatment.

The iMATCH programme (Innovate UK, Manchester Advanced Therapy Centre) was completed this year incorporating our research into a Cytokine Release Syndrome predictive algorithm and Decision Support System to assist management of immune related toxicities.

The IN-HOME study assesses feasibility of Acute Kidney Injury (AKI) detection in the patient's home. Having demonstrated feasibility, the study is open to recruitment and evaluates potential for earlier diagnosis of AKI/change in renal function in cancer patients with intensive home monitoring.

The A-EYE study completed recruitment at the Manchester Royal Eye Hospital. Development of a new AI model to detect adverse retinal abnormalities associated with cancer treatment, using the OCT scans collected, has begun and performance and clinical acceptability of the model will be assessed (Figure 6).

We are working with colleagues in Italy and Spain within the *UpSmart CRUK Accelerator Award* to enable SMART Experimental Cancer Medicine Trials. PROACT 2.0, a mobile and web app to manage patients-to-medical-team communications across early phase trials was developed within UpSMART and a patient usability study was completed. A protocol and research package for APACE, a multi-national feasibility study evaluating use of accelerometers to capture physical activity levels in cancer patients on early phase clinical trials, was developed and submitted for ethical review.

In EU Horizon 2020 funded CCE_DART

(Building Data Rich clinical Trials) we are part of a consortium (Vall d'Hebron, Barcelona led) and lead on two of 18 work packages. digital ECMT are driving discovery of multi-layer complex biomarkers and development of online tools for Patient Reported Outcome (PRO) measurements (with the National Cancer Institute, Amsterdam).

Digital Inclusivity Guidance has been developed following Manchester Academic Health Sciences Centre funded focus group meetings with diverse populations to ensure our research and technology clinical trials processes and practice are more inclusive. This piece of work won 'Commitment to Equality Award' at the Greater Manchester Cancer Awards in October 2022.

The Quality Assurance, Operations and Administrative Teams

provide significant, professional and essential support for all CBC research.

Publications listed on page 56

CANCER INFLAMMATION AND IMMUNITY

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Agrin Moeini
Laetitia Nobel-Brat[2]
Massimo Russo[2]

Scientific Officer
Shih-Chieh Chiang

Graduate Students
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[1]left in 2022
[2]joined in 2022

Inflammation is an established cancer hallmark linked to aggressive tumour growth, poor prognosis and treatment resistance. The clinical success of immune checkpoint blockade (ICB) therapies across multiple cancer types has however, uncovered a type of intratumoural inflammatory response with potent tumour-suppressive features. This type of inflammation, less prevalent in established tumours, is characterised by increased infiltration by select immune cell types, particularly cytotoxic T cells and conventional dendritic cells.

Our group at the Cancer Research UK Manchester Institute investigates the signals and pathways that regulate the establishment of inflammatory tumour environments that favour natural anti-cancer immunity and the response to ICB. We combine pre-clinical cancer models with the analysis of patient samples to delineate the cellular and molecular determinants that underpin immunotherapy success. Our basic and translational research underpins two upcoming clinical trials, and our ultimate goal is to develop more effective targeted interventions to boost tumour immunity and improve cancer treatment responses.

Efforts on immune cell profiling at the tumour site have largely focused on T cell-mediated inflammation, which is also the target of established and emerging immune biomarkers. Tumours that exhibit signs of T cell infiltration and increased levels of inflammatory mediators associated with T cell cytotoxic activity are referred to as 'hot' or 'inflamed', and patients with such tumours tend to respond better to ICB therapy. However, inflammation is a broad term that encompasses the functional consequences of both innate and adaptive immune cell activity and is a characteristic of essentially all solid cancers. Even 'immune desert' tumours, which lack immune cell infiltration, typically show upregulation of inflammatory signalling in cancer or stromal cells. However, the most common type of inflammatory response found in clinically apparent tumours is hostile to cytotoxic T cells and favours cancer progression, spread and therapy resistance. Therefore, rather than being inflamed or not, tumours display quantitatively and qualitatively different inflammatory profiles. Under this working paradigm, a very active area of research of our group is centred on the study of the cyclooxygenase (COX)-2/prostaglandin E2 (PGE2) axis, which our past work underscores as a key determinant of whether the tumour inflammatory 'flavour' is pro- or anti-tumourigenic.

Expanding this prior research identifying PGE2 as a key factor suppressing the immune response within the tumour microenvironment, we have recently examined its role in inflammation and tumour immunity during cytotoxic therapy treatment. Earlier studies had linked PGE2 release from cancer cells undergoing apoptosis after chemotherapy or radiotherapy with enhanced cancer cell proliferation and tumour repopulation. We sought to investigate the prevalence, mechanisms, and implications of increased PGE2 synthesis by cancer cells following cytotoxic therapy, particularly with respect to their inflammatory properties.

We first examined the kinetics of activation of the COX-2/PGE2 pathway in multiple cancer cell lines treated with chemotherapy. In doing so, we found that transcriptional upregulation of Ptgs2, the gene encoding COX-2, and consequent release of PGE2 happens for both murine and human cancer cells, but only in those with pre-existing COX-2 expression. We could attribute the increase in PGE2 production to transcriptional upregulation of Ptgs2 post-chemotherapy. Cancer cells that were genetically modified to express COX-2 from a constitutive unrelated promoter did not display pathway enhancement following chemotherapy. These results have significant clinical implications, as measuring the baseline activity of the COX-2/PGE2 pathway in tumour cells could aid in identifying patients who are most likely to benefit from the addition of a COX-2 inhibitor to chemoimmunotherapy protocols.

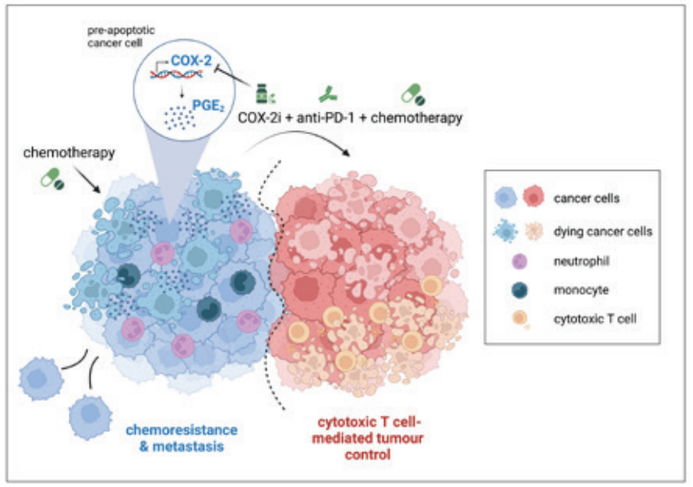

Figure 1.
Cytotoxic therapy activates the COX-2/PGE_2 pathway and modulates tumour-associated inflammation.
Chemotherapy upregulates COX-2 transcription and downstream PGE2 production in dying cancer cells, inducing an inflammatory response that limits the efficacy of chemoimmunotherapy combinations. Inhibition of COX-2 alongside chemoimmunotherapy treatment enhances immune-mediated tumour growth control and prevents metastasis in pre-clinical models. Adapted from Bell & Zelenay, *Cell Stress*, 2022.

We generated and validated a COX-2 reporter cell line and used it to monitor the kinetics of COX-2 transcriptional upregulation alongside cancer cell growth following treatment by live cell imaging. This allowed us to screen a library of over 1200 market-approved drugs. Interestingly, all classes of chemotherapeutic agents or drugs that induced growth arrest in cancer cells also led to increased COX-2 transcription, indicating that activation of the COX-2/PGE2 pathway is a universal phenomenon that occurs regardless of the cytotoxic drug's mechanism of action. Furthermore, chemotherapy drugs that induce immunogenic cell death (ICD), and those that do not, showed equal increase in COX-2 expression and PGE2 synthesis, ruling out the possibility that this phenomenon is responsible for the differential ability of ICD and non-ICD inducers to stimulate cancer immunity.

By using inhibitor and siRNA-based approaches, we investigated the mechanism behind COX-2/PGE2 upregulation following chemotherapy treatment. Our findings suggest that caspase activity, reactive oxygen species, and various transcription factors, such as NF-κB, C/EBPβ, Sp1, and AP-1, do not play an essential role. In contrast to previous reports, we showed that the increase in COX-2 expression and PGE2 release occur before and independently of the activation of caspase-3/-7 but coincide with the arrest in cancer cell proliferation. Indeed, no transcriptional upregulation of COX-2 was detected for any of the library screen compounds tested unless they also induced cancer cell arrest. Given the prevalence of COX-2 upregulation following chemotherapy across different cancer cell lines and the variety of treatments that could trigger it, we hypothesised that there is a conserved, yet unidentified, cell stress response pathway underlying the acute upregulation of COX-2 transcription. Further investigation is warranted to characterise this pathway and determine whether it also contributes to COX-2 upregulation post-cytotoxic treatment in non-tumour cells.

We then evaluated the impact of increased COX-2/PGE2 pathway activity on the inflammatory response of cancer cells post-chemotherapy *in vivo* by examining the recruitment of immune cells and the production of soluble inflammatory mediators after injecting chemotherapy-treated cells into the peritoneum of mice. This experimental model, which has been used previously to study the inflammatory response to dead or dying cells, allowed us to selectively assess the response driven by the treated cancer cells and exclude any confounding effects that systemic chemotherapy may have on non-tumour cells. Our results indicate that cancer cells that upregulate COX-2 expression post-chemotherapy stimulate much more profound recruitment of neutrophils and monocytes compared with untreated control cancer cells. Notably, cancer cells that lack or express basal levels of COX-2, but cannot upregulate its transcription post-chemotherapy, behaved similarly to untreated cells, highlighting upregulation of COX-2 as a major determinant of the inflammatory properties of chemotherapy-treated cancer cells.

Given these findings and our previous work highlighting a crucial contribution for cancer cell-intrinsic COX-2/PGE_2 activity to tumour immune evasion, we next examined if pharmacological inhibition of COX-2 could alter the efficacy of chemoimmunotherapy combinations. Crucially, mice bearing poorly immunogenic tumours formed by 4T1 breast cancer cells failed to respond unless treated with the triple drug combination of chemotherapy, PD-1 blockade and COX-2 inhibition. This was also the case in an adjuvant therapy experimental model where orthotopically-implanted mammary tumours were surgically removed prior to the initiation of therapy. The addition of a COX-2 inhibitor alongside chemoimmunotherapy was again essential to limit tumour relapse and metastatic spread to the lung.

In conclusion, our recent findings support a model whereby increased COX-2 expression and the resulting augmented PGE^2 synthesis, by modulating the inflammatory features of cancer cells post-cytotoxic treatment, limit T cell-mediated tumour immunity (see Figure). Therefore, pharmacologically targeting COX-2 could represent a viable strategy to unleash the efficacy of combinations of cytotoxic therapy and immunotherapy. This possibility will be tested in the clinic in the upcoming clinical trial LION (**L**ifting **I**mmune **C**heckp**O**ints with **N**SAIDs), a basket trial funded by the J P Moulton Charitable Foundation and The Christie NHS Foundation Trust where we will investigate if addition of a selective COX-2 inhibitor enhances the efficacy of standard of care (ICB or ICB plus chemotherapy) in lung, breast and kidney cancer.

Publications listed on page 58

CELL DIVISION

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[1]Maternity leave
[2]Left in 2022

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. The DNA damaging approaches and microtubule disrupting agents of irradiation and chemotherapy owe much of their success to the checkpoint pathways that ensure transition through the cell division cycle only occurs when genome integrity is guaranteed.

We study these checkpoint pathways and the control of commitment to the physical process of genome segregation, known as 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 that frames the questions to ask of the more complex context of human cell division.

In a typical cell division cycle the G1 gap phase precedes DNA replication in S phase, before a second gap phase, G2, separates S from genome segregation with the mitotic spindle in Mitosis (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). Once cells are through the Restriction Point, they are committed to the cycle and progress around the cycle even if the pro-division cues that pushed them through this regulatory step are removed. Successive waves of Cdk-cyclin activities then drive different events as cells transit the cycle.

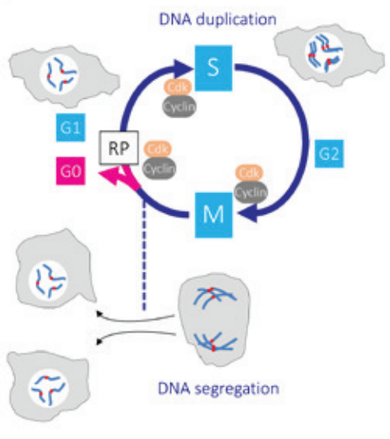

Figure 1.
The human cell cycle with Cdk1-Cyclin B control of the G2/M transition
Passage through the restriction point (RP) in G1 phase commits a cell to passage through the cell division cycle. DNA replication in S phase is separated from mitosis by a gap phase, G2. Transition through the major rate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

Defects in DNA integrity and the ability to form the mitotic spindle 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 change chromosome number, cancer cells become more reliant upon these checkpoints than their normal neighbours. Consequently, agents that enhance DNA damage or perturb mitotic spindle function are widely used in the clinic as they increase the level of damage in the already stressed cancer cells to a point where checkpoint defences 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 ways that will selectively eliminate cancer cells.

Commitment to mitosis from G2 phase is driven by activation of the Cdk1-Cyclin B protein kinase. Because the Wee1 family kinases – Wee1 and Pkmyt1 – inhibit Cdk1-Cyclin B, removal of this phosphate by Cdc25 drives cells into mitosis. 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. There has therefore been considerable interest in developing drugs that inhibit Wee1 to weaken checkpoints that protect the DNA damage laden tumour cells. However, as Wee1 targets Cdk2 complexes alongside Cdk1-Cyclin B, Wee1 inhibitors perturb S phase progression to cause general toxicity that has proven dose limiting in clinical trials. As the second Wee1 family member Pkmyt1 only regulates Cdk1-Cyclin B and Pkmyt1 can be completely removed from untransformed cells without affecting viability, there is keen interest in the outcome of a current series of Pkmyt1 inhibitor trials. To refine the use of such Pkmyt1 inhibitors in the clinic we are asking how, when and why Pkmyt1 is used to regulate mitotic commitment?

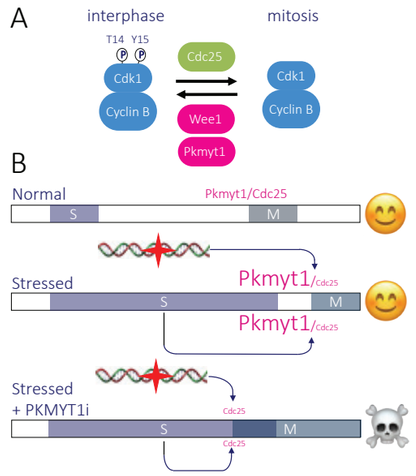

Figure 2.
Pkmyt1 in Cdk1-Cyclin B regulation in checkpoint control
A Cdk1-Cyclin B activity is held in check in interphase as a consequence of phosphorylation of Cdk1 by Wee1 family kinases. Cdc25 removes the inhibitory phosphate to trigger mitosis. **B** DNA damage or incomplete DNA replication trigger checkpoint pathways that boost inhibitory phosphorylation of Cdk1 and reduce counteracting Cdc25 activity. Inhibition of the inhibitory kinases, e.g., Pkmyt1, abolishes this restraint to initiate division before DNA integrity is restored, leading to death.

Cdk1-Cyclin B promotes mitosis by activating downstream mitotic kinases that target many substrates to generate and control the mitotic spindle. As each stage of mitosis is completed, each phosphate these kinases have put on targets to modify target function must be removed to enable transition to the next stage of division. It therefore goes without saying that blocking this phosphate removal will trap the cell at an intermediary mitotic stage. As extended mitotic arrest triggers cell death, there is considerable interest in finding ways to manipulate mitotic phosphatase function to delay cells in division and so trigger death in the tumour cells that are already struggling to divide properly.

PP1 and Protein Phosphatases 2A (PP2A) determine the timing and rate of mitotic progression. PP1 is recruited to docking sites from where it dephosphorylates local targets. Hetero-trimeric PP2A enzymes comprise single scaffolding and catalytic subunits, alongside one of four different types of regulatory subunit. Multiple, alternatively spliced, genes give the potential to generate hundreds of PP2A complexes in humans, whereas fission yeast can live on one of each, or in the case of PP2A-B55, none. This makes fission yeast an ideal system in which to uncover the principles of mitotic phosphatase control.

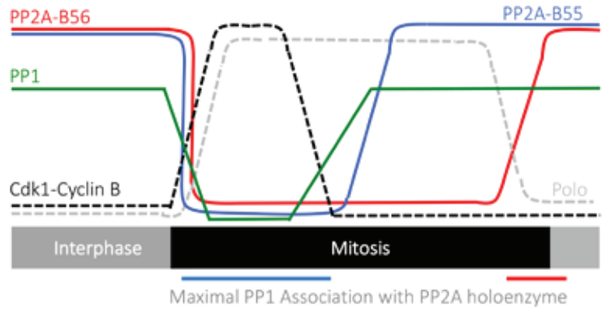

Figure 3.
The fission yeast mitotic PP1-PP2A mitotic phosphatase relay
See text for details.
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We have previously described how the direct recruitment of PP1 to PP2A-B55 and PP2A-B56 re-activates these PP2A phosphatases to support timely mitotic progression in fission yeast. In this cascade PP1 acts as a master regulator. The inhibition of PP1 by Cdk1-Cyclin B as cells commit to mitosis is auto-catalytically reversed as soon as Cdk1-Cyclin B activity is lost when the Cyclin B subunit is destroyed because the complex has fulfilled its function. The reactivated PP1 then reactivates PP2A-B55 to drive cells towards mitotic exit. Phosphorylation of the PP1 docking site of PP2A-B56 by Polo kinase initially blocks PP1 binding to PP2A-B56, however, once polo activity declines in the final stages of mitosis, PP2A-B55 dephosphorylates the Polo phosphorylation site on B56 to allow PP1 to now reactivate PP2A-B56 (Figure 3). Thus, while mitotic onset depresses the activity of all the phosphatases so that the protein kinases can most efficiently change protein behaviour, PP1 plays an overseeing role in ensuring the timely reversal of phosphorylation by PP2A enzymes.

One prediction of this model is that both PP2A-B55 and PP2A-B56 are subjected to inhibitory phosphorylation events that are reversed by PP1. We have therefore mapped sites of phosphorylation on both fission yeast holoenzymes to find phosphorylation events whose amplitude is PP1 dependent. Encouragingly, both B55 and B56 possess sites where phosphorylation is enhanced when PP1 activity is compromised. In both cases the sites are candidates for being direct targets of PP1 because their phosphorylation reduces PP2A activity. The conservation of each site in human cells has prompted us to focus upon the analogous phosphorylation events in human cells and determine whether abolition of this form of control in humans may alter mitotic progression.

These studies are complemented by our team's analyses of other mitotic checkpoints and the centrosome's influence over cell cycle control. We hope that the cumulative findings of these complementary programmes will contribute to the development of novel strategies that exploit the inherent genome instability of tumours for therapeutic benefit.

CELL PLASTICITY & EPIGENETICS

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During cancer development, malignant cells are constantly exposed to a myriad of selective forces – intrinsic and extrinsic – that shape the evolutionary trajectory of most tumours and contribute to the establishment of extensive genetic heterogeneity. Based on this widely accepted view of cancer evolution, the cancer research community has focused most of its efforts on the study of genetic changes, though an important wealth of evidence obtained from various cellular systems suggests that non-genetic heterogeneity may play an equally fundamental role fuelling cancer evolution.

Notably, phenotypic plasticity, the ability of a single genotype to produce a variety of phenotypes, has been documented as a core biological process underlying numerous molecular and cellular events ranging from unicellular adaptation to multi-cellular organism development. Translating this concept onto cancer cell populations, phenotypic plasticity may lead to the establishment of co-existing phenotypically distinct metastable states that in turn, may grant populational adaptation to fast-paced environmental conditions (exposure to drugs, hypoxia, invading new niches, etc.), even in the absence of genetic divergence. Given the crucial role that non-genetically encoded phenotypic states play in biology, our research aims to unravel the molecular mechanisms underlying such a phenomenon and to address its role as a key determinant in cell plasticity during cancer progression/evolution.

Over the past few years, by means of single-cell analysis, our lab has shown that clonal populations of a variety of cellular systems of diverse origin display multiple non-genetically encoded metastable states that can be ascribed to dramatically different cell phenotypes. Following our observations, in order to unravel the molecular mechanisms underlying the observed cellular plasticity, we have developed a lineage tracing technology termed Barcode decay Lineage Tracing-Seq (BdLT-Seq). Our method (accepted for publication in *Nature Communications*) allows the building of directional lineage trees that link its individual branches to metastable states, thus uncovering the molecular "rules" of transcriptome plasticity in comparable genomic backgrounds. BdLT-Seq relies on a high-complexity library of non-genetically episome-encoded molecular identifiers that is transfected into cells, and which sets a unique fingerprint to each cell contained within the population (Figure 1a and b). Notably, though episomal vectors undergo scheduled replication and therefore are stably maintained within transfected cells, they are also randomly inherited upon cell division. This important feature results in a decay in the number of unique barcodes in daughter cells while maintaining unique lineage-specific fingerprint, which relates each cell to its ancestor (Figure 1a and c).

Importantly, applying BdLT-Seq to an HRAS-G12V driven clonal HA1ER cell model, we found that transcriptome states are replenished upon cell division/s but do also transition into distinct states resulting in a progeny displaying different transcriptome profiles (Figure 1e), thus fuelling the generation of non-genetically heterogeneous populations. Moreover, we have shown that the plastic capacity of transcriptome states to generate progeny residing in distinct states is not random but is somehow encoded in non-genetic networks and restricted in a lineage-linked manner. Notably, restricted transcriptome plasticity and inheritance is not unique to tumorigenic models and has also been observed in immortalised cell systems, suggesting its widespread and perhaps universal nature. Interestingly, subcloning a parental – clonal – population gives rise to populations of cells that are enriched in subsets of states and only partially recapitulate the heterogeneity observed in the parental population, thus validating our observations related to lineage-linked transcriptome plasticity. Importantly, subclones enriched in distinct states show significant variations in their response to various environmental cues (e.g., anchorage-independent growth, anticancer drugs, oncogenic transformation) suggesting that metastable states play a key role in shaping cancer onset, progression, and evolution.

Following those lines and based on our findings, we used BdLT-Seq to explore if and how lineage-linked transcriptome plasticity plays a role in oncogene-induced cellular transformation. For that we built a multiplexed cell system in which six RAS mutant variants from different families (HRAS-G12C, HRAS-Q61K, NRAS-Q61R, NRAS-Y64D, KRAS-G12V, KRAS-E62K) are individually expressed in an otherwise isogenic clonal population of immortalised cells (HA1E) and performed a simultaneous analysis of the impact of distinct oncogenes on lineage-linked transcriptome evolution in a single assay. Notably, we found that cells expressing different RAS oncogenes are readily enriched in distinct clusters, suggesting that the expression of RAS variants promote remodelling of the population heterogeneity in an oncogene-specific manner (Figure 1d). Furthermore, we showed that the evaluated RAS variants have a distinct impact on transcriptome plasticity dictating the degree of population heterogeneity. Interestingly, despite a similar degree of non-genetic heterogeneity observed by specific RAS variants (HRAS-G12C versus HRAS-Q61K), a subset of states displays distinct patterns of transcriptome plasticity (for example, cluster 1 to cluster 4 transitions) that may affect population dynamics, resulting in variations in phenotypic output (Figure 1d).

Finally, it is worth stressing that to further elucidate the molecular mechanisms underlying transcriptome transitions and hence phenotypic remodelling, our lab has looked into the identification of the potential molecular players involved in such process. Interestingly, we have identified that a large subset of long non-coding RNAs (lncRNAs) and a small fraction of intrinsically disordered proteins (IDRs) underlies clonal phenotypic divergence, thus suggesting these molecular compartments as key players in cell plasticity. Notably, the identified lncRNAs and IDR-containing proteins colocalise within perinuclear structures and form granule-like precipitates, allowing us to hypothesise that IDR proteins together with lncRNAs may act as architectural scaffolds in liquid-liquid phase separations, a process which may provide temporal and spatial control of signalling transduction, thus unravelling an exquisite mechanism by which non-genetic information may fuel evolutionary processes.

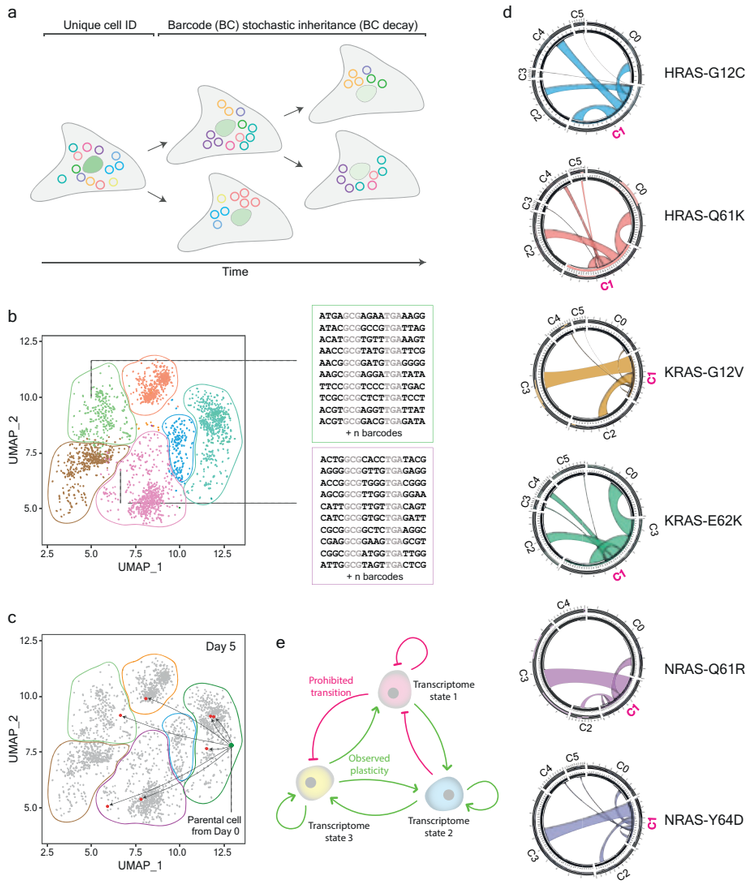

Figure 1.
A Scheme representing BdLT-Seq conceptual framework that can be applied to any model system susceptible to being transfected with the episomal library. The combination of barcodes per cell sets its unique fingerprint ID. Barcode decay is exemplified as a function of time. **B** Toy UMAP plot depicting scRNA-Seq data exemplifying cell ID and the identified transcriptome states. Two cells are individualised and shown with a subset of their corresponding BdLT-Seq barcode identifiers, which provides unequivocal information about cell identity. **C** Toy UMAP plot of scRNA-Seq data displaying an example of the lineage relationship for a cell at Day 0 (green dot) and its progeny (red dots) at Day 5 of tracing as determined by BdLT-Seq. Gene expression state boundaries are shown as coloured lattices. **D** Chord diagrams representing transcriptome state dynamics for the RAS variant multiplexed cell population. All detected clusters are depicted (C0 to C5) and integrate the collapsed behaviour of all cells that belong to a particular gene expression state for each RAS variant. Transcriptome divergence from C1 is shown as an example of transcriptome plasticity. Origin cluster is shown in pink (Day 0) and chords represent end point cluster association (Day 5). **E** Scheme representing an example of lineage-linked transcriptome state transitions as determined by BdLT-Seq.

Credits for artwork: Lisa Shlyakhtina, Katherine Moran and Maxi Portal.

CELL SIGNALLING

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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. Moreover, other KRASm isoforms lack targeted therapies in the clinic. 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.

RAC is a member of the RHO-like family of GTPases and cycles between a GDP- and a GTP-bound state. When GTP-bound, it interacts with various 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. In particular, 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 Commun 2016; Woroniuk *et al.,* Nat Commun 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 Commun 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 that 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. They perform these contrasting roles in cell migration by selecting RAC effectors (Marei *et al.,* Nat Commun 2016). Over-expression of specific GEFs, which occurs commonly in many cancers, can therefore drive different oncogenic signalling pathways. 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 are evaluating the requirement of two RAC GEFs, TIAM1 and STEF (also known as TIAM2) in KRASm-LUAD formation and progression for the following reasons: 1) both are RAC-specific GEFs; 2) they are both non-essential in mouse; and 3) and they are the only two RHO-GTPase GEFs that possess RAS-binding domains (Muller *et al.,* Nat Cell Biol 2020), suggesting that they are effectors of RAS. In fact, TIAM1 is a known RAS effector required for the activation of RAC by RAS (Lambert *et al.,* Nat Cell Biol 2002), and TIAM1 is required for RAS-induced skin tumours in mouse (Malliri *et al.,* Nature 2002).

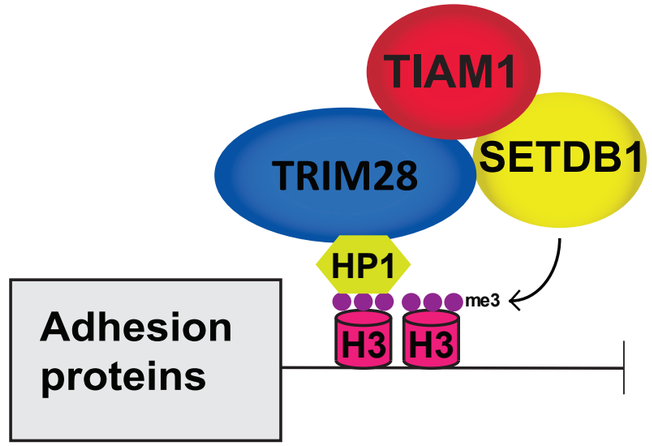

Figure 1.
Model depicting the role of TIAM1 in regulating cell-cell adhesion of LUAD cells.
TIAM1 is part of the TRIM28-SETDB1 transcriptional co-repressor complex that co-occupies genomic targets such as the clustered protocadherins promoting H3K9me3 deposition. These epigenetic changes suppress gene transcription and lead to a more mesenchymal phenotype, promoting the migration of NSCLC cells.

Role of RAC signalling in KRASm NSCLC

To begin investigating the role of TIAM1 in LUAD, we probed a human LUAD tumour microarray for TIAM1. Consistent with our previous publication in colorectal cancer (CRC) (Diamantopoulou *et al.,* Cancer Cell 2017), we revealed not only cytoplasmic but also nuclear TIAM1. However, in contrast to CRC, nuclear TIAM1 prevalence was associated with advancing lung cancer stage and decreased survival of patients, suggesting a pro-malignancy role for nuclear TIAM1 in LUAD. To investigate the role of TIAM1 in the malignant progression of LUADs, we depleted TIAM1 in several LUAD cell lines, which also showed inducible expression of nuclear TIAM1. Interestingly, TIAM1-depletion suppressed the migration of LUAD cells, an effect rescued by nuclear-localised TIAM1 (NLS-TIAM1).

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), while nuclear TIAM1 overexpression increased 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) in LUAD. 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.,* In revision).

Role of RAC signalling in SCLC

SCLC is a highly aggressive malignancy, broadly divided into neuroendocrine (NE, >80% of SCLC) and non-NE subtypes. Analysis of expression data from SCLC tumours, patient-derived models, and established cell lines, showed that the expression of TIAM1 is associated with a neuroendocrine gene programme. Moreover, we showed that TIAM1 depletion reduces viability and tumorigenicity of SCLC cells by increasing apoptosis associated with conversion of BCL2 from its pro-survival to pro-apoptotic function via BH3 domain exposure. We showed that this conversion is dependent upon cytoplasmic translocation of Nur77, an orphan nuclear receptor. Like in other cell types, TIAM1 is present in the nucleus of SCLC cells, where it interacts with Nur77 sequestering it in SCLC cell nuclei. TIAM1 depletion promoted Nur77 translocation to the cytoplasm. Importantly, mutant TIAM1 with reduced Nur77 binding failed to suppress apoptosis triggered by TIAM1 depletion. We are currently following up on these published data (Payapilly *et al.,* Cell Rep. 2021) by evaluating the role of additional GEFs for RAC and RAC itself in SCLC biology.

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LEUKAEMIA BIOLOGY

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[1]Left in 2022
[2]Joined in 2022

The goal of the group is to develop understanding of disease mechanisms in myeloid lineage blood cancers, such as acute myeloid leukaemia (AML), and through doing so to identify candidate therapeutic targets for development through to the clinic. In 2022 we completed two projects in the disease mechanisms area. In the first (Maiques-Diaz et al., 2022, Oncogene) we report our new insights into how inhibitors of the histone demethylase LSD1 function to promote differentiation in myeloid leukaemia through disrupting the interaction of the CoREST complex with chromatin; and in the second we report our discovery of a non-coding transcript arising from intron 8 of FTO, which regulates expression of a gene called IRX3 (Camera et al., due to be published in 2023). IRX3 is highly and inappropriately expressed in ~30% of cases of human acute myeloid leukaemia and contributes to the cellular differentiation block that is the pathognomonic feature of the disease.

Much of the focus of the group continues to be on understanding disease mechanisms in acute myeloid leukaemia (AML). This is a blood cancer characterised by a block to normal myeloid lineage differentiation leading to accumulation of myeloid blast cells in bone marrow (BM), with failure of normal blood cell production. Despite much progress in recent years, including the FDA approval of several novel therapies, it remains the case that long term survival from AML remains poor, especially in those over the age of 60.

Inhibitors of the histone demethylase LSD1 including iadademstat and bomedemstat, which are derivatives of the monoamine oxidase inhibitor tranylcypromine, continue in clinical trial evaluation in myeloid blood cancers. Iadademstat has been evaluated in a now completed phase 2 combination study with azacitidine in elderly patients with AML and, in collaboration with colleagues at Vall d'Hebron University Hospital, Barcelona, we reported encouraging results at an oral presentation at the American Society for Hematology meeting in New Orleans in December 2022. Bomedemstat has been evaluated in AML and myelofibrosis and has now moved into a phase 3 trial with registrational intent in the setting of hydroxycarbamide-intolerant essential thrombocythaemia. Imago Biosciences, which has developed bomedemstat, was recently acquired by Merck in a $1.4 billion deal, such is the excitement and promise over the drug for the future.

LSD1 forms a corepressor complex with RCOR1 (CoREST), histone deacetylase (HDAC1/2) and other components and has enzymatic capacity to demethylate monomethyl and dimethyl lysine 4 of histone H3 (H3K4) in a flavin adenine dinucleotide (FAD) dependent manner. In addition, LSD1 has a critical scaffolding function: it binds the N-terminal sequence of SNAG domain transcription factors such as GFI1 through a peptide binding cleft formed by the amine oxidase domain. This interaction is essential for the transcription repressor function of these DNA binding proteins, and we have previously shown that it is the predominant target for the activity of LSD1 inhibitors in inducing leukaemia cell differentiation. To identify LSD1 protein binding partners that facilitate the stability of LSD1's interaction with GFI1 on chromatin, and to further understand how inhibitors of LSD1 induce leukaemia cell differentiation, in a study led by Alba Maiques-Diaz (Maiques-Diaz et al., 2022, Oncogene) we made use of *MLL*-rearranged leukaemia cell models. These are dependent on the physical interaction of LSD1 with GFI1 to maintain their proliferative, undifferentiated cellular state. Making use of cutting-edge mass spectrometry approaches, and in collaboration with Tony Whetton and his team (now at

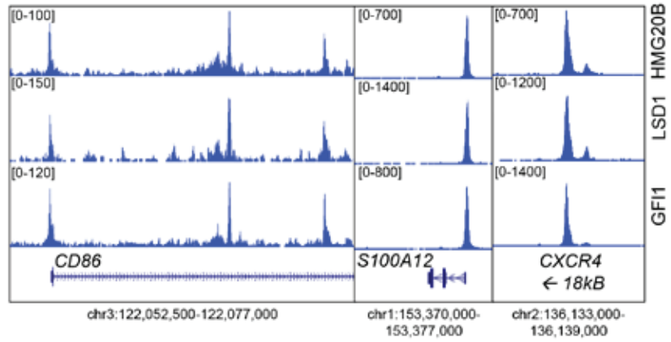

Figure 1.
Co-localised binding on chromatin in THP1 AML cell at the indicated sites of GFI1, LSD1 and HMG20B.

University of Surrey), we performed LSD1 immunoprecipitation analyses in the presence and absence of LSD1 inhibitors and discovered that the interaction of the HMG-box protein HMG20B with LSD1 was also disrupted by LSD1 inhibition. Downstream investigations revealed that HMG20B is co-located on chromatin with GFI1 and LSD1 genome-wide (Figure 1); the strongest HMG20B binding co-locates with the strongest GFI1 and LSD1 binding. Functional assays demonstrated that HMG20B depletion induces leukaemia cell differentiation and further revealed that HMG20B is required for the transcription repressor activity of the essential myeloid transcription factor GFI1 through stabilising LSD1 on chromatin at GFI1 binding sites. Interaction of HMG20B with LSD1 is through its coiled-coil domain. Thus, HMG20B is a critical component of the GFI1:LSD1 transcription repressor complex which contributes to leukaemia cell differentiation block.

Another area of focus for the lab in recent years has been our discovery that mis-expressed transcription factors, such as the Iroquois homeodomain protein IRX3 and the Forkhead factor protein FOXC1, contribute frequently to the differentiation block which is the pathognomonic feature of the disease. *IRX3* is highly expressed in the developing nervous system, limb buds, kidney and heart and with its paralog *IRX5* makes essential contributions to cardiac and skeletal development. Importantly, non-coding variation within introns 1 and 2 of *FTO* (for fat mass and obesity-associated) which sits 200-500KB downstream of *IRX3*, provides the strongest genetic association for risk of human obesity. Adult *Irx3*$^{-/-}$ mice have increased basal metabolic rate and reduced fat mass, with browning of white adipose tissue, attributable to loss of hypothalamic or preadipocyte *Irx3* expression. A specific variant inside the obesity-associated region abrogates the binding of the ARID5B repressor, leading to activation of a strong enhancer that promotes the expression of *IRX3* and *IRX5* during adipocyte development.

As already mentioned, *IRX3* is highly expressed in 20-30% of cases of AML and its misexpression contributes to the differentiation block. Targeting the cellular mechanisms by which these factors operate represents an attractive candidate therapeutic approach; drugs which induce leukaemia cell differentiation are already components of effective therapeutic regimens, most notably in acute promyelocytic leukaemia. It remains unclear how *IRX3* is aberrantly mis-expressed in human AML and so in a study led by Francesco Camera we searched the topologically associating domain within which *IRX3* sits for candidate regulatory elements that might control IRX3 gene expression. Making use of long-range chromatin interaction analyses, genome editing, enhancer-function studies and diverse bioinformatics tools in both AML cell lines and primary patient samples, we identified a region of four clustered elements in intron 8 of *FTO* in primary human AML cells sited 220kB downstream of *IRX3* whose differential histone acetylation, DNA methylation and contacts with the *IRX3* promoter correlated with *IRX3* expression. Genetic deletion of the region's sub-components confirmed their role in positively regulating *IRX3* expression. By RNA sequencing of primary AML samples, we also identified hitherto unannotated long non-coding transcripts arising from this locus, which we term *FTO*-lncAML, and characterised their sequence by rapid amplification of cDNA ends (RACE). Knockdown (KD) of *FTO*-lncAML induced phenotypic differentiation (Figure 2), loss of clonogenic activity of AML cells, reduced *FTO* intron 8 histone acetylation and reduced *FTO* intron 8:*IRX3* promoter contacts. While both *FTO*-lncAML KD and *IRX3* KD induced molecular differentiation as determined by RNA sequencing, *FTO*-lncAML KD but not *IRX3* KD led to down regulation of HOXA genes indicating transcript activity in trans. In keeping with this, patient AML samples expressing *FTO*-lncAML also expressed higher levels of HOXA genes and lower levels of genes associated with differentiation. Thus, a regulatory module in the final intron of *FTO* consisting of clustered enhancer elements and a non-coding RNA is active in human AML, impeding myeloid differentiation.

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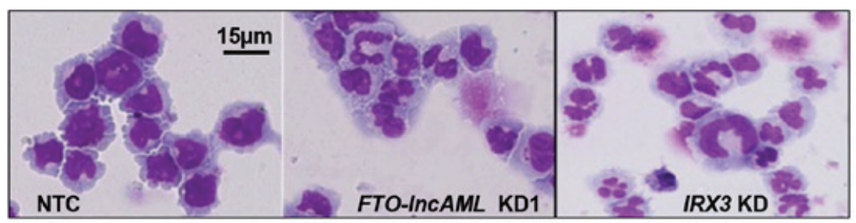
Figure 2.
Fujioka AML cell differentiation induced by knockdown of *FTO*-lncRNA or *IRX3*.



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[1]Joined in 2022

Allogeneic haematopoietic stem cell transplantation is the only curative therapy for many patients with acute myeloid leukaemia (AML) and other poor-risk haematological malignancies. Recipients are 'conditioned' with chemo/radiotherapy before receiving blood-forming stem cells harvested from a donor. These stem cells repopulate the bone marrow and provide a new immune system, which eliminates the cancer. However, disease relapse remains the most common cause of death and is due to failure of donor T cells to eliminate residual leukaemia. Donor T cells are often dysfunctional at relapse and leukaemic cells frequently exhibit reduced immunogenicity.

The Leukaemia Immunology and Transplantation group formed in autumn 2022 with the aim of developing a comprehensive strategy to prevent post-transplant relapse (Figure 1). We are developing novel biomarkers to identify patients at risk of relapse, defining the critical drivers of T-cell dysfunction and exploring the potential of pharmacological induction of leukaemic differentiation to augment donor T-cell responses.

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

To prevent relapse, at-risk patients must first be identified. Early recognition is essential, because established relapse compromises graft function and likely forecloses the possibility of influencing donor immune responses. Existing methods, such as minimal residual disease detection, can only be applied to a minority of patients and convey no information regarding relapse mechanism. We believe that biomarkers of immune dysfunction could predict disease recurrence in the majority of patients and guide manipulation of the donor immune response to avert relapse.

Recent studies have found that exhausted T cells (T_{EX}) are detectable in the blood and bone marrow of patients who go on to experience relapse. We have established 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. The study has now received ethical approval and will open in spring 2023. We are currently developing a mass cytometry panel that allows deep profiling of T-cell heterogeneity, dysfunction and exhaustion. This will be applied to hundreds of samples from PM-SCT to confirm detection of T_{EX} as a relapse biomarker and determine the time point(s) and cell surface markers that allow optimal prediction.

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. Several tissue leakage proteins can be found in plasma following transplantation, reflecting sub-clinical organ damage that can occur weeks before the onset of clinically apparent GvHD. We are currently applying different MS- and affinity-based proteomic methods to longitudinal blood samples from transplant recipients to discover novel biomarkers that predict GvHD onset. Recent studies have also identified plasma proteomic signatures of anti-leukaemic T-cell activity. We therefore plan to use samples from PM-SCT, and the proteomic methods refined through our investigation of GvHD, to discover novel protein biomarkers of impending relapse. Our ultimate ambition is to combine cytomic and proteomic data from these studies and develop multi-modal signatures and algorithms capable of predicting multiple transplant outcomes.

Identifying drivers of post-transplant T-cell exhaustion

Exhaustion is a distinct state of T-cell differentiation characterised by impaired effector function. Immune checkpoint inhibitors can reinvigorate or prevent the exhaustion of T cells and have revolutionised the management of several solid tumours. Evidence now implicates T-cell exhaustion as a mechanism of post-transplant AML relapse. Leukaemia-reactive exhausted T cells are present at relapse, typically expressing multiple inhibitory receptors, whose cognate ligands are expressed by AML cells (Figure 2A). Checkpoint inhibitors can induce post-transplant AML remissions, suggesting that T-cell exhaustion is a modifiable mechanism of relapse, but the increased risk of GvHD has limited its use. It is therefore necessary to identify context-specific drivers of T-cell exhaustion to inform treatments that re-establish anti-leukaemic T-cell responses without causing GvHD.

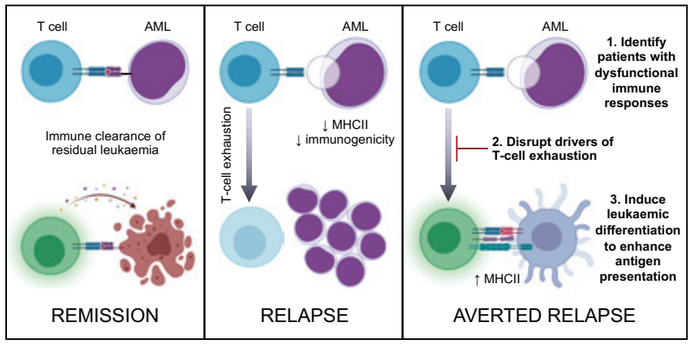

Figure 1.
A strategy for preventing post-transplant AML relapse by identifying patients with early evidence of immune dysfunction then intervening to modify both T cell- and AML-mediated mechanisms of relapse, namely T-cell exhaustion and leukaemic immune evasion through downregulation of MHC class II (MHCII).

Exhausted T cells are highly diverse; both gene expression and immunophenotype vary with clinical context. There are multiple potential drivers of exhaustion, including inhibitory cell signalling, suppressive cytokines and hypoxia. DNA sequences termed 'enhancers' are critical regulators of cell lineage specification, which integrate cell signals and environmental cues to determine context-specific gene expression. We are applying single-cell sequencing approaches to patient samples to map enhancer activity and transcription factor binding at exhaustion-associated genes. We believe that the pattern of activation and transcription factor binding will identify the pathways most responsible for driving post-transplant T-cell exhaustion (Figure 2B). These pathways can then be targeted to prevent exhaustion and subsequent relapse.

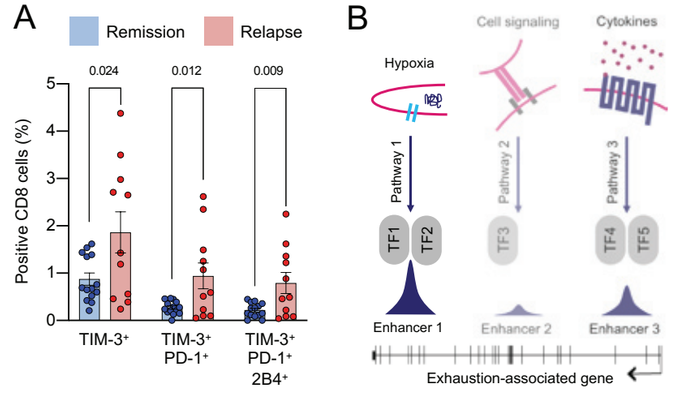

Figure 2.
A Donor T cells expressing multiple inhibitory receptors are more abundant at post-transplant relapse compared to time-matched remission controls. **B** We hypothesise that the pattern of enhancer activation and transcription factor binding at exhaustion-associated genes will reflect the activity of the pathways driving post-transplant T-cell exhaustion.

Inducing leukaemic differentiation to augment donor T-cell responses

In addition to T-cell exhaustion, murine studies identify poor antigen presentation as detrimental to anti-leukaemic T-cell responses. Professional antigen-presenting cells (APCs) activate CD4+ T cells by displaying antigenic peptides on major histocompatibility complex class II (MHCII) molecules together with co-stimulatory signals. AML often expresses MHCII and both genomic loss and transcriptional downregulation are common at post-transplant relapse, suggesting a strong selective pressure and a possible mechanism of immune evasion. We are investigating the potential of compounds that induce leukaemic differentiation to drive expression of MHCII and co-stimulatory molecules to enable robust $CD4^+$ T-cell activation, enhance $CD8^+$ T-cell effector function and promote successful disease clearance. We have several compounds 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. Drug-induced differentiation produces cells that share many morphological, transcriptional and phenotypic similarities with APCs, but they also retain a core leukaemic transcriptional programme and we term them leukaemia-derived APCs. We are currently investigating their ability to process and present antigens, and to engage and activate T cells.

The unique biology of AML presents an opportunity to enhance anti-tumour immune responses by manipulating leukaemic differentiation, rather than targeting normal immune populations. This is a very appealing strategy in the context of stem cell transplantation, where there is often widespread immune activation caused by the mismatch between donor immune cells and healthy recipient tissue. Immunotherapies that promote generalised donor immune cell activation are liable to trigger additional toxicity in the form of graft-versus-host disease, as has been observed with the use of immune checkpoint inhibitors. By contrast, compounds that induce leukaemic differentiation appear to have no effect on normal T-cells or monocytes, ensuring that enhanced antigen presentation and T-cell activation is specific to leukaemia-reactive populations.

MOLECULAR ONCOLOGY

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[1]Group to close in early 2023

2022 was a year of transition for Molecular Oncology. In November, working with Cancer Research Horizons, Caroline Springer and I founded Oncodrug Ltd, a CRUK MI biotechnology start-up company and in spring 2023 I will move to the company as Chief Scientific Officer.

A major activity of 2022 was therefore the managed closure of the Molecular Oncology laboratory. I am delighted that all the members of my group found new positions within CRUK MI or at other organisations, but the task of archiving and ensuring that all our important sample collections and reagents that will continue to be available to the research community should not be underestimated. I am very grateful to Valeria and Megan for their tireless hard work to achieve this.

We also worked to finish ongoing projects and submit our results for our last few papers, focussing on the causes of human disease and how our discoveries can improve patient diagnosis and treatment. We explored the genomes of melanomas from patients with the rare genetic disorder Xeroderma Pigmentosum (XP) and discovered that they have unique signatures linked to specific XP subtypes. Our data explain in part why XP patients have such a high lifetime risk of melanoma and may guide future treatment selection for melanomas in these individuals. We also explored variants of unknown clinical significance (VUS) in patients with congenital disorders of glycosylation (CDG), another rare genetic disease. CDG patients present with very broad symptoms, and we developed an assay to measure the biological activity of the VUS to provide a robust and rapid functional test that can complement sequencing data on the CDG diagnosis pipeline. We also published our final melanoma review, which we hope will be a valuable resource to those within and outside the field.

As part of our interest in understanding how ultraviolet radiation (UVR) causes melanoma, we collaborated with colleagues from the UK National Xeroderma Pigmentosum (XP) Service at Guy's and St Thomas' NHS Foundation Trust (London) to sequence the genomes of melanomas from XP patients. XP is a rare genetic disorder with seven subtypes (depending on which gene is mutated) whose underlying cause is a defect in DNA repair. XP patients present a variety of symptoms, but because the pathways involved are important for repairing UVR-induced DNA damage, about half of the patients suffer extreme UVR sensitivity, can burn after just a few minutes in the sun, and have a 2,000-fold increased lifetime risk of melanoma. We found that XP melanomas have very high mutation rates in cancer genes and in particular the majority have mutations in *NF1*, a subtype that accounts for only ~15% of non-XP melanomas. Notably, the XP melanoma genomes have a predominance of C>T mutations at pyrimidine dimers, a validated UVR-signature, and an observation that is consistent with these melanomas being UVR driven. Curiously however, when assessed within a trinucleotide context, the XP melanoma mutation signatures are distinct from non-XP melanoma signatures and moreover, the specific signatures in individual patient melanomas are inextricably linked to their XP subtype. We also noted that in addition to the typical UVR mutations at pyrimidine dimers, the XP melanoma signatures include an increased number of atypical UVR-induced mutations at non-pyrimidine dimer sites. Thus, XP melanomas have unique features that set them apart from non-XP melanomas and our work advances our understanding of how mis-repair of UVR-damaged DNA accelerates melanoma development. We also describe how specific XP subtypes affect UVR susceptibility and note that the specific mutation signatures in XP melanomas could guide clinical management of these patients.

In a separate project, we examined the relationship between cause and effect in patients with congenital disorders of glycosylation, another rare genetic disorder with a difficult diagnosis pathway. The underlying mechanisms cause abnormalities in *N*-glycosylation on cell surface and secreted proteins, but patients present with a multitude of symptoms, including developmental delays, and definitive diagnosis relies on detection of abnormal glycosylation of the protein transferrin in the serum. Over 130 genes are implicated in this disease and next-generation sequencing is adding to the list, but discriminating pathogenic from non-pathogenic variants is laborious and requires complex model systems. Our longstanding interest in the enzyme lysyl oxidase (LOX) led us to study CDG because we performed a proximity ligation screen to identify proteins that transiently interact with LOX in the cell. LOX is a secreted protein that stiffens the extracellular matrix by cross-linking collagen and elastin. In 2017, we reported that LOX drives tumour progression by trapping the epidermal growth factor receptor (EGFR) at the cell surface, and we have worked with Caroline Springer, then the Director of the CRUK MI Drug Discovery Unit, to discover inhibitors of LOX for cancer treatment. Our proximity labelling screen identified OST48 as a protein that interacts with LOX. OST48 is a non-catalytic component of the oligosaccharyltransferase (OST) complex, a molecular machine that glycosylates cell surface and secreted proteins. OST48 is encoded by the gene *DDOST*, and DNA sequencing has revealed over 40 candidate mutations in *DDOST* in CDG patients, but only two are validated as clinically significant. The remainder are uncharacterised and designated as variants of unknown clinical significance. We used our discovery that LOX interacts with OST48 to develop a functional assay to test the biological activity of *DDOST* in cells. We confirmed that the two known clinically significant *DDOST* variants are inactive, identified two other inactive variants, and showed that the remaining candidates are active. Thus, we developed a robust assay that can explore the biology of the OST complex and provide a rapid functional test for candidate mutations to support genome sequencing in the diagnosis pathway of individuals with CDG.

Being Director of CRUK MI for nine years was a privilege, and it was pleasure being a Group Leader at the Institute for eleven years. We faced many challenges, not least the fire that forced us into temporary accommodation at Alderley Park and the pandemic that affected us all. However, we were equal to those challenges, and I am proud of what we achieved during my tenure. I am particularly pleased by the achievements of my own lab and thank the members of the Molecular Oncology group who, over the years, made important contributions to our understanding of cancer biology and improvements in patient care. We raised over £13m to fund our research directly and contributed to collaborative grants worth over £30m, which included a CRUK Grand Challenge. We published over 60 primary research papers, and 16 reviews, commentaries and book chapters. We published four patents and contributed to four biomarker-led clinical trials that seek to change clinical practice. Finally, we received several prizes and awards that recognised our work internationally.

I wish also to thank all the group leaders, postdoctoral fellows, students, and scientific officers at the Institute for the breakthroughs that drove our research strategy. They created an excellent research environment and culture in which our science could thrive. One of my aims when I became Director was to increase the Institute's international profile further and that required the Group Leaders to take on more travel, committee work, and roles in national and international scientific organisations. I thank all the Group Leaders, particularly Caroline Dive, the Deputy Director at the time, who accepted this challenge with gusto and helped to raise our profile. I am also very grateful to the management team whose dedication, loyalty and hard work kept the Institute running through the challenges, great and small. I especially thank Caroline Wilkinson (Chief Operating Officer) and Stuart Pepper (Chief Laboratory Officer) with whom I worked closely to provide an effective research environment. I also thank the members of the core facilities and Operations teams who, without fuss, make the wheels of the Institute turn. I thank The University of Manchester for its support, particularly in the aftermath of the fire. Finally, thank you to CRUK for providing the core funding that underpinned our activities, and also to the many other organisations (listed in Table 1) that also funded CRUK MI during my tenure as Director; you allowed us the freedom to follow the science and aspire to improve patient care, and I am very grateful for your generosity.

Early in 2023, CRUK MI will move into the new Paterson Building, and I wish you all the best for continued success in your new home. Truly, CRUK MI is a phoenix risen from the ashes. For me, the next chapter is equally exciting as I work with Caroline Springer and our other Oncodrug colleagues to discover new drugs to treat cancer and other diseases. We continue, of course, to collaborate with our former CRUK MI colleagues, particularly Iain Hagan, and no doubt together we will face many new challenges; to meet those I am sure that I will draw on my time and the lessons I learned at CRUK MI.

Publications listed on page 59

Table 1.
List of all additional funding sources that have supplemented the research across the CRUK Manchester Institute over the past 10 years since 2012.

Other organisations that have funded CRUK Manchester Institute since 2012			
Abbott Laboratories	CellCentric	Institut de Cancerlogie Gustave Roussy	Novartis
Academy of Medical Sciences	Christie Hospital NHS Foundation Trust	John Swallow Fellowship	Neuroendocrine Cancer UK
Amgen	Chugai	Kay Kendall Leukaemia Fund	Ono Pharmaceuticals
Angle Inc	Clearbridge Biomedicals	Leo Pharma Foundation	Pancreatic Cancer Research Fund
Astex Pharmaceuticals	CRT Pioneer Fund	Leukaemia & Lymphoma Research Fund	Parsortix
Astra Zeneca	David & Ruth Lewis Trust	Lung Cancer Research Foundation	Perfusion Biotech
BBSRC	Euclises Pharmaceuticals Inc	Medical Research Council	Pickering Leukaemia Research
Bioven	European Commission	Medimmune LLC	Prostate Cancer UK
Bloodwise	European Organisation for Cancer Research and Treatment of Cancer	Menarini Biomarkers Singapore	Roche
Boehringer Ingelheim	European Research Council	Merck	Rosetrees Trust
British Lung Foundation	Fondation ARC pour la Recherche sur le Cancer	Moulton Charitable Trust	Roy Castle Lung Cancer Foundation
Cambridge University	GlaxoSmithKline	My-T Bio Ltd	Taiho Oncology Inc
Carrick Therapeutics	Harry J Lloyd Charitable Trust	National Institute of Health Research	The US Department of Health and Human Services

SKIN CANCER AND AGEING

Institute Fellow
Amaya Virós

Postdoctoral Fellows
Timothy Budden
Karthik Mallela[1]
Cian Vyas[2]

Clinical Fellow
Sarah Craig[2]

Graduate Students
Shilpa Gurung
Pedro Durao

[1]Joined in 2022
[2]Left in 2022

The Skin Cancer and Ageing lab studies the mechanisms that underpin greater metastatic burden in skin cancer patients who are older. The incidence and mortality rates continue to rise, as does the proportion of the population who is over 50 years old. Importantly, many elderly patients with skin cancer develop other primary skin cancers that complicate 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 looking at why there is a survival discrepancy between old and young patients. We have focused efforts in tumour biology of elderly patients and published genomic signatures of why some tumours grow faster in the skin than others. Since then, we have discovered that the role of the ageing stroma has a greater effect on patient survival than the genetic differences we have found in the tumours.

First, we are following our previous findings that collagen density is a key predictor of patient outcome. We showed that collagen degradation in the aged dermis, following chronic UV exposure and damage to the connective tissue, inhibits melanoma invasion. A subset of patients who develop melanoma over very sun damaged skin, like the face, have a very good prognosis. These findings help explain why epidemiological studies have shown that melanomas arising over sun exposed sites have better survival than melanomas that arise over sun-protected sites. We showed that collagen has the necessary physical properties in connective matrices to allow cancer cell invasion. However, some tumours can reconstitute collagen synthesis at the edge of tumour growth, which then allows melanoma cells to squeeze in, invade, metastasise, and lead to early patient death (Budden et al, *Nature Communications* 2021). This year Tim Budden has looked at the mechanisms that underpin new collagen synthesis at the invasive front of tumours. Tim has shown, first that specific tumour cues in the bulk of the invasive primary melanoma will switch on collagen synthesis by local tissue fibroblasts. Second, he describes the mechanisms that distinguish extracellular matrix (ECM) remodelling in fibroblasts that have endured high levels of sun damage. Briefly, he shows UV damages dermal fibroblasts as well as collagen, and this effect is heterogeneous. Critically, some dermal fibroblasts retain the capacity to respond to tumour cues instructing new ECM synthesis. Finally, Tim is exploring the correlation between the rate of collagen degradation and collagen synthesis, and the recruitment of T cells to the invasive front of melanoma in the dermis. We are looking at animal model results and have exciting data in our human cohort of patients.

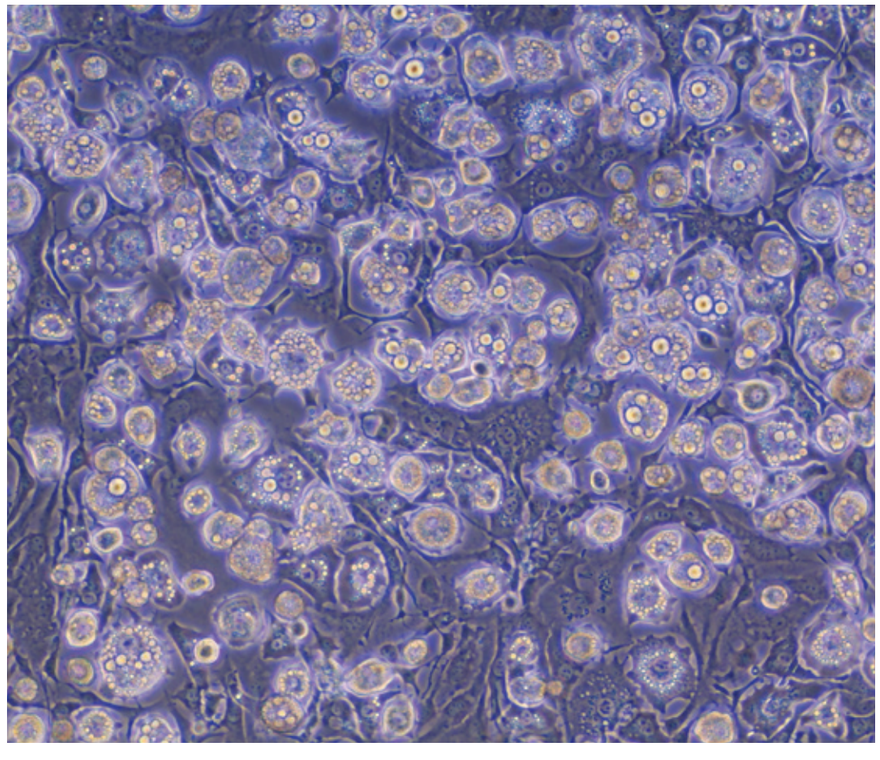
Our second scientific breakthrough this year has been building the *in vivo* models to study sex bias in immunotherapy response for inoperable, locally advanced, or metastatic cutaneous squamous cell carcinoma (cSCC) skin cancer. Our previous work shows aged men have more skin cancer compared to age-matched women. We published that men and male mice are more susceptible to primary aggressive and metastatic cSCC, and that female and male animals and women challenged with epithelial cell carcinogens (UV light) have different transcriptomic responses; women and female mice activate distinct transcriptomic pathways linked to improved cancer immunity. This protection was lost in immunosuppressed women, who have the same rate of disease as men (Budden et al, *Clinical Cancer Research* 2021).

Following this exciting preliminary data, Tim is looking at immunotherapy response differences by sex, and exploring sex-specific strategies of adjuvant therapy for cSCC prevention. We have initial models showing topical 5-fluorouracil and anti-PD1 *in vivo* have a strong sex bias favouring male response. We are working with clinical collaborators to collate clinical evidence of sex bias in cemiplimab treated patients and human tissue to validate our findings.

Lastly, Shilpa Gurung has studied how aged and young subcutaneous fat contribute differently to melanoma metastatic potential. She submitted her thesis this year and has continued to work on this project. Her work is under review. Her study and thesis describe the plethora of changes that occur in melanoma cells after exposure to different adipocyte-secreted lipids. We have found evidence that extracellular lipids can affect the metastatic course of disease. We will continue focusing efforts to exploring the role of lipid metabolism in primary melanoma. In parallel to this project, we will shortly submit for publication our work, in collaboration with the Sanz-Moreno Lab, led by Sarah Craig, who successfully passed her PhD viva this year. Sarah's work details one novel aspect of how over-the-counter antioxidants affect melanoma metastasis.

Finally, we have been working on a new project funded through CRUK's ACED scheme to look for biomarkers of melanoma progression in early-stage melanoma. This work will focus on human sample research from clinical practice and the use of imaging algorithms to predict patient outcome. We are collaborating with Oregon University to drive the study forward.

Publications listed on page 59



Brightfield image of differentiated 3T3-L1 mouse adipocytes. Differentiation is marked by the development of intracellular lipids (lipid bubbles inside the cell) upon administration of differentiation media.

Image supplied by Shilpa Gurung (Skin Cancer and Ageing).

STEM CELL BIOLOGY

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[1]Left in 2022
[2]Funded on an MRC Doctoral Training Partnership; joint with the Division of Developmental Biology and Medicine, University of Manchester
[3]CRUK Manchester Centre funded Clinical Research Training Fellowship

Cytotoxic therapy has been the standard of care over the last 30 years for acute myeloid leukaemia (AML). Unfortunately, more often than not it fails to cure patients, and the five-year survival rate is only around 20%.

Therefore, there is a pressing need to develop more specific and efficient therapies. 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. Our overarching goals are to improve patient outcomes by identifying and validating therapeutic targets for leukaemia treatment and developing robust protocols for in vitro production of clinical-grade blood cells for adoptive cancer immunotherapies.

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. A critical barrier to the widespread use of current CAR-T cell products is their autologous nature. These cellular products are patient-selective and therefore very costly and challenging to manufacture. In contrast, allogeneic cell products can be scalable and readily administrable. However, they face critical concerns of graft-versus-host disease (GvHD), a life-threatening adverse event in which therapeutic cells attack host tissues and rejection by host immune cells, limiting their antitumour efficacy. 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 from haematopoietic stem cells (HSCs), recapitulating adult haematopoiesis. Alternatively, they are also produced during embryonic development directly from haemogenic endothelial cells. We aim to understand the processes of generating HSCs and immune cells to ultimately replicate them and establish cell production platforms of these blood cells for therapeutic production.

Embryonic waves of haematopoiesis

Murine and human haematopoietic systems develop in the embryo in at least three spatiotemporally overlapping waves. The first wave, also called primitive haematopoiesis, gives rise at E7.5 in the extraembryonic yolk sac to primitive erythrocytes, primitive megakaryocytes, and primitive macrophage progenitors. This wave generates tissue-resident macrophages such as microglia found in the adult brain. The second wave, termed pro-definitive haematopoiesis, starts in the extraembryonic yolk sac vasculature at E8.25, where a specific subpopulation of specialised endothelial cells – haemogenic endothelial cells – give rise to haematopoietic cells through an endothelial-to-haematopoietic transition (EHT), a process unique to developmental haematopoiesis. This wave first gives rise in the extraembryonic yolk sac to erythro-myeloid progenitors (EMPs) at E.25 and then to the first lymphoid cells as multipotent lymphoid-myeloid progenitors (LMPs) in both extraembryonic yolk sac and intraembryonic para-aortic splanchnopleura region. The final wave of haematopoiesis, called definitive haematopoiesis, arises from haemogenic endothelial cells present in the dorsal aorta in the intraembryonic aorta-gonad-mesonephros (AGM) region. This wave mainly generates from E9.5 to E11.5 long-term multilineage adult engrafting haematopoietic stem cells (HSCs). HSCs also emerge in the vitelline and umbilical arteries and then seed the foetal liver, where they expand and mature. They next colonise the bone marrow, where they remain throughout life, and provide a continuous supply of all blood cells via adult haematopoiesis.

Overall, embryonic haematopoiesis is very different from adult haematopoiesis. In adult haematopoiesis, all the differentiated haematopoietic cells originate from HSCs, whereas in embryonic haematopoiesis, most of the differentiated haematopoietic cells originate independently from HSCs.

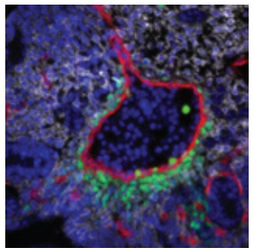

Figure 1.
Immunofluorescent staining of mouse E10.5 dorsal aorta (DA). RUNX1 (green), CD31 (red), PDGFRa (grey). RUNX1+ cells in the endothelial lining of the DA are hemogenic endothelial. Mesenchymal RUNX1+ cells, which support haematopoiesis, are abundant in the ventral subaortic region.

Investigating the generation of HSCs

Haematopoietic stem cells (HSCs) sit at the apex of the blood system and are powerful treatment modalities for cancer and blood malignancies. Understanding the molecular programmes underpinning their formation during embryogenesis is critical for the development of efficient protocols to generate and amplify HSCs *in vitro*. As indicated above, the first transplantable HSCs are generated in mice intra-embryonically in the region of the aorta-gonad-mesonephros (AGM) between embryonic day (E) E10.5 to E11.5. Within this limited time window, haemogenic endothelium cells transition to non-adherent haematopoietic cells via a process termed the endothelial-to-haematopoietic transition (EHT). Morphologically, the EHT produces intra-aortic haematopoietic clusters (IAHC) protruding from the endothelium into the lumen of the dorsal aorta and other major arteries. Although HE is established as the cellular source of the first blood cells *in vivo* and *in vitro*, our knowledge of the molecular and cellular mechanisms regulating HSC emergence from HE remains incomplete.

The transcription factor RUNX1 controls the initiation and completion of EHT and is essential for establishing definitive haematopoiesis. Downstream RUNX1 target genes *Gfi1/Gfi1b* are critical regulators of EHT that recruit histone-modifying complexes to silence the endothelial programme in HE. The sequential expression of the transcriptional repressors GFI1/1B marks distinct stages of the EHT. GFI1 is expressed in HE, while GFI1B is mainly found in IAHC. In contrast, RUNX1 expression is detected during all stages of EHT. Additionally, RUNX1 expression is also found in subaortic mesenchyme. The exact identity and the potential role of these mesenchymal RUNX1+ cells are currently unknown.

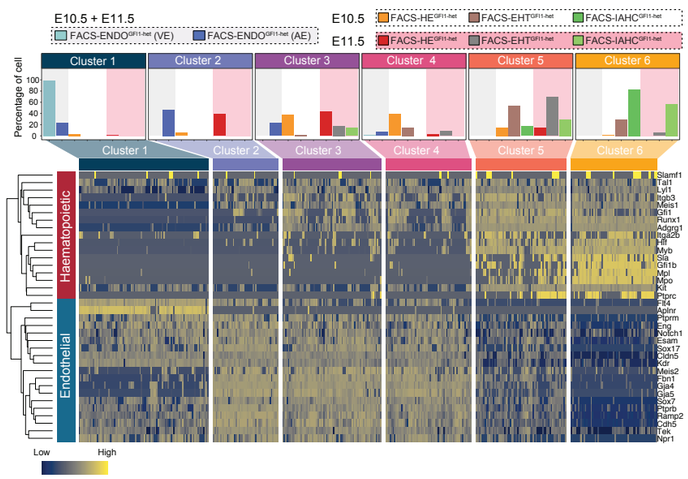

Figure 2.
Distribution of FACS isolated populations in each cluster with background shaded according to the embryonic days (top). Heat map illustrating haematopoietic and endothelial genes expression all *silico* clusters (bottom). Genes were clustered based on Pearson correlation while the cells were grouped according to the *in silico* clusters. VE: venous endothelium, AE: arterial endothelium.

Single-cell RNA sequencing (scRNA-seq) is a powerful tool to profile developmental pathways. Although this can now be done globally on whole organs and organisms, understanding pathways involving rare cell populations still greatly benefits from targeted approaches using enriched cell populations. We and others have previously taken the latter approach to start resolving molecular events leading up to HSC formation in the murine AGM. Much emphasis has been on the final steps of HSC commitment within the IAHC. However, the intricacies of dorsal aortic HE differentiation before the initiation of EHT are not well defined. Reasons for this lack of clarity are the rarity of the HE population but also the lack of suitable cell surface markers for HE purification.

To gain insight into how dorsal aortic HE progresses towards EHT, we used two transgenic reporter mouse models (*Runx1bRFP* and *Gfi1Tomato/Gfi1bGFP*) to isolate and profile a wide variety of phenotypic HE. We reasoned that targeted scRNA-seq of Runx1b:RFP+ and Gfi1:Tomato+ phenotypic HE populations are essential to profile them in-depth as they represent only a tiny fraction of the total CDH5+ endothelial populations in the AGM (approximately 6% and 0.6% respectively, Figure 1). We also profiled Runx1b:RFP+ AGM mesenchymal cells. To maximise data recovery, we adopted a full-length scRNA-seq protocol. The resulting dataset contains nearly 1,200 FACS-sorted cells, covering nine E10.5 mouse AGM populations.

Our data captured a detailed HE differentiation continuum, covering pre-HE and HE stages, giving rise to the first HSCs. We found that this continuum is marked by angiotensin-I converting enzyme (ACE) expression and that pre-HE and HE can be discerned based on cell cycle status. Additionally, we established that the rare Runx1b:RFP+ sub-aortic mesenchymal population supports haematopoiesis and consists of smooth muscle and PDGFRa+ cells. Altogether, our finding provides new insight into the generation of HSCs, and we have made our high-resolution single-cell data set covering HE and its surrounding niche in the mouse dorsal aorta accessible online.

Investigating the generation of other blood lineages

We are now leveraging our expertise in evaluating the generation of HSCs in the AGM to examine at the molecular and cellular levels how EMPs and LMPs are produced in the earliest wave of haematopoiesis. Comparing and contrasting both processes will provide new clues to produce HSCs and immune cells for cellular immunotherapies.

Publications listed on page 60

SYSTEMS ONCOLOGY

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Seung Hyun Lee[3]

[1]Left in 2022
[2]Joint with Caroline Dive, CBC and Juan Valle, Christie NHS FT
[3]Joint with Juan Valle, Christie NHS FT and Lucy Foster, MFT

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 signalling pathways by which the tumour cells conscript the 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. Collectively, these results suggest that interactions between tumour and host can be both tumour restrictive and tumour promoting. Recent advances in single cell approaches have accelerated the characterisation of tumour host cell populations, where specific subsets have been identified across both immune and mesenchymal cells.

We recently used single cell mass cytometry to annotate the microenvironmental composition in a commonly used murine model of PDA (pdx-1 Cre; $KRas^{LSL\text{-}G12D/Wt}$; $p53^{LSL\text{-}R172H/Wt}$). 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 CAFs distinguished by the expression of CD105 (Endoglin). Isolation and characterisation of both $CD105^{pos}$ and $CD105^{neg}$ CAFs revealed distinct expression of immune-regulatory signals. Moreover, the two stromal subsets expressed CD105 in a noninterchangeable manner and responded differentially to most exogeneous signals tested, suggesting the subsets may have distinct functional roles in the tumour microenvironment. Indeed, tumour cells co-implanted with $CD105^{pos}$ fibroblasts grew only slightly faster than tumour cells implanted in isolation, suggesting a tumour permissive role of $CD105^{pos}$ fibroblast. In contrast, co-implanted $CD105^{neg}$ fibroblasts restrict tumour growth. This effect is dependent on functional immunity. Moreover, we identified both $CD105^{pos}$ and $CD105^{neg}$ fibroblasts in all normal and tumour-bearing tissues analysed. These data demonstrate that tumour permissive and restrictive fibroblast subsets co-exist throughout PDA development and provide molecular insights into the signals governing tumour development.

Development of a fully synthetic 3D model of human pancreatic cancer

Although tumour cells constitute less than 15% of the tumour volume in patients, most *in vitro* models do not support the study of tumour cells within an equally complex microenvironment. To improve how tumours can be modelled *in vitro*, we have worked with Prof Linda Griffith (MIT) and Prof Martin Humphries (UoM) to adapt a fully synthetic scaffold that supports 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 engage 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. We observe that tumour cells exhibit different growth patterns 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.

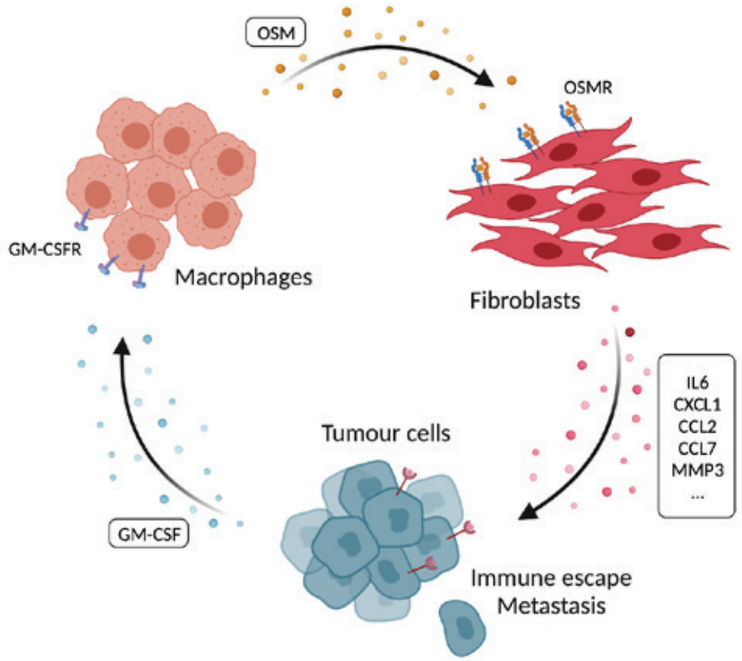

Figure 1.
Tumour and host interactions drive immune escape and metastasis. Tumour cells recruit macrophages by GM-CSF, which in turn secrete OSM to activate inflammatory signalling in pancreatic fibroblasts. The altered signalling milieu drives immune escape and metastasis.

Created with BioRender.com

Tumour stromal interactions control tumour growth and metastasis

In deciphering interactions between tumour and host, emphasis has commonly been on direct interactions. We have recently described an additional control layer between host cell populations. Specifically, macrophages recruited to PDA secrete Osm, which in turn engages an inflammatory signalling programme in Osmr expressing pancreatic fibroblasts. This inflammatory programme engages tumour cells directly by activating migration and EMT to drive metastasis. Thus, tumour cells implanted in animals deficient for *Osm* exhibit reduced metastatic capability. Moreover, the immune microenvironment in *Osm* deficient animals also appears more primed, suggesting that targeting of Osm may activate an anti-tumour immune response.

Publications listed on page 60

TRANSLATIONAL LUNG CANCER BIOLOGY

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Scientific Officer
Anthony Oojagger

Graduate Student
Julia Ogden

The Translational Lung Cancer Biology group addresses the barriers to progression in lung squamous cell carcinoma medicine. Lung squamous cell carcinoma (LUSC) is an aggressive type of lung cancer that originates in bronchial basal cells with limited therapeutic options. Apart from chemotherapy, only immunotherapies result in marginal improvement of survival in LUSC patients. 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.

Forty percent 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. Hence, preventing deaths by LUSC requires improving therapeutic modalities and early detection methods. 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 (Figure 1C). However, existing LUSC models do not recapitulate those complexities, and this is a barrier to reversing the dismal landscape of the disease.

Modelling the complexity of lung squamous cell carcinoma (LUSC)

Lung squamous cell carcinoma has been historically difficult to model using genetically engineered mouse models (GEMMs) and to this date they are not sufficiently developed. 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, LUSC genomics is much more complex than *SOX2* amplification and additional modelling strategies are needed to develop models that represent this heterogeneity.

There is not a single targetable pathway that dominates the genomic landscape of LUSC. 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.

Understanding this extensive heterogeneity in LUSC involves addressing four key questions:

- Can these pathways drive intrinsically LUSC tumourigenesis?
- Do they cooperate in driving LUSC tumourigenesis?
- Are LUSC cells addicted to these pathways?
- Are these pathways mutually dependent?

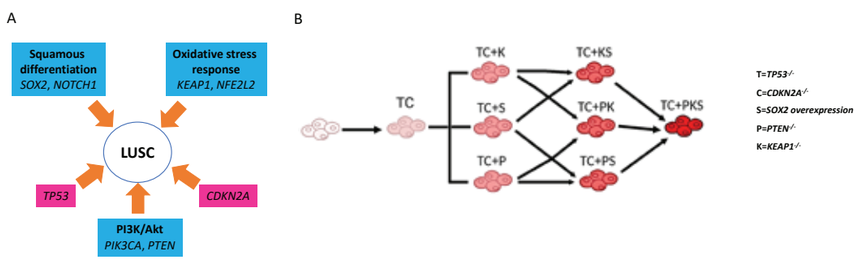


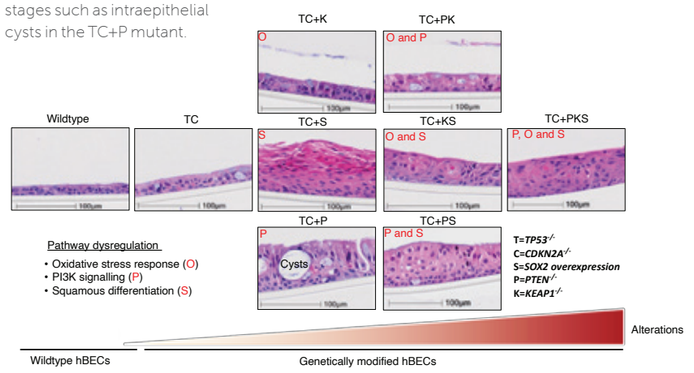
Figure 1.
A Summary of the most relevant tumour suppressors (pink boxes) and pathways (blue boxes) involved in LUSC development, with examples of pathway components altered in LUSC. **B** Summary of the mutant HBCs that we are developing. All mutants contain *TP53* and *CDKN2A* mutations (almost universally inactivated in LUSC) and all possible combinations of the three pathways in order to assess individual and combined effects.

Figure 2.
Haematoxylin-eosin-stained sections or air-liquid interface (ALI) HBC cultures from wild-type and the mutant HBCs following the strategy shown on Figure 1B. ALI cultures are bronchial organotypic cultures widely used to investigate bronchial epithelial morphology. The morphology of wild-type and TC mutants is very similar, whereas addition of *SOX2* overexpression induces squamous metaplasia, a type of low-grade preinvasive lesion. *KEAP1* and *PTEN* inactivation induce an epithelial morphology characterised by loss of apical-basal asymmetry, consistent with high grade premalignant lesions. Other allele combinations induce near normal morphologies or other morphologies with unclear correlation with premalignant stages such as intraepithelial cysts in the TC+P mutant.

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 availability of more relevant mouse models will increase the number of projects involving animal research.

Building a human LUSC model by genetic manipulation of human basal cells (HBCs) as an alternative to murine models

Human basal cells – the LUSC cells of origin – are a feasible and versatile alternative to mouse models to interrogate LUSC evolutionary history and develop multiple allele combinations that reflect LUSC inter-patient heterogeneity. Current methodologies permit efficient expansion of HBCs, genome editing and development of organoids mimicking bronchial morphology. Using HBCs in combination with organotypic cultures (organoids) and other *in vitro* assays, we can disentangle how driver alterations induce epithelial perturbations indicative of LUSC initiation and progression.



Using HBCs to model LUSC presents multiple advantages beyond the replacement of mouse models. Importantly, modelling inter-patient heterogeneity is simpler in HBCs, as they can be easily manipulated. They reflect human diversity better than mouse models and constitute a more adequate system to investigate the effect of exposures, mainly smoking.

In the Translational Lung Cancer Biology laboratory, we have designed, implemented and characterised a genome engineering strategy whereby, using genetically modified HBCs, we intend to capture a large extent of the inter-patient heterogeneity that we observe in LUSC patients (Figure 1B). To do this, we have generated increasingly complex mutant HBECs bearing inactivating mutations in the tumour suppressors *TP53* and *CDKN2A* 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).
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 2C). Addition of *PTEN* and *KEAP1* mutations results in complete loss of epithelial polarity consistent with transition to high-grade preinvasive stages. Our observations have enabled us to define a genetic roadmap that describes the LUSC developmental stages, from a normal bronchial epithelium to high-grade premalignant stages.

Design and development of XTABLE, an open-source tool to interrogate the transcriptomes of endobronchial lesions

In collaboration with the Bioinformatics and Biostatistics Team in the Cancer Biomarker Centre (Matt Roberts, Alastair Kerr) we have developed XTABLE, an open source bioinformatic application that enables the user to investigate the biology of the LUSC premalignant stages (Figure 3). To this end, XTABLE integrates multiple modalities of statistical analysis (gene-centred analyses, pathway and ontology analyses, custom made transcriptional signatures, etc.) and four different publicly available transcriptomic databases of LUSC premalignant lesions. XTABLE allows the stratification of samples by stage, progression potential and surrogates of chromosomal instability (CIN signatures). Guidelines for the download and use of the application will be published in *Elife* in early 2023. The application can be downloaded from https://gitlab.com/cruk-mi/xtable.

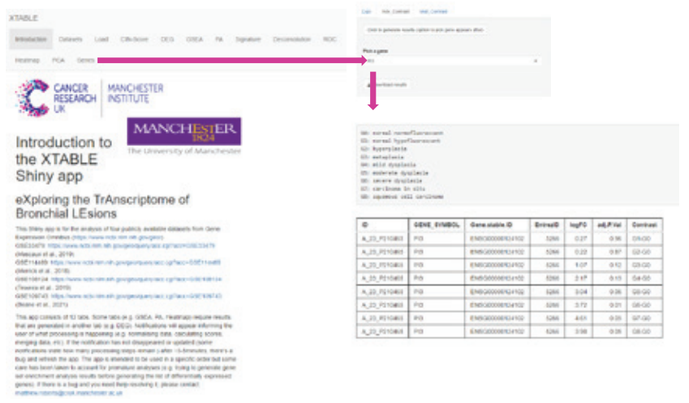


Figure 3.
An example of the use of XTABLE to interrogate endobronchial lesion transcriptomes.
After selecting the cohort of interest in the 'Dataset' tab, the expression of a gene of interest can be interrogated using different modalities of analysis. These functions retrieve the normalised expression of the gene of interest for all samples in the study (Expr function), compare the different groups of samples defined in the study (*Indiv_Contrast* function, shown in the figure), or groups defined by the user (*Mult-Contrast* function).

www.cruk.manchester.ac.uk/Our-Research/Translational-Oncogenomics

TRANSLATIONAL ONCOGENOMICS

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[1]Joined in 2022
[2]Left in 2022

Since the 1990's the number of UK residents diagnosed with prostate cancer each year has steadily risen. The latest figures show that there are now over 52,000 new diagnoses per year and incidence rates are increasing amongst younger men. Managing this widespread disease continues to be a major challenge for health professionals due to the clinical heterogeneity seen between patients.

The majority of men present with disease that is localised to the prostate and is potentially curable by either surgery or radiotherapy or indeed, by active surveillance alone. However, in some patients with high-risk disease (men with a 15-fold increased risk of dying of prostate cancer), the disease will progress to become metastatic and incurable. The challenge faced by clinicians is to therefore accurately assess the risk of death for each patient and triage them to best treatment.

Our research focusses on the genetic and microenvironmental features (i.e., hypoxia) shared by high-risk, aggressive prostate cancers. 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.

High risk prostate cancers in families with germline BRCA2 mutation

Over recent years there has been a growing appreciation of the role of DNA repair genes in the biology of prostate cancer. In-depth analyses of the prostate cancer genome have shown that somatic mutations in DNA repair genes are relatively frequent and are more common in incurable, castrate-resistant disease (mCRPC) than in primary cancers. Concordantly, it has been shown that men carrying germline mutations in such genes are at a higher risk of developing prostate cancers that progress to become metastatic. The most frequently observed DNA repair defect is germline mutation of the breast cancer susceptibility-2 (BRCA2) gene, which confers an 8 to 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.

Given the high-risk nature of these germline-driven cancers, model systems based on prostate cells harbouring BRCA2 deficiency are needed for pre-clinical analysis. However, BRCA2 is required for cellular proliferation and viability, and this essential nature has hampered the development of cell-level genetic models which, whilst lacking BRCA2 function, can nevertheless be cultured and stably expanded. The development of prostate epithelial cell models engineered to undergo BRCA2 loss of heterozygosity (LOH) would be advantageous. This would allow for detailed probing of BRCA2 tumour suppressor functions and shed new light on how tumour genomes evolve over time when homologous recombination (HR) is impaired. Such models could also be used to address pertinent clinical questions. For example, given numerous trials have demonstrated the utility of PARP inhibitors or platinum drugs in targeting BRCA2 deficient tumours, there are now questions as to how best predict which patients are likely to respond or be resistant to such treatments.

Recently, we have successfully immortalised prostate epithelial cell cultures (PrEC) derived from BRCA2 germline carrier patients undergoing prostatectomy at the Christie NHS Foundation Trust. To do this, we employed a methodology involving stable expression of the human telomerase (hTERT) gene combined with careful passaging of the cells over several months. Although laborious, this method leads to the outgrowth of immortal cells with a relatively unperturbed and stable genome. Having achieved this milestone, we've gone on to use gene-editing techniques to target the wild-type BRCA2 allele in the carrier cells and thus generate a unique BRCA2 LOH model system. Importantly, these BRCA2 mutant cells can be cultured permitting the study of drug responses, DNA repair and the long-term consequences of HR deficiency for genome stability. We hope these new cellular models will aid development of new, personalised treatments and provide insights into resistance mechanisms where germline deficiencies in DNA repair are implicated.

Hypoxia and links to genetic instability

In many solid tumours, regions of acute or chronic hypoxia develop as proliferating tumour cells outstrip the ability of a poorly organised vasculature to supply oxygen. Hypoxia is considered to be an adverse feature, present in high-risk prostate cancers, and the relationship between oxygen depletion and radio-resistance has long been appreciated. Moreover, our work has shown previously that tumour hypoxia is frequently correlated with high rates of genome instability and that both features are often present in tumours that recur following treatment.

To better understand the relationship between hypoxia and metastatic spread, we have initiated the HYPROGEN study (see Figure 1) in which patients receive the hypoxia tracer molecule, Pimonidazole prior to undergoing either radical prostatectomy or biopsy. This allows the detection of hypoxic areas within tissue specimens and will be combined with the latest genomics and spatial transcriptomic analyses to understand in greater detail the drivers of metastasis. We are indebted to all the patients participating in the study, including those with early metastatic disease who have voluntarily donated bone biopsy material. These unique samples will allow us to match genomic features present in these metastatic lesions with those in the primary tumour to provide new insights into the metastatic process. Recruitment to HYPROGEN is now well underway and we acknowledge the valuable contribution made by our colleagues working in the Christie NHS Foundation Trust, MCRC Biobank and CRUK MI core facilities in ensuring this unique, multi-disciplinary study is a success.

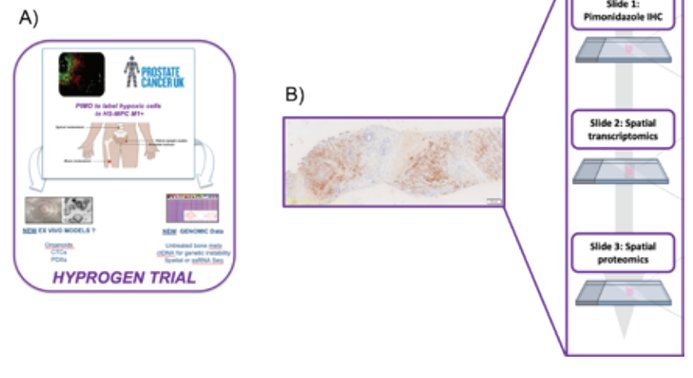
In addition to our work with clinical samples, we are also carrying out a detailed analysis of the hypoxic-response in cellular systems that employ hTERT-immortalised PrECs. To achieve a comprehensive picture, we have taken a 'multi-omic' approach and collected proteomic, transcriptomic, metabolomic and methylomic data. These data represent the hypoxia phenotype observed at the molecular level and we hope this will provide clues as to the mechanistic links between hypoxia and genomic instability.

Additionally, given the co-occurrence of genome damage and hypoxia in tumours, we are also investigating whether the metabolic response to hypoxia is impacted by the presence of oncogenic mutations. This study could also reveal promising new ways to track hypoxia in high-risk patients. Novel hypoxia biomarkers are much needed to better stratify patients undergoing hypoxia-directed therapies as part of clinical trials. Although strategies to better target hypoxic tumours already exist, such approaches are unlikely to fulfil their promise unless practitioners can reliably identify those patients most likely to benefit. We are currently interrogating this large dataset using multifactorial analysis techniques in partnership with Prof David Wedge (Division of Cancer Sciences, The University of Manchester). We believe detailed studies such as this will help identify the novel hypoxia biomarkers used to direct future trials.

We have enjoyed great success in using gene expression as a marker to measure hypoxia in tumour samples. Until recently, we have used bulk RNA preparations as the source of transcriptomic information, which allows us to assign each tumour a single, overall hypoxia score. However, hypoxic regions are rarely uniform in nature. Loco-regional differences in oxygenation can occur in tissues as growth and re-modelling processes take place, and such effects will be masked in transcriptomes obtained from entire tissue biopsies. Recent advances in spatially resolved transcriptomic techniques now permit the analysis of gene expression across multiple, microscopic capture regions within a tissue section. In this way, hypoxia-dependent gene expression can now be mapped and coordinated with important clinic-pathological features. Working with our colleagues in the CRUK MI core facilities, we have established a workflow to quantify hypoxia in fixed prostate cancer tumour specimens using the 10X Visium platform. We have demonstrated the utility of this technique in quantifying hypoxia across microscopic gradients in which oxygenation levels, as measured by a gene expression score, varied significantly within 100µm. Furthermore, new computing techniques permit the use of transcriptomic data to infer the presence of copy-number changes at the DNA level, opening up the exciting possibility of examining *in situ* the relationship between hypoxia and genome instability. We will now deploy these methods to samples collected as part of the HYPROGEN trial in order to maximise the information we obtain from these precious patient samples.

Publications listed on page 60

Figure 1.
A The HYPROGEN trial aims to characterise the role of hypoxia in driving the early metastatic spread of tumours. Patients with oligo-metastatic disease are recruited to the study prior to receiving any treatment. Hypoxia can be assessed by means of the tracer molecule, pimonidazole, which is administered before biopsies are taken from both the primary tumour and selected bone metastases. Genomics and transcriptomics will then be applied to provide insights into the relationship between hypoxia, genomic instability and metastatic spread. **B** A needle core prostate biopsy from a patient recruited to the HYPROGEN trial. The sample shown has been stained by immunohistochemistry to detect pimonidazole adducts and illustrates the loco-regional distribution of hypoxic areas. We believe this degree of heterogeneity is typical of high-risk primary prostate tumours and highlights the need for spatial analysis to fully understand the impact of hypoxia on tumour metabolism and metastasis.



CANCER RESEARCH UK MANCHESTER INSTITUTE

RESEARCH SERVICES

RESEARCH SERVICES

Chief Laboratory Officer
Stuart Pepper

During 2022, there has been considerable effort in preparing for our relocation to the new building early next year. Some significant procurements have been made to provide new equipment, particularly for the Biological Resources Unit and the Scientific Computing data centre; in total over £5million has been invested in new equipment for the building. Aside from planning the move, we have also carried out recruitment, with extra posts recruited into three core facilities.

Chief Laboratory Officer
Stuart Pepper

There have been some changes in our FACS facility this year with the retirement of Jeff Barry, one of our long serving members of staff. Jeff has made a great contribution to the Institute over many years, and I wish him well in his retirement. Toni Banyard has now stepped up to become the manager of the team and has already made an impact with the purchase of a major upgrade of our mass cytometer and recruitment of new staff.

Whilst the building has neared completion, the core facilities have been busy expanding the support to our scientists with new applications, as detailed in the sections below. Expansion has included the introduction of new applications, such as the new Data Independent Acquisition applications in mass spec; procurement of new equipment, such as the GeoMX platform to support spatial genomics applications; or the expansion of existing equipment, such as the cytometer upgrade in FACS to provide greater multiplex capability and greater throughput.

Overall, it has been a very successful year with the core facilities ready to take advantage of the further opportunities for collaborative working that the new building will provide next year.

Biological Mass Spectrometry
Duncan Smith, Yvonne Connolly

The Biological Mass Spectrometry facility has seen significant growth in the use of both isobaric tags for protein profiling and targeted quantification across the Institute this year. To maximise both the utility and performance of tandem mass tagging, we have introduced TMTpro to our portfolio of options. This technology allows the profiling of up to 16 different protein samples within any experiment. In concert, we have developed and optimised a novel gas phase fractionation method that streamlines the TMT approach whilst also improving sensitivity and reducing cost.
The team has successfully developed a Parallel Reaction Monitoring (PRM) targeted approach to facilitate the profiling of a panel of transcription factors in human bone marrow from AML patients and healthy donors in collaboration with the Leukaemia Biology group and the Computational Biology Support facility. This project demonstrates the power of targeted analysis and can be transferred to panels of proteins of interest without the requirement of having to generate specific panels of antibodies. The field of Data Independent Acquisition (DIA) mass spec has many advantages over the classic Data Dependent Acquisition (DDA) approaches we have used for the last two and a half decades. DIA has the potential to be more comprehensive and offers more accurate quantitation than the conventional DDA approaches. However, challenges with data handling and processing have limited the utility of this approach for the last few years, making it of restricted utility within a core facility. We have seen a step change in the capability of commercial software to address these limitations over the past year. We have therefore recently invested in the leading package for DIA analysis to unlock its potential within the Institute. Over the coming year, many of our protein profiling approaches will be transitioned to DIA approaches, bringing significant enhancements to what can be achieved by our research groups.

Biological Resources Unit
Transgenic Breeding
Team Leader: Jennifer Hughes
Irana Bakhtiari-Cunado, Daniel Bennett, Tim Bloor, Carl Conway, Wesley Moore, Victoria Preston, Rose Storey, Martin Vincent

The BRU Transgenic Breeding Team breeds mice for CRUK Manchester Institute researchers under the authority of a central breeding project license held by the team manager. The team provides husbandry, pairs mice for breeding, monitors timed matings, records and weans litters, takes ear biopsies for genotyping and identification purposes, manages the outsourced genotyping and genetic background monitoring services, translates and transfers genotyping results, checks lines with deleterious phenotypes for onset of symptoms and provides the Named Animal Care and Welfare Officer (NACWO) service for CRUK MI mice in the facility. As well as breeding particular transgenic lines for specific researchers, we also batch breed immune compromised NSG mice, which the BRU Experimental Team then allocate for use as required. Having completely refreshed the breeding colony in 2021, this year we went on to produce the majority of NSG mice used for tissue and experiments within the Institute, resulting in a welfare improvement for these mice due to the shorter transport times between breeding and experimental facilities.

The breeding facility is housed in a clean unit with a high health status and is kept free from common mouse pathogens. We regularly screen the mice to check that no infections have been introduced and now only use non sacrificial sampling methods for this purpose. In order to protect this high health status, new transgenic lines coming from external sources have to be transferred into the facility as either embryos or sperm by the GEMM team, and then thoroughly health screened to ensure that the resulting offspring are specific pathogen free. At present mice required by researchers are transferred in weekly shipments to the BRU Experimental Team at Alderley Park upon request; after transfer a minimum of one week acclimatisation is required before mice can be enrolled in experiments.

Eight staff members currently provide day-to-day care for 74 different transgenic mouse lines that are spread across approximately 900 cages in a facility located within the main university campus. Our rooms in the facility are covered directly by the CRUK MI Establishment License, meaning that we benefit from being able to use the same National Veterinary Service as the team at Alderley Park, and allowing consistency of practice. In accordance with Home Office requirements all of our mice are closely monitored in order to ensure high welfare standards.

Images left to right: Building a relationship; Mouse in its house. These photos are two of the winning photos submitted by Biological Resources Unit animal technicians as part of Institute of Animal Technology (IAT) technician's month celebrations.

Images supplied by BRU technicians.

Over the last year the number of mice used in production and supply to CRUK MI researchers has increased by 4.5% compared to the previous year. Cage numbers have however been reduced, due to both increased efficiency of use resulting in a decreased duration of holding animals within the facility, and also because of a reduction in the number of animals which are either singly or pair housed. The total number of live breeding lines was reduced by 10.6% overall in 2022, despite being able to introduce 25 new lines for the researchers. The lines closed were mainly those belonging to research groups leaving the Institute and the new breeding lines include some that have either been rederived in or produced by the GEMM Team, and others that have been generated by crossing existing lines.

Experimental Services

Team Leader: Lisa Doar

Lisa Dique, Jo Roberts, Laura Dean, Rachel Walker, Eirini Syemon, Tom Bosley, Emma Playle, Jacqui Clayton, Lewis Woolley, Diane Beeston, Lisa Flynn, Jacek Kruza[1], Gary Cooke, Pat Ellison

[1]Left in 2022

2022 got off to a slow start in Q1, but the rest of the year has been extremely busy for the Experimental Team. The two new team members who joined at the end of 2021 have settled in well and have developed their technical skill sets over the course of the last year, which has allowed us to continue to be flexible in supporting the research groups with their *in vivo* work.

Although some minor refinements still need to be made, we have set up three new models this year. Image guided injection of tumour cells into the bladder is much less invasive than our current bladder cancer model. The final refinement we are still working on here is trying to encourage the mice to empty their bladders pre-injection, which makes the injections much easier to perform.

Intra-tracheal inoculation of cells directly into the lung is the second method, which should give better tumour development than our current method. The third model is photoconversion of cells using UV light so we can track the destination of migrating cells. For this model, we had to alter the positioning of the light and the depth of tumour implantation, but we are now starting to see some promising results.

There has been many new students and researchers requesting training in a range of techniques this year. We have expanded the pool of trainers in the BRU team and streamlined our procedures for management of training to improve the efficiency of our training processes. The main improvement was the introduction of a dedicated mailbox for training so that requests are clearly visible and get processed promptly.

Work on the new BRU facility has been significant this year. All the new equipment we ordered has been delivered, installed and commissioned. The facility is almost completed and looks fantastic. It consists of five holding rooms of different sizes, a quarantine and containment room, 10 different types of procedure rooms, which will allow us to carry out a range of work from routine dosing to imaging, X-ray irradiation, UV-irradiation and tissue sampling. We also have a dedicated surgery suite with three theatres. The cage wash area has a robot to dismantle the cages, which is an improvement from a manual handling perspective and reduces the risk of exposure to animal allergens, which is an important consideration when building a new animal research facility. We also have a large cage washer, autoclave and decontamination chamber to keep everything in the facility as sterile as possible.

Although the animal areas are at basement level, the changing rooms and staff break out area in the new BRU are on the ground floor, which means we have access to natural light. The ceilings are much higher, and the corridors are wider, so the whole area feels brighter and more spacious than the old facility. The process of designing and kitting out the facility has been intense over the last few years but also highly rewarding and will be especially pleasing next year when all the hard work comes to fruition and the facility starts to come to life.

Flow Cytometry

Antonia Banyard, Jeff Barry[1], Yosra Elagili[2]

[1]Retired in 2022, [2]Left in 2022

The Flow Cytometry facility has been going through some major changes. Toni Banyard was appointed the manager in April 2022, following the retirement of former facility manager Jeff Barry.

Since this management change, the facility has procured the latest upgrade of mass cytometer allowing for overnight runs of samples to maximise its capabilities. This instrument is currently involved in organoid analysis, clinical trial samples and bone marrow samples to better understand the immune interaction within the tumour microenvironment by labelling cells with up to 50 different markers. We are working closely with the Computational Biology Support and Scientific Computing facilities for the downstream analysis.

Flow cytometry is a technology that is pivotal to all our research groups in the Institute and as such the need for multiple platforms is being realised, with the procurement of a 24-colour analyser to complement our already impressive portfolio of instruments. This new cytometer will benefit our researchers by reducing the time involved and maximising the samples, along with access to state-of-the-art software for easy analysis.

We are also upgrading our sorting platforms with the procurement of a user-friendly sorter, which can be utilised by researchers for slightly more simple panels, and incorporating fluorescent reporters, again reducing the time constraints on our researchers.

The facility also has a two-camera imaging cytometer, which is being used by our research groups for analysis such as phagocytosis, where dendritic cells engulf cancer cells pre and post radiotherapy, and quantifying γH2AX in clinical breast samples post radiation to assess its effectiveness on varying genetic backgrounds.

The Flow Cytometry facility continues to grow in strength in terms of the equipment available but also in the training provided to the researchers on the hardware and software. Further, the facility aims to be involved at the start of projects to ensure maximum information can be attained from the samples and to create more efficient experiments.

We are now at the end stages of the move back to our original site in Withington and looking forward to being back amongst our colleagues at the OCRB and the Christie NHS Foundation Trust.

Genome Editing and Mouse Models

Natalia Moncaut, Athina Papaemmanouil, Lauren Street

The Genome Editing and Mouse Models (GEMM) core facility is an advanced technology platform responsible for providing new genetically engineered mouse models. Working together with researchers at CRUK MI, GEMM delivers strategic advice to generate forefront cancer mouse models to study mechanisms of tumour initiation, progression, and response to therapy.

The way we understand the biology of tumour microenvironment has been transformed by single cell RNA sequencing (scRNAseq). This approach provides a higher resolution of the transcriptional state of individual cells and allows the identification of new cell populations with potential functions in normal and transformed tissue. Based on the transcriptome information, we are generating several mouse models to specifically modify these different cell subsets and understand their function within a variety of tumours. Conditionally expressing diphtheria toxin- or fluorescent marker-based approaches, the new mouse models will allow researchers to specifically ablate or label these cells. Combining the unprecedented possibility of introducing precisely and efficiently modifications into the mouse genome with the exhaustive transcriptomic information provided by scRNAseq allows us to design new experimental models to strengthen our understanding of cancer biology in ways unimaginable before.

We continuously work to maintain a safely archived stock of all the mouse strains being bred at CRUK MI. Sperm and embryo cryopreservation are performed routinely, providing some insurance against loss of strains caused by adverse events, such as breeding failure, genetic drift, environmental adversities, and disease outbreaks.

Histology

Garry Ashton, Caron Abbey, Deepti Wilks (Haematological Malignancy Biobank)[1], Nicola Tonge, David Millard, Amy Lawrence[2], Peter Magee[2]

[1]Left in 2022
[2]Joined in 2022

The Histology core facility continues to offer a full range of both routine and advanced histological services that underpin oncology research across the CRUK MI, allowing basic and translational research groups to adopt various tissue-based experimental approaches.

In 2022, recruitment continued with a new scientific officer appointed to help develop the routine and specialised services offered. Continued focus on the training and professional development of staff ensured the unit continues to be at the forefront with technological developments whilst also offering a comprehensive and flexible service relevant across all research themes.

In routine practice, both human and mouse tissue, in addition to organotypic assays, spheroids, agar plugs and cell pellets, continue to be evaluated together with fresh vibratome tissue sections (50–250µm) for *ex vivo* cultures of tumours to evaluate and develop three dimensional studies. Requests for special stains have included Masson Trichrome, Picro Sirius Red, Alcian Blue, Cresyl Violet and PAS.

The facility has continued to play a key role in the development of sophisticated labelling techniques. Multiplex mRNA *in situ* hybridisation, immunofluorescence and combinations of protein and RNA labelling have been employed. Automation has resulted in these techniques becoming popular. In addition, the facility has been instrumental in the development and optimisation of high number spatial phenotyping allowing for the study of cellular interactions. Focus on maximising the use of precious tissue samples has allowed high numbers of samples to be analysed consecutively. Spatial transcriptomics – molecular profiling on a tissue section – has been evaluated and a workflow developed in collaboration with the Molecular Biology and imaging core facilities together with the Translational Oncogenomics group. The success of this work has resulted in several other groups eager to employ this technology in their research.

Both the Leica and Roche IHC platforms ensure consistency, reproducibility and standardisation allowing for access to high throughput routine immunohistochemistry, antibody validation and troubleshooting services, which again have proved popular in tissue expression evaluation and the phenotyping of CDX models.

Research projects involving the use of biobank material processed through the facility remains high. A dedicated scientific officer is responsible for ensuring the unit is compliant with current human tissue legislation.

Laser capture continues to prove popular with several groups using the system over the year. Protocols continue to be developed to improve the quality and quantity of the material captured and extracted for downstream analysis.

Tissue microarrays allow for high sample number throughput and analysis. The construction of new and modified TMAs has been used in both standard and advanced labelling techniques. A new TMA platform allowing for accurate core sampling by incorporating a digitised H&E overlay will be arriving early in 2023.

In collaboration with the Targeted Therapy Group (within the Division of Cancer Sciences, The University of Manchester), four multiplex IHC panels have been developed, which are being used to probe the tumour immune microenvironment in patients before and after radiotherapy to identify dynamic immune biomarkers of radiotherapy. The panels use the opal technology and are run on the Roche automated Ventana platforms. The panels are used to quantify different types of T cells, macrophages, monocytes, neutrophils and MDSCs in tumour and stromal areas. The group are also investigating immune changes after radiotherapy in murine models using 3-plex mIHC panels run on the Leica Bond system.

In another collaboration, laser capture microdissection (LCM) and immunohistochemistry (IHC) have both been used to identify and capture specific areas of expression of a metabolic protein. The novel subcellular locations remain to be explored for its biomarker (prognostic, diagnostic, therapeutic) potential. The samples are being processed for RNA seq and differential gene expression analysis will identify potential differences between cells with/without this protein at different subcellular locations.

Similarly, the Translational Radiobiology Group (DCS) are using both IHC and LCM to identify and capture specific regions of interest in xenografts. Further analysis using IHC to identify cell surface proteins is underway. Standard RNA/DNA extraction and H&E staining for morphological evaluation are techniques commonly used in around ten different projects using precious human samples.

Working with the Histology core facility, the Genito-Urinary Cancer Research (DCS) group published three papers employing a high throughput multiplex immunofluorescence (mIF) workflow. Using this method, they published methodology for an automated quantitative single-cell level assessment of mitochondrial alterations in formalin fixed paraffin embedded tissue (FFPE) (Sachdeva et al, Scientific Reports, PMID: 35459777). This leveraged tyramide signal amplification on the Ventana Discovery Ultra platform coupled with automated multispectral imaging on the Vectra 3 platform. They further developed this workflow to investigate the influence of EphA2 signalling (Sachdeva, Hart et al, British Journal of Cancer, PMID: 35869144) and the Type 1 interferon regulator IRF7 (Pearson et al, bioRxiv 2022) on prostate cancer progression and long-term survival.

Molecular Biology Core and Computational Biology Support

Wolfgang Breitwieser, Andzhela Abu Rashed, Christopher Clark[1], Dave Lee, John Weightman, Lucy Goodman, Rachel Horner, Robert Sellers, Yannick von Grabowiecki[1], Sudhakar Sahoo

[1]Left in 2022

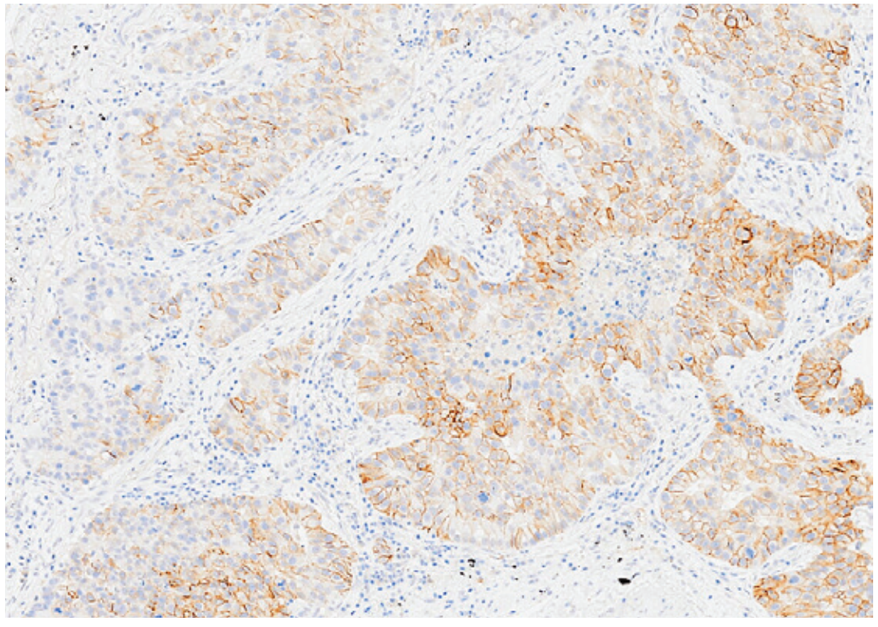
Of great importance to the technological advancement of the service, MBC is putting significant efforts into method validation for novel and improved next generation sequencing applications. One major focus has been on the support of projects that are restricted with low sample quality as well as input, such as those derived from tumour or liquid biopsies. In a recent validation, low quality and quantity RNA input samples extracted from exosome vesicles were processed using Ampliseq-for-Illumina methodology. This uses a highly multiplexed PCR approach to provide whole transcriptome data information. Bioinformatic analysis of this project has shown that typically more than 15,000 transcribed genes could be reproducibly detected, demonstrating that Ampliseq is a promising approach for transcriptome profiling of previously problematic samples.

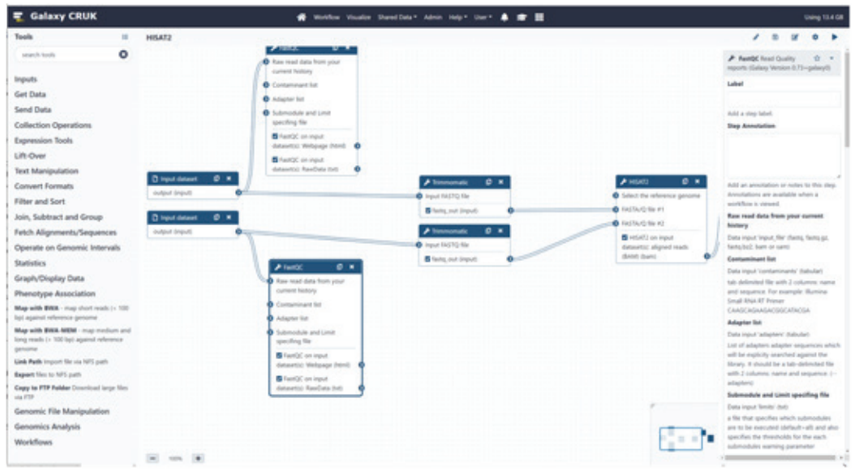
The most widely used method for human cell line authentication (HCLA) involves short tandem repeat (STR) analysis. STRs are microsatellite sequences in the human genome, and the number of repeating units of a given microsatellite varies widely between individuals. Recently we introduced a novel HCLA methodology by using clonal sequencing. For this we adopted a kit from a commercial technology provider, Verogen Forenseq. Performing highly multiplexed sequencing runs on the service's MiSeq NGS platform, we are now able to determine >50 autosomal as well as X/Y-chromosome STR markers. In addition, the technology has the potential to identify >90 single nucleotide polymorphisms, to enable further characterisation of cell lines. All STR profiles are cross checked against our extensive in-house database of previously tested samples as well as public reference databases. In addition, we are continuously expanding our database by adding profiles of newly established cell lines as well as their derivatives.

Spatial biology describes the multi-modality analysis of biological materials in the context of their tissue environment. Recent technological advances, including spatial transcriptomics, spatial proteomics and mass spectrometric imaging, have led to unprecedented possibilities for interrogation of cellular phenotypes. However, they also present new challenges in bioinformatics analysis stemming from the scale and complexity of the data output. In recent months we adopted the 10X Genomics Visium Spatial Transcriptomics platform for the interrogation of gene expression profiles in a tissue environment context at high resolution. This technology is based on the extraction of mRNA molecules from fresh or FFPE tissue sections, while maintaining the information on their spatial origin within the tissue context through molecular barcoding. While not at single cell level, the resolution of the barcode spots (currently 50 mm) allows for the reliable delineation of cell types and cell communities through the application of advanced

Lung adenocarcinoma, 40x whole tissue. Stained for Tbet.

Image supplied by Victoria Fife (Cancer Biomarker Centre)





Screenshot CRUK MI Galaxy Portal.
In 2021, SciCom introduced Docker container technology for hosting shiny web applications, allowing developers to make their R scripts widely available via graphical user interfaces. This approach proved far superior to the traditional way of hosting shiny applications. Therefore, SciCom has started migrating already existing Shiny apps with their programmers to the new hosting system. We have also started developing Shiny training so that MI researchers can develop their Shiny applications directly using Docker containers. Using this technology, SciCom also developed the Cytofkit2 web app (see Figure 2) in close cooperation with the Flow Cytometry core facility. The app allows the simple analysis of mass cytometry data utilising the Phoenix High-Performance Computing resource by submitting jobs using a graphical interface.

bioinformatics tools. Depending on the tissue type, typically we expect to identify up to 3,000-5,000 individual expressed genes within each spot. In addition, advances in the technology also allow for the simultaneous detection of multiple tissue protein biomarkers alongside transcriptomics data providing further refinement of cell type annotation and additional tools for the detection of novel tissue biomarkers.

Scientific Computing

Marek Dynowski, John Campion, Kevin Doyle, Anoop Sanalkumar, Stephen Kitcatt, ZhiCheng Wang

In 2022, SciCom focused on the future of computing at the CRUK Manchester Institute by designing and building a modern data centre for hosting the Core IT and SciCom computing and storage. CRUK MI's new data centre will be located on the 4th floor of the new Paterson Building and is specially designed to support high-density computing and provide efficient cooling to maintain optimal temperatures to reduce the TCO. Power is backed up via two independent 135kW uninterruptible power supplies (UPS) and access is tightly controlled and monitored via CCTV to ensure maximum uptime, redundancy, and security. The UPS can run the entire Core IT/SciCom infrastructure in the data centre for a minimum of 15min. This gives the generator time to kick-in to provide the power for all the IT systems, including the High-Performance Computing (HPC) cluster. This design ensures that even in an event of a total power loss, computations and data transfers are not interrupted and all the services will continue to operate.

The data centre will host a new HPC cluster designed by SciCom with a focus on performance, security, and redundancy. As a result, this system can also be used for business-critical services and clinical trial analysis. The installation of the cluster will begin in March 2023. It consists of 100 x standard compute servers, redundant management and login servers, a NVIDIA "Redstone" based GPU server for high-throughput AI/ML based applications, and image analysis applications, as well as two large memory servers with 4TB RAM each and 96 x compute-cores. All components are connected via a high-speed/low-latency InfiniBand network. Application servers such as the fully integrated RStudio server, which is essential for the downstream analysis of the results, are replaced by more powerful hardware. Behind the scenes, SciCom has continuously improved the storage infrastructure for large research data sets to be able to operate them efficiently in the new building while ensuring a high level of data security and protection. The security of all the servers in the Institute was significantly increased by the introduction of new software that conducts regular security scans of all the virtual and physical servers.

To fully utilise the great potential that such an extraordinary infrastructure offers, SciCom conducts mandatory personal introductions for the usage of the Phoenix platform for every new user and conducted training sessions in 2022 in close cooperation with the Computational Biology Support team. Additional potential

Screenshot Cytofkit2 App.
The new data centre, the new HPC cluster, the Galaxy web portal and the Shiny service are important components of the Phoenix high-throughput data analysis platform and ensure that our scientists have the computing power and the right tools to advance their outstanding research.

provides the introduction of the Galaxy web-based workflow portal (see Figure 1). It allows users to easily design, automate and submit workflows to the HPC system. Galaxy is currently set up in cooperation with the Bioinformatics and Biostatistics team in the Cancer Biomarker Centre, the Institute's Computational Biology Support team and selected users.

Visualisation, Irradiation & Analysis

Steve Bagley, Alex Baker, Jianhua Tang, Kang Zeng[1]

[1]Left in 2022

The facility supports the research initiatives of the Institute in microscopy, high content screening, histology imaging, data processing and analysis of in vivo and in vitro projects and irradiation (X-ray and UV) support. Support for imaging and irradiation is also provided for our researchers based at the Oglesby Cancer Research Building.

Assistance can take the form of project design, training (initial and ongoing), instrument development, troubleshooting, and quality control of outputs. Following a period of successful training, the facility makes the equipment available for use.

The year has been one where several major projects have been undertaken and new workflows introduced:

- Support of CODEX projects across the Institute (with Histology)
- Assisting in spatial transcriptomic projects (Molecular Biology & Histology)
- Multiview labelling and analysis, development of hi-plex workflows and analysis (with Histology)
- Developing new methods for the image alignment and analysis of cyclic staining using IHC (with the Cancer Biomarker Centre)
- Introduction of SOPs for the quality control and monitoring of imaging outputs
- Developing metadata output for assistance in reporting
- Introducing new image deconvolution methods for super-resolution data
- Development of routines for low-impact super-resolution time lapse

In microscopy, 23K hours have been logged, high content screening logged 4.5K hours; in histology 71K slides have been imaged, and 14K hours of image analysis time logged. 103 training sessions have been held to assist in microscopy, the use of irradiation, and data analysis. In addition, support of imaging equipment in research groups has been ongoing, including the installation of a new microscope and the training of researchers.

In the new year, the facility will purchase a phosphor imager and gel documentation system to support the move away from the use of photographic darkrooms in the new building.

Planning for the relocation to the new site has reached a crescendo, as early next year we will be installed in new laboratories designed to accommodate our current equipment and future developments. The design includes temperature/air handling that will provide a constant environment around the systems, which includes a separate room for heat sources and overhead power/gas conduits allowing a range of imaging equipment and automation. The open-plan, future-proofed lab design will adapt to evolving research practices and technologies.

CANCER RESEARCH UK MANCHESTER INSTITUTE

PUBLICATIONS AND OPERATIONS

RESEARCH PUBLICATIONS

Cancer Biomarker Centre

(page 14)

Caroline Dive

Refereed research publications

Wysocki O, Zhou C, Rogado J, Huddar P, Shotton R, Tivey A, Albiges L, Angelakas A, Arnold D, Aung T, Banfill K, Baxter M, Barlesi F, Bayle A, Besse B, Bhogal T, Boyce H, Britton F, Calles A, Castelo-Branco L, Copson E, Croitoru A, Dani SS, Dickens E, Eastlake L, Fitzpatrick P, Foulon S, Frederiksen H, Ganatra S, Gennatas S, Glenthøj A, Gomes F, Graham DM, Hague C, Harrington K, Harrison M, Horsley L, Hoskins R, Hudson Z, Jakobsen LH, Joharatnam-Hogan N, Khan S, Khan UT, Khan K, Lewis A, Massard C, Maynard A, McKenzie H, Michielin O, Mosenthal AC, Obispo B, Palmieri C, Patel R, Pentheroudakis G, Peters S, Rieger-Christ K, Robinson T, Romano E, Rowe M, Sekacheva M, Sheehan R, Stockdale A, Thomas A, Turtle L, Viñal D, Weaver J, Williams S, Wilson C, Dive C, Landers D, Cooksley T, Freitas A, Armstrong AC, Lee RJ, On Behalf Of The Esmo Co-Care. (2022)
An International Comparison of Presentation, Outcomes and CORONET Predictive Score Performance in Patients with Cancer Presenting with COVID-19 across Different Pandemic Waves.
Cancers (Basel) 14(16):3931.

Chemi F, Pearce SP, Clipson A, Hill SM, Conway AM, Richardson SA, Kamieniecka K, Caeser R, White DJ, Mohan S, Foy V, Simpson KL, Galvin M, Frese KK, Priest L, Egger J, Kerr A, Massion PP, Poirier JT, Brady G, Blackhall F, Rothwell DG, Rudin CM, Dive C. (2022)
cfDNA methylome profiling for detection and subtyping of small cell lung cancers.
Nature Cancer 3(10):1260-1270.

Lee RJ, Wysocki O, Zhou C, Shotton R, Tivey A, Lever L, Woodcock J, Albiges L, Angelakas A, Arnold D, Aung T, Banfill K, Baxter M, Barlesi F, Bayle A, Besse B, Bhogal T, Boyce H, Britton F, Calles A, Castelo-Branco L, Copson E, Croitoru AE, Dani SS, Dickens E, Eastlake L, Fitzpatrick P, Foulon S, Frederiksen H, Frost H, Ganatra S, Gennatas S, Glenthøj A, Gomes F, Graham DM, Hague C, Harrington K, Harrison M, Horsley L, Hoskins R, Huddar P, Hudson Z, Jakobsen LH, Joharatnam-Hogan N, Khan S, Khan UT, Khan K, Massard C, Maynard A, McKenzie H, Michielin O, Mosenthal AC, Obispo B, Patel R, Pentheroudakis G, Peters S, Rieger-Christ K, Robinson T, Rogado J, Romano E, Rowe M, Sekacheva M, Sheehan R, Stevenson J, Stockdale A, Thomas A, Turtle L, Viñal D, Weaver J, Williams S, Wilson C, Palmieri C, Landers D, Cooksley T; ESMO Co-Care, Dive C, Freitas A, Armstrong AC. (2022)
Establishment of CORONET, COVID-19 risk in oncology evaluation tool, to identify patients with cancer at low versus high risk of severe complications of COVID-19 disease on presentation to hospital.
JCO Clinical Cancer Informatics 6:e2100177.

Shue YT, Drainas AP, Li NY, Pearsall SM, Morgan D, Sinnott-Armstrong N, Hipkins SQ, Coles GL, Lim JS, Oro AE, Simpson KL, Dive C, Sage J. (2022)
A conserved YAP/Notch/REST network controls the neuroendocrine cell fate in the lungs.
Nature Communications 13(1):2690.

Zhou C, O'Connor J, Backen A, Valle JW, Bridgewater J, Dive C, Jayson GC. (2022)
Plasma Tie2 trajectories identify vascular response criteria for VEGF inhibitors across advanced biliary tract, colorectal and ovarian cancers.
ESMO Open 7(2):100417.

Orlando F, Romanel A, Trujillo B, Sigouros M, Wetterskog D, Quaini O, Leone G, Xiang JZ, Wingate A, Tagawa S, Jayaram A, Linch M; PEACE Consortium, Jamal-Hanjani M, Swanton C, Rubin MA, Wyatt AW, Beltran H, Attard G, Demichelis F. (2022)
Allele-informed copy number evaluation of plasma DNA samples from metastatic prostate cancer patients: the PCF_SELECT consortium assay.
NAR Cancer 4(2):zcac016.

Wu Y, Biswas D, Usaite I, Angelova M, Boeing S, Karasaki T, Veeriah S, Czyzewska-Khan J, Morton C, Joseph M, Hessey S, Reading J, Georgiou A, Al-Bakir M; TRACERx Consortium, McGranahan N, Jamal-Hanjani M, Hackshaw A, Quezada SA, Hayday AC, Swanton C. (2022)
A local human Vδ1 T cell population is associated with survival in nonsmall-cell lung cancer.
Nature Cancer 3(6):696-709.

Frost H, Graham DM, Carter L, O'Regan P, Landers D, Freitas A. (2022)
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Burke H, Freeman A, O'Regan P, Wysocki O, Freitas A, Dushianthan A, Celinski M, Batchelor J, Phan H, Borca F, Sheard N, Williams S, Watson A, Fitzpatrick P, Landers D, Wilkinson T; REACT COVID group. (2022)
Biomarker identification using dynamic time warping analysis: a longitudinal cohort study of patients with COVID-19 in a UK tertiary hospital.
BMJ Open 12(2):e050331.

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Tivey A, Church M, Rothwell D, Dive C, Cook N. (2022)
Circulating tumour DNA - looking beyond the blood.
Nature Reviews Clinical Oncology 19(9):600-612.

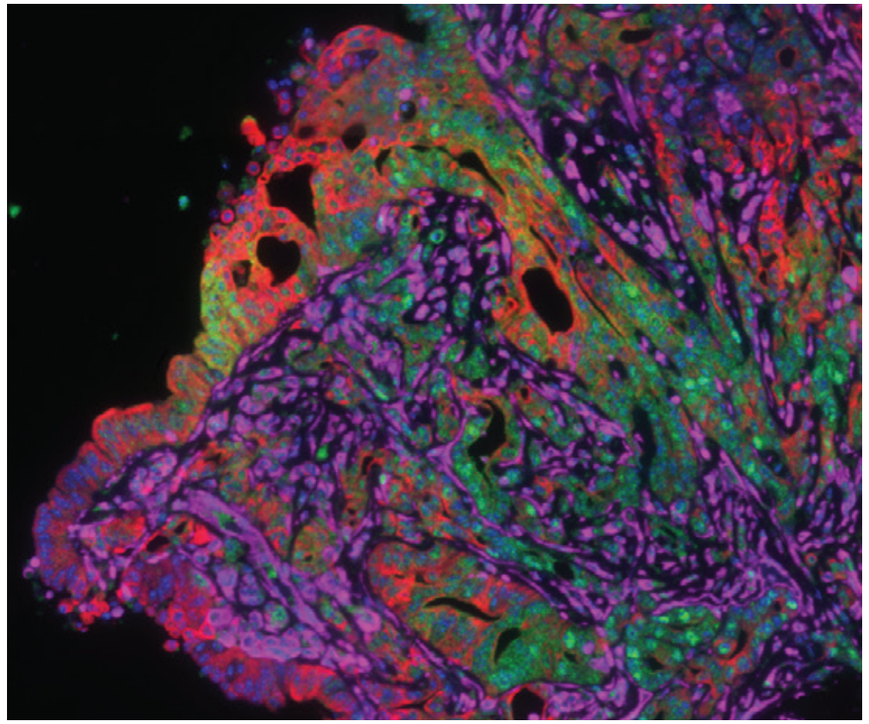
Gardner L, Kostarelos K, Mallick P, Dive C, Hadjidemetriou M. (2022)
Nano-omics: nanotechnology-based multidimensional harvesting of the blood-circulating cancerome.
Nature Reviews Clinical Oncology 19(8):551-561.

Frizziero M, Kilgour E, Simpson KL, Rothwell DG, Moore DA, Frese KK, Galvin M, Lamarca A, Hubner RA, Valle JW, McNamara MG, Dive C. (2022)
Expanding therapeutic opportunities for extrapulmonary neuroendocrine carcinoma.
Clinical Cancer Research 28(10):1999-2019.

Crosby D, Bhatia S, Brindle KM, Coussens LM, Dive C, Emberton M, Esener S, Fitzgerald RC, Gambhir SS, Kuhn P, Rebbeck TR, Balasubramanian S. (2022)
Early detection of cancer.
Science 375(6586):eaay9040.

PI3K signalling activation (green) in murine pancreatic precancerous lesions (red, ductal marker), infiltered with fibroblasts (purple).

Image supplied by Celia Cintas (Systems Oncology). Credit to Caron Behan for 3-plex staining (Histology facility)



Cancer Inflammation and Immunity

(page 20)
Santiago Zelenay

Refereed research publications

Bell CR, Pelly VS, Moeini A, Chiang SC, Flanagan E, Bromley CP, Clark C, Earnshaw CH, Koufaki MA, Bonavita E, Zelenay S. (2022)
Chemotherapy-induced COX-2 upregulation by cancer cells defines their inflammatory properties and limits the efficacy of chemoimmunotherapy combinations.
Nature Communications 13(1):2063.

Nalio Ramos R, Missolo-Koussou Y, Gerber-Ferder Y, Bromley CP, Bugatti M, Núñez NG, Tosello Boari J, Richer W, Menger L, Denizeau J, Sedlik C, Caudana P, Kotsias F, Niborski LL, Viel S, Bohec M, Lameiras S, Baulande S, Lesage L, Nicolas A, Meseure D, Vincent-Salomon A, Reyal F, Dutertre CA, Ginhoux F, Vimeux L, Donnadieu E, Buttard B, Galon J, Zelenay S, Vermi W, Guermonprez P, Piaggio E, Helft J. (2022)
Tissue-resident FOLR2+ macrophages associate with CD8+ T cell infiltration in human breast cancer.
Cell 185(7):1189-1207.e25.

Stok JE, Oosenbrug T, Ter Haar LR, Gravekamp D, Bromley CP, Zelenay S, Reis E Sousa C, van der Veen AG. (2022)
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EMBO Journal 41(6):e109760.

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Bell CR, Zelenay S. (2022)
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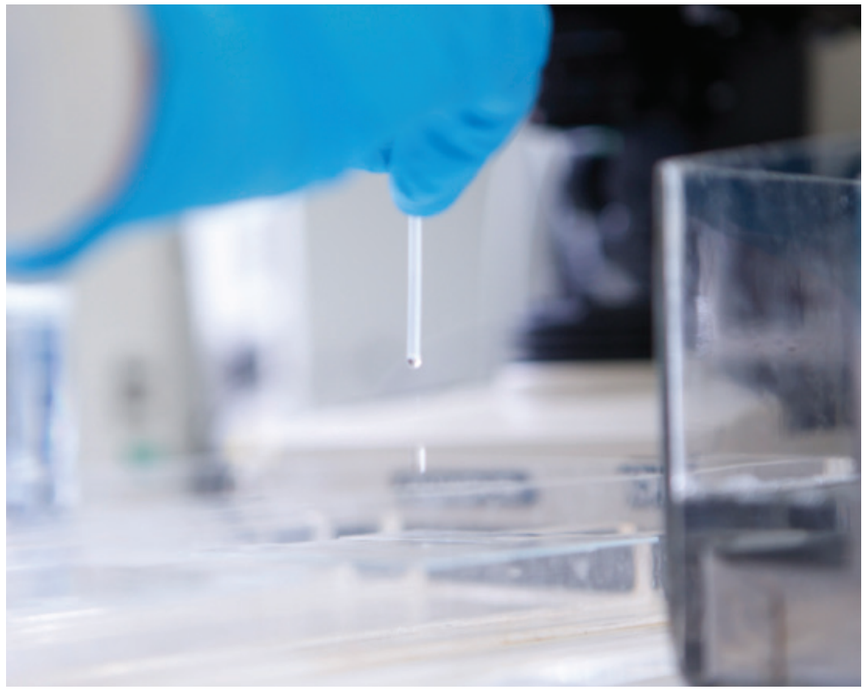
Cell Stress 6(9):76-78.

Cell Signalling

(page 26)
Angeliki Malliri

Refereed research publications

Banka S, Bennington A, Baker MJ, Rijckmans E, Clemente GD, Ansor NM, Sito H, Prasad P, Anyane-Yeboa K, Badalato L, Dimitrov B, Fitzpatrick D, Hurst ACE, Jansen AC, Kelly MA, Krantz I, Rieubland C, Ross M, Rudy NL, Sanz J, Stouffs K, Xu ZL, Malliri A, Kazanietz MG, Millard TH. (2022)
Activating RAC1 variants in the switch II region cause a developmental syndrome and alter neuronal morphology.
Brain 145(12):4232-4245.



Leukaemia Biology

(page 28)
Tim Somervaille

Refereed research publications

Maiques-Diaz A, Nicosia L, Basma NJ, Romero-Camarero I, Camera F, Spencer GJ, Amaral FMR, Simeoni F, Wingelhofer B, Williamson AJK, Pierce A, Whetton AD, Somervaille TCP. (2022)
HMG20B stabilizes association of LSD1 with GFI1 on chromatin to confer transcription repression and leukemia cell differentiation block.
Oncogene 41(44):4841-4854.

Mead AJ, Butt NM, Nagi W, Whiteway A, Kirkpatrick S, Rinaldi C, Roughley C, Ackroyd S, Ewing J, Neelakantan P, Garg M, Tucker D, Murphy J, Patel H, Bains R, Chiu G, Hickey J, Harrison C, Somervaille TCP. (2022)
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Molecular Oncology

(page 32)
Richard Marais

Refereed research publications

Valpione S, Campana LG, Weightman J, Salih Z, Galvani E, Mundra PA, De Rosa F, Gupta A, Serra-Bellver P, Lorigan P, Germetaki T, Dynowski M, Kitcatt S, Sahoo S, Lee D, Dhomen N, Lord G, Marais R. (2022)
Tumour infiltrating B cells discriminate checkpoint blockade-induced responses.
European Journal of Cancer 177:164-174.

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MiR-378a inhibits glucose metabolism by suppressing GLUT1 in prostate cancer.
Oncogene 41(10):1445-1455.

Lee KA, Thomas AM, Bolte LA, Björk JR, de Ruijter LK, Armanini F, Asnicar F, Blanco-Miguez A, Board R, Calbet-Llopart N, Derosa L, Dhomen N, Brooks K, Harland M, Harries M, Leeming ER, Lorigan P, Manghi P, Marais R, Newton-Bishop J, Nezi L, Pinto F, Potrony M, Puig S, Serra-Bellver P, Shaw HM, Tamburini S, Valpione S, Vijay A, Waldron L, Zitvogel L, Zolfo M, de Vries EGE, Nathan P, Fehrmann RSN, Bataille V, Hospers GAP, Spector TD, Weersma RK, Segata N. (2022)
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Eden M, Hainsworth R, Gordon LG, Epton T, Lorigan P, Rhodes LE, Marais R, Green AC, Payne K. (2022)
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British Journal of Dermatology 187(1):105-114.

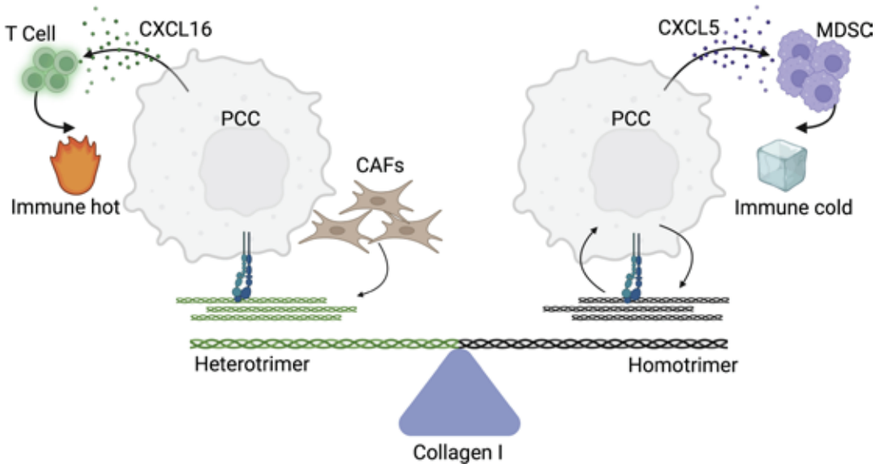
Skin Cancer and Ageing

(page 34)
Amaya Virós

Other publications

Fernandes S, Vyas C, Lim P, Pereira RF, Virós A, Bártolo P. (2022)
3D Bioprinting: An Enabling Technology to Understand Melanoma.
Cancers (Basel) 14(14):3535.

Darmawan CC, Ohn J, Mun JH, Kim S, Lim Y, Jo SJ, Kim YG, Kim B, Seong MW, Kim BJ, Lee C, Kwak Y, Chung HJ, Virós A, Lee DY. (2022)
Diagnosis and treatment of nail melanoma: a review of the clinicopathologic, dermoscopic,



Collagen I trimer composition balances pro- and anti-tumour signals in the microenvironment. Collagen I homotrimers produced by pancreatic cancer cells (PCCs) activate tumour cell autonomous signalling to produce CXCR2 ligands (CXCL5), attract myeloid-derived suppressor cells (MDSCs), and thereby induce an immune cold microenvironment that is refractory to immune checkpoint blockade treatment. Collagen I heterotrimers produced by cancer-associated fibroblasts (CAFs) restrain pancreatic ductal adenocarcinoma (PDAC) progression through production of CXCL16 to attract CD8pos T cells and induce sensitivity to immune checkpoint inhibitor treatment.

Image from Jorgensen Cancer Cell. 2022 Aug 8;40(8):802-804, created with BioRender.com

and genetic characteristics.
J Eur Acad Dermatol Venereol. 36(5):651-660.

Stem Cell Biology

(page 36)
Georges Lacaud

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Selkirk E, Patel R, Hoyle A, Lie-A-Ling M, Smith D, Swift J, Lacaud G. (2022)
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Heliyon 8(11):e11362.

Systems Oncology

(page 38)
Claus Jørgensen

Refereed research publications

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Pancreatic ductal adenocarcinoma cells employ integrin α6β4 to form hemidesmosomes and regulate cell proliferation.
Matrix Biology 110:16-39.

Halbrook CJ, Thurston G, Boyer S, Anaraki C, Jiménez JA, McCarthy A, Steele NG, Kerk SA, Hong HS, Lin L, Law FV, Felton C, Scipioni L, Sajjakulnukit P, Andren A, Beutel AK, Singh R, Nelson BS, Van Den Bergh F, Krall AS, Mullen PJ, Zhang L, Batra S, Morton JP, Stanger BZ, Christofk HR, Digman MA, Beard DA, Viale A, Zhang J, Crawford HC, Pasca di Magliano M, Jorgensen C, Lyssiotis CA. (2022)
Differential integrated stress response and asparagine production drive symbiosis and therapy resistance of pancreatic adenocarcinoma cells.
Nature Cancer 3(11):1386-1403.

Other publications

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Translational Oncogenomics

(page 42)
Rob Bristow

Refereed research publications

Fletcher CE, Deng L, Orafidiya F, Yuan W, Lorentzen MPGS, Cyran OW, Varela-Carver A, Constantin TA, Leach DA, Dobbs FM, Figueiredo I, Gurel B, Parkes E, Bogdan D, Pereira RR, Zhao SG, Neeb A, Issa F, Hester J, Kudo H, Liu Y, Philippou Y, Bristow R, Knudsen K, Bryant RJ, Feng FY, Reed SH, Mills IG, de Bono J, Bevan CL. (2022)
A non-coding RNA balancing act: miR-346-induced DNA damage is limited by the long non-coding RNA NORAD in prostate cancer.
Mol Cancer. 21(1):82.

Other publications

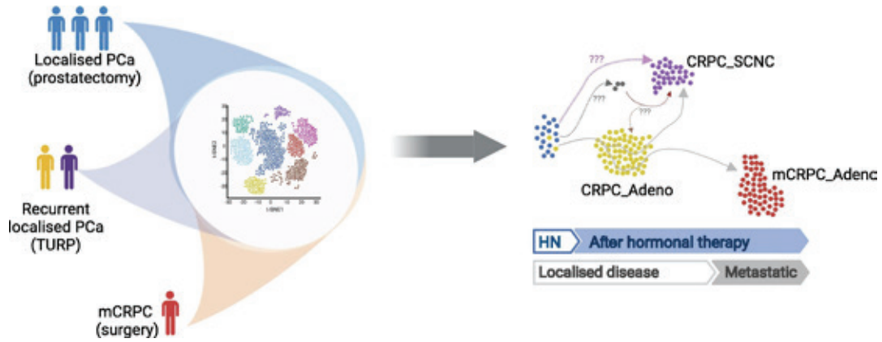
Bristow RG, Engel J, Jayasinghe I, Kampmann M, Sansom OJ, Bryant DM. (2022)
Conversations with LGBT+ scientists about visibility, leadership and climbing the career ladder.
Journal of Cell Science 135(4):jcs259880.

Select additional publications

Lane B, Khan MT, Choudhury A, Salem A, West CML. (2022)
Development and validation of a hypoxia-associated signature for lung adenocarcinoma.
Scientific Reports 12(1):1290.

Epithelial cells were isolated from three patients with localised prostate cancer, two with localised recurrent disease, and one with soft-tissue metastatic prostate cancer. The identification of a pre-existing CRPC stem-like population opens many questions regarding the progression path of prostate tumours towards CRPC-Adeno or CRPC-SCNC phenotypes and their implications for patient treatment. HN=hormone-naïve disease; TURP=transurethral resection of the prostate; CRPC-Adeno=castration-resistant prostate cancer, adenocarcinoma phenotype; CRPC_SCNC=CRPC, small-cell neuroendocrine phenotype; mCRPC=metastatic CRPC.

Image from Ali A, Baena E. Eur Urol. 2022 May;81(5):456-457.



Ali A, Baena E. (2022)
The Needle in the Haystack: The Presence of Castrate-resistant Prostate Cancer Cells in Hormone-naïve Prostate Cancer.
European Urology 81(5):456-457.

Dolma L, Muller PAJ. (2022)
GOF Mutant p53 in Cancers: A Therapeutic Challenge.
Cancers (Basel) 14(20):5091.

EXTERNAL SEMINAR SPEAKERS 2022

The seminar series that we run is vital for the Institute, connecting world-class researchers across the broad spectrum of cancer research. Following the challenges of maintaining connectivity with the research community remotely during the aftermath of the COVID-19 pandemic, this year we resumed more in person scientific interactions with 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.

George Vassiliou
Wellcome-MRC Cambridge Stem Cell Institute

Brian Huntly
Department of Haematology, University of Cambridge

David Cortez
Vanderbilt University

Lisa Coussens
Oregon Health & Science University

Carla Kim
Harvard Stem Cell Institute

Pedro Beltrao
EMBL European Bioinformatics Institute

Michael Boemo
Department of Pathology, University of Cambridge

Richard White
Sloan Kettering Institute

Joerg Mansfield
Institute of Cancer Research

Ian Collins
Institute of Cancer Research

Elisa Laurenti
Wellcome-MRC Cambridge Stem Cell Institute

Simon Leedham
Queens College, Oxford

Manuel Valiente
Spanish National Cancer Research Centre

Sean Morrison
UT Southwestern Medical Center

Axel Behrens
Institute of Cancer Research

Alejandra Bruna
Institute of Cancer Research

Sarah-Jane Dawson
Peter MacCallum Cancer Centre

Olaf Heidenreich
Princess Maxima Centre, Netherlands

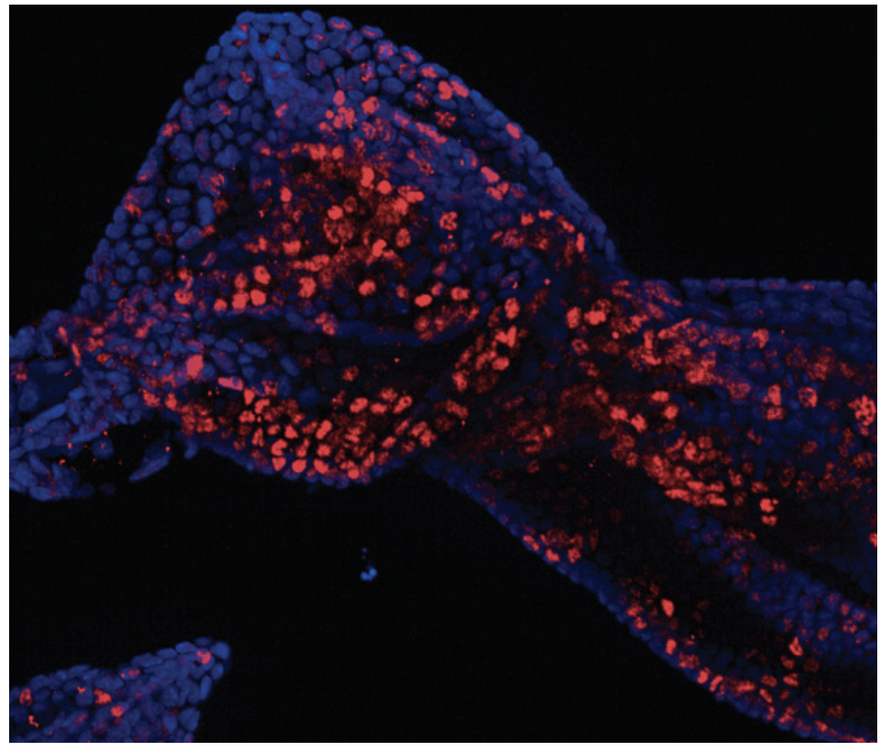
Johanna Joyce
University of Lausanne

Richard Tothill
University of Melbourne

John Diffley
Francis Crick Institute

Intersection between Science and Art. Murine pancreatic organoids cultured in Matrigel. Cyan: nuclei, red: PI3K activation signalling.

Image supplied by Celia Cintas (Systems Oncology)



James Brenton
Cancer Research UK Cambridge Institute

Ben Z. Stanger
University of Pennsylvania

Jess Butler
University of Aberdeen

Anguraj Sadanandam
Institute of Cancer Research

Chiara Francavilla
University of Manchester

OPERATIONS

Chief Operating Officer
Caroline Wilkinson

Chief Laboratory Officer
Stuart Pepper

Chief Finance Officer
Mike Berne

Chief Human Resources Officer
Rachel Powell

The year was dominated by ongoing work to contribute to the design of the new research facility on the site of the Christie NHS Foundation Trust, which replaces the former Paterson Building. Increasingly, this has been complemented by detailed discussions regarding operational arrangements for the building which we shall share with our Trust colleagues. Towards the end of 2022, work started on the relocation programme, which is due to start in early Spring 2023 and will see us move from our current base at Alderley Park over a few weeks. The Institute will be split across the new building, to be called the Paterson Building for the immediate future, and the Oglesby Building for Cancer Research located over the road. We look forward to reuniting with our colleagues across the Christie campus and to the scientific synergies and collaborations that both buildings will facilitate.

Chief Operating Officer
Caroline Wilkinson

Janet Watson retired from the Operations team in February. She had chaired the Institute's Animal Welfare and Ethical Review Body for several years and had an enormous impact on the Institute's animal research culture of care. She was instrumental in relocating our experimental facility to Alderley Park after the 2017 Paterson Building fire and was an influential and insightful mentor to many across CRUK MI.

We were very fortunate to recruit Sally Robinson as our new AWERB Chair. Like Janet, Sally comes to the role with an abundance of experience having been Director of AstraZeneca's Animal Sciences and Technology team at Alderley Park and a member of the Animals in Science Committee amongst other significant appointments.

Maria Belen Conti Vyas left the Institute in October after several years, first as Administrative Services Coordinator and then as Executive Assistant to myself and Stuart Pepper. Amongst many contributions to the Institute during this time, Belen played a key role in the Institute's communications activities through running our social media accounts and organising many scientific as well as social events to bring the MI community together. Belen and her family relocated to Singapore where we wish them all the best for their new life. Karen Lee, who covered Belen's role during maternity leave, has stayed on as permanent Executive Assistant to myself and Stuart Pepper, providing much needed continuity as we build up to the move. We welcomed Helen Jones who joined Karen in the Administration team as Administrative Services Co-ordinator.

The Human Resources team launched our Equality, Diversity and Inclusion strategy in the autumn and will seek to work on its implementation as 2023 unfolds. The Finance and Logistics teams have worked hard to find ways to help alleviate issues caused by COVID-19 related supply chain and inflationary pressures. A special mention should go to Purchasing Officer David Jenkins who arranged a suppliers' fair which offset the costs of holding our annual colloquium. IT, Health and Safety, Logistics and Lab Services teams have also worked tirelessly on the design of the new building as well as logistics of the relocation and operational arrangements for the new building.

We welcomed Jeff Barry as our Wellbeing and Engagement Adviser and our increased emphasis on this area was further recognised with the formal inclusion of Wellbeing in our Health and Safety Committee, which is now the Health, Safety and Wellbeing Committee.

The first in-person Institute colloquium since 2019 was a major boost for all and was well organised by the Scientific Operations and administration teams. We took the opportunity to launch the Institute's Equality, Diversity and Inclusion strategy to our staff and students and which you can read more about this below.

Another major project was the wind down of the Institute's Drug Discovery Unit concomitant with the spin out of a company Oncodrug, which is based at Alderley Park, to continue to pursue various targets. This involved a huge effort across the Operations team in a short space of time together with colleagues in the former DDU, University of Manchester and CRUK. We wish them all the best in their new venture.

The coming year promises to be incredibly busy as we relocate to the new Paterson Building, but we are all excited to be returning to our original location in Withington on the Christie NHS Trust Foundation site. We look forward to reuniting with our clinical and scientific colleagues based there, and working with operational colleagues from across the Christie and wider University of Manchester as we help establish the Institute in its new home.

Institute Administration Team
Ruth Cox, Naomi Samuels[1], Helen Jones[2], Soraya Francis[3], Maria Belen Conti[3], Karen Lee

[1]Maternity cover, [2]Joined in 2022, [3]Left in 2022

Ruth Cox is Executive Assistant to the Institute Director and her role is being covered by Naomi Samuels during her maternity leave. Karen Lee is Executive Assistant to the Senior Management Team. Helen Jones joined us towards the end of the year as our Administration Services Coordinator.

This year the Administration Team have organised many meetings, seminars and workshops in a virtual and hybrid format. Notably, our internal seminar series returned to in person, which has been a great way to reconnect with each other.

As a team they support the Director and the Institute Faculty day-to-day and have helped to organise the first in person Institute Colloquium since 2019, alongside a range of education and engagement events for staff and students throughout the year.

The team has also supported high profile visits to the Institute this year, including members of our Scientific Advisory Board and the CRUK Trustees. Both visits were a great success.

We have hosted a varied programme of national and international speakers in our External Seminar Series, with an increasing number giving their talks in person. We are grateful to all our invited speakers for committing their time to give talks. Details can be found at cruk.manchester.ac.uk/seminars.

We were delighted to be able to end the year with a memorable Christmas party, held in a Manchester city centre venue with interactive games such as table tennis, indoor curling and junkyard golf, to celebrate getting together and having fun after some challenging years.

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

This year we began to see some positive changes in the charitable funding availability following the pandemic. Although we have not quite returned to original levels, it is nevertheless encouraging to see the sector recovering.

One of the biggest challenges we have faced this year is the rising price of consumables. However, we continue to maintain good relationships with our suppliers that still allows us to maximise our scientific activities within the budgetary constraints.

The Finance team continues to support the Institute Director and the management of the

overall Institute budget, while providing costs and advice for new research proposals and contracts for all our groups. Despite ongoing global financial pressures, we continue to be successful in winning numerous new awards.

The Institute's move to the new Paterson Building is imminent and the Finance team are looking forward to the move.

Human Resources

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

[1]Joint with Administration

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.

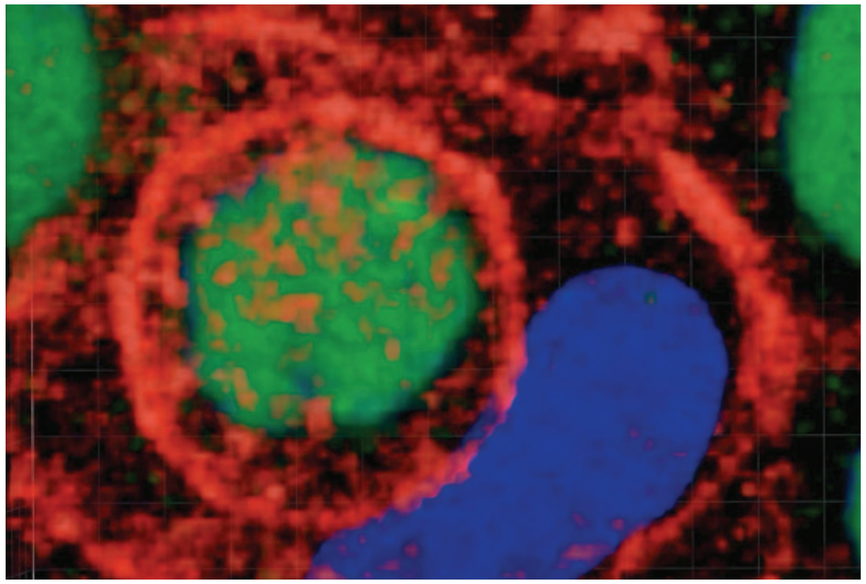
During 2022, we were successful in appointing and onboarding 48 people into the Institute, and we look forward to continuing to support the growth of the Institute over the next couple of years. The department reviewed where we advertise our vacancies and as a result, we have widened our audience, with the increased use of social media, to obtain maximum exposure to attract high quality candidates.

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 changed the grading ratings and developed the process to place more of an emphasis on wellbeing, training and development, which has become more motivational for staff. The department also facilitated the successful promotion of seven individuals. 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.

Over the year, there has been a drive on wellbeing and supporting mental health within the Institute. We are delighted to have appointed a Wellbeing & Engagement Adviser to complement our commitment to the wellbeing of our staff. We have also established a Wellbeing Committee and in the coming year we will be recruiting Wellbeing Champions. In addition, we have been supporting staff through the cost-of-living crisis with additional financial support and increased promotion of the Employee Assistance Programme. We are also pleased to announce that the Institute now has four trained Mental Health First Aiders.

The department has been actively involved with the launch of the Institute's Equality, Diversity and Inclusion (EDI) Strategy along with the Institute's vision and aims, which are available to view on our external website. The vision is to create a diverse and inclusive culture which develops, attracts, and maintains a positive environment for staff and students whilst achieving the Institute's aim to deliver world class cancer research. The department supported the Institute in devising a two-year action plan in addition to establishing an EDI Strategic Steering Committee and an EDI Subcommittee to achieve this vision.

Next year, the department will continue its focus on Equality, Diversity and Inclusion and wellbeing, in addition to the relocation to the new building and a review of our onboarding and induction processes.



Cell-in-cell.
3D image shows an A431 p53 KO cancer cell (green) inside an A431 mutp53 cancer cell. Membrane stained with ezrin (red).

Image supplied by Lobsang Dolma (former group, Tumour Suppressors)

Information Technology

Steve Royle, Matthew Young, Brian Poole, Krar Haider

The CRUK Manchester Institute Core IT team is a small group of four experienced IT professionals. We provide a wide range of IT support services to over 300 research and support staff, currently spread across several sites. We manage IT service desks on our two main sites at Alderley Park in Cheshire and the Oglesby Cancer Research Building in south Manchester.

We currently manage over 600 desktop computers, comprising a mixture of Windows 10 and Windows 11 PCs and laptops, Apple iMacs and Mac Books, plus a growing number of tablet devices, mainly Apple iPads and iPhones. All these devices are centrally authenticated, with access to cloud-based services plus an on-site central file-store, a server farm and network printing. The Core IT infrastructure comprises a 400Tb enterprise-class file storage facility for our research data. This is based on a replicated design and is hosted in two geographically separate datacentres to provide a resilient, high availability, redundant and fit for purpose storage facility. They are connected by a dedicated CRUK MI resilient wired and wireless network infrastructure across all CRUK MI research facilities at Alderley Park and the OCRB.

This year has been another busy year for IT. Over the past 12 months, planning work has accelerated as we have developed detailed, multi-faceted plans to return to our original site in Withington. Plans include moving our datacentre IT equipment i.e., servers, storage and network equipment, plus our office furniture and associated computer equipment, including desktop PCs, monitors and printers.

Whilst all the move planning has been gathering pace this year, we have also been busy delivering our catalogue of professional IT services to all Institute staff and students. The post-COVID-19 era has reshaped the way we work. Some of our staff are now hybrid working, from home and onsite. We have continued to provide the same high level of IT support using a selection of new and existing remote support tools. Teams and Zoom platforms continue to be our virtual meeting rooms of choice and our staff have adapted well to these video conferencing platforms. Further digital transformation work continues in the form of the implementation of 'Teams Calling' (telephony) across The University of Manchester campus network, of which CRUK MI and the new Paterson Building are a part. This will provide a telephony service to all CRUK MI staff through their MS Teams accounts using their existing desktops and laptops, thereby saving the cost of buying hundreds of additional desk phones.

The new Paterson Building (based on the Christie NHS Foundation Trust site) is a new custom-built cancer research building shared between the Christie and The University of Manchester, which will be the main location for the Institute from spring 2023 onwards. All three organisations' IT networks will be available throughout the building.

Safety and Facilities Management

Colin Gleeson

Health and Safety

Colin Gleeson, Chris Bamber, Jeff Barry[1]

[1]Joined in 2022

The Health and Safety team has concentrated on three major strands of work over the past twelve months, covering our relocation and occupancy of the new building on the Christie NHS Foundation Trust site, development and roll-out of the Institute's wellbeing initiative, and normal work activities around monitoring health and safety performance.

Throughout these past twelve months we have been preparing for our relocation and occupancy of our new building. This involved attending several residual late stage planning meetings, contributing to the planning of the complex relocation of the Institute, focusing on health and safety requirements for the move to, and subsequent occupancy of, the new building, and ensuring operational readiness of the building. Alongside these essential relocation-related tasks, there has been much checking of legal requirements and procedural arrangements around the transport of the Institute's biological material and chemical agents. Likewise, there has been development and production of several documents and risk assessments related to the move and occupancy of the new building.

We have made considerable progress in the development and roll out of the Institute's wellbeing initiative. This has encompassed regular meetings of the Wellbeing Focus Group, resulting in the establishment of wellbeing priorities and strategies that have subsequently been rolled out. For example, we have developed new wellbeing pages on the Institute's intranet providing a valuable resource for staff and students.

In the background, we have continued with our normal work streams, especially around the proactive monitoring of health and safety performance via inspection programmes and reactive investigations of accidents and incidents. We have also continued with risk controls assurance programmes around local exhaust ventilation, gases, fire safety and lone working.

We anticipate that the imminent move to the new building and subsequent occupancy will be challenging in many respects. But it also affords us the opportunity to re-evaluate some health and safety policies with an aim to review, and where necessary refine, the overall management of health and safety for the Institute.

Electronics

Yunis Al-hassan

As part of the Institute's electrical and fire safety strategies, the electronics engineer continues to undertake PAT testing and equipment repairs where required. The repair facility continues to provide a significant economic benefit to the Institute in that unnecessary expenditure on replacement equipment is avoided. The Institute's electronics engineer also tracks Institute equipment which is under warranty, service contract or in-house repair. Again, this provides a significant economic benefit to the Institute.

Laboratory Services

Mark Craven, Christine Williams, Corinne Hand, Tony Dawson, Busola Atuegbe, Petra Kubinova Dilfi[1]

[1]Left in 2022

During 2022, Lab Services has continued to supply the various sites with sterile fluids, glassware, plastics and microbiological media. These operational sites are currently at Alderley Park, Oglesby Cancer Research Building, Proton Beam Therapy Centre, MCRC Biobank and the Incubator building.

Within the past year, the department has accommodated a new glass wash service provider at AP and has worked with the new team to minimise any issues upon its launch.

The department continues to offer additional services such as access to photographic dark rooms. In addition, the team oversees the delivery of clean lab coats across the site, as well as operating a monthly pipette clinic for the researchers.

Lab Services manage the supply and maintenance of some portable safety related items, such as the lab and office based first aid, hand-held 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 as requested by research groups.

We will open the Lab Services department in the new Paterson Building early next year and, alongside the Core Facility departments, we are preparing to deliver a full service once the building opens in 2023.

The new department will have a full range of glass washers and autoclaves and offer a media production to the Paterson Building.

Once the Paterson Building opens and we transfer from the Alderley Park site, we will operate across the Paterson Building and the OCRB.

Logistics

Andrew Lloyd, Michael Alcock, Edward Fitzroy, Nigel Fletcher, Sedia Fofana, Wayne Howarth Jonathan Lloyd, Robin Sherratt (with support from Tony Dawson, Laboratory Services)

Over the past year, the Logistics team based at OCRB and Alderley Park have continued to deliver successfully a high-quality reactive service to the Institute. This includes a daily transport service of samples and goods between various research locations and core facility departments. The team also collect time- and temperature-sensitive samples from the Christie NHS Foundation Trust CTU department and support the transportation of mice from the Incubator at The University of Manchester campus to Alderley Park. The team have worked closely with Lab Services by supporting the delivery of sterile media and glassware between sites, including the returning of empties and recyclable plastics.

The team operate a back of house service, taking in the Institute's deliveries. Items are receipted and distributed accordingly. Undelivered items are stored in the department at the appropriate temperature.

The team have continued to facilitate the delivery of dry ice, gas cylinders and liquid nitrogen. The Institute's cell lines and other key biological samples that are stored in nitrogen is also monitored, and the nitrogen levels topped up as required by the team.

Researchers can order central stores stock items via the intranet, with the option of collection in person or distribution by the Logistics team. We have a catalogue of over 100 stores items from tissue culture essentials to cleaning products. Included in this system are the enzymes and media stored in the Institute's freezers (Sigma, Life tech, Promega, New England Bio labs, and Qiagen). On the occasion when stocked items have become unavailable, we have sourced alternatives. We continue to buy in bulk from our trusted suppliers using quotes, producing savings for the Institute.

In preparation for relocation to the new building, the team have been working hard to remove and dispose of unwanted, old or broken equipment and furniture. They have also worked closely with the IT team helping to collect old desktop computers and monitors.

Next year, the focus will be on supporting the relocations, whilst also developing new operational procedures in the new Paterson Building.

Scientific Administration

Caroline Wilkinson, Gillian Campbell, Julie Edwards, Christopher McCauley, 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 preparation of the relocation of the Institute in addition to their normal role. There was a welcome return to in person interviews of PhD students and in person vivas across our Postgraduate Education Programme (overseen by Postgraduate Education Manager Julie Edwards– see the Education section of this report).

Gill Campbell 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 the Harry J Lloyd Charitable Trust, Pancreatic Cancer UK, Medical Research Council, Rosetrees Trust, The Lady Tata Memorial Trust and Cancer Research UK. 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 saw a welcome return to an in-person Institute colloquium held at Alderley Park which Gill coordinated ably assisted by members of the Scientific Administration and general administration teams. She has also started to work on plans to refresh our external website in 2023.

Andrew Porter has continued to develop his recently created role of Research Integrity and Training Adviser and has assisted the Institute's scientific community through a supportive manuscript review process and bespoke

training sessions covering topics such as statistical analysis, meta data recording and assisted with bioinformatics training. He continues to deliver his research integrity induction sessions to new starters and has benefitted from and contributed to discussions in a network with other research integrity officers across the CRUK Institutes. Andrew is also a core part of the Institute's Communications team providing content for our social media platforms as well as liaising with the press teams at CRUK and The University of Manchester over relevant opportunities to publicise our research. Andrew drove a new initiative in 2022 to put together a team to visit local schools to engage students with science through hands on experiments, presentations and quizzes.

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. This year the Institute's Information Governance Committee coordinated by David has explored how best we can adopt Microsoft Teams, which has been rolled out by The University of Manchester, to ensure best Information Governance practice is being followed across the Institute. David has also contributed to the Institute's relocation planning as one of several "Move Champions" to coordinate furniture moves, telephony changes and is part of a group developing our operational arrangements for the new Paterson Building.

Chris McCauley is the Institute's web developer who produces and maintains our various online platforms including the intranet, external website as well as the staff recruitment and PhD application portals. This year he updated the platforms underpinning these applications and has continued to refine our staff recruitment portal - JobMarker - and the PhD recruitment portal to optimise the experience for applicants and recruiting managers. He has created new functionality on the Institute's intranet, the hub, to support PhD student leave recording. He has started to explore options for our external website refresh as well as contributing to the Scientific Computing team's activities.

Animal Welfare

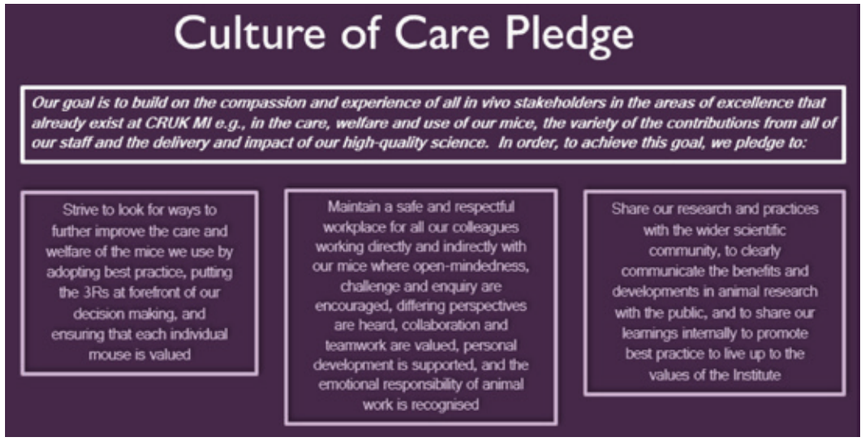
Caroline Wilkinson, Establishment Licence Holder; Simon Poucher, Regulatory Liaison and Training Officer; Sally Robinson[1], Animal Welfare & Ethical Review Body (AWERB) Chair; Janet Watson[2] (former AWERB Chair); Stuart Pepper, Chief Laboratory Officer

[1]Joined in 2022, [2]Left in 2022

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 the 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, ensuring the provision of appropriate management structures and processes, staff training, and facilities for the care and use of mice, while encouraging implementation of the replacement, reduction and refinement (3Rs) of the use of animals. It also reviews proposed collaborations and grant applications involving animal research.

Mother and litter. One of the winning photos submitted by Biological Resources Unit animal technicians as part of Institute of Animal Technology (IAT) technician's month celebrations.

Image supplied by BRU technician.



During 2022, a Culture of Care subgroup was established under AWERB. This group has developed a Culture of Care pledge that represents the Institute's values in relation to animal research.

We have turned some of the challenges presented by the pandemic and lockdowns throughout 2020/21 into opportunities for care of our animals whilst preserving our research activity. For example, 19 of 41 site visits by our vet in 2022 were conducted through video links. This meant that two different sites could be 'visited', and animals inspected on the same day without the 48-hour quarantine required when visiting different mice facilities.

The number of mice used in the production and supply to CRUK MI researchers was 14,663 (an increase of 4.5% over 2021). The duration of time which mice are kept in the facility has been decreased by 2.3%, reflecting an increased efficiency in use. We have been able to revise husbandry practices to reduce numbers of mice that are either singly or pair housed, so enhancing the potential for interaction between mice. The total number of live breeding lines was reduced by 10.6% overall, despite introducing 25 new lines for the researchers. This was driven by cryo-preserving lines not currently needed. Overall, there were 18,291 mice used in regulated procedures, including production and supply, under the Act in 2022 (a reduction of 5.5% compared to 2021).

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 quickly resolved with ASRU. AWERB reviewed six new project licence applications and fourteen amendments 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 have introduced many refinements to procedures to improve the welfare of the mice.

These include:

- The use of ultra-sound imaging to guide injections of cancer cells into the liver and bladder wall. This removes the need for surgical procedures.
- Development of a non-invasive method of administering analgesia to mice experiencing embryo transfer
- Initiating the development of a process for the use of inhalation anaesthesia rather than injectable anaesthetic for mice undergoing irradiation in enclosed units
- Development of a pictorial guide for the identification and management of skin sores

We regularly share our best practices with other establishments. For example, our Named Animal Welfare and Care Officers have commenced regular meetings with those from other CRUK Institutes.

Our scientists have taken part, by invitation, in online forums and conferences and contributed to expert groups arranged by

national bodies such as the National Centre for Replacement, Refinement and Reduction of Animals in Research (NC3Rs), Royal Society for the Prevention of Cruelty to Animals (RSPCA) and the Laboratory Animals Science Association (LASA) to further the sharing of knowledge and advice on laboratory animal use.

- For example, individuals at CRUK MI are part of an expert working group that are collaborating to update the 2010 guidelines on the welfare of animals used in cancer research (https://www.nc3rs.org.uk/our-portfolio/revision-guidelines-animal-use-and-welfare-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

- Our Establishment Licence Holder and AWERB Chair both sit on the national PEL holders forum which provides feedback to ASRU and which helps to train other Establishment Licence holders

To encourage more internal sharing of activities that have a beneficial effect, however small, on the experience of the mice, we have introduced a Culture of Care 'Recognition Reward' for anyone involved in the process of animal research at the Institute. Eight rewards were given in 2022.

Much energy has been put into the planning of our return to the new research facilities in the re-built Paterson Building. We expect this to be in the building by early 2023 and will be very excited to return 'home'.

Cancer Research UK Commercial Partnerships

Martyn Bottomley, Nathalie Dhomen[1]

[1]Joined in 2022

The Cancer Research UK Commercial Partnerships team is a specialist oncology-focused development and commercialisation team that is part of Cancer Research Horizons, Cancer Research UK's innovation engine. Launched in April 2022, Cancer Research Horizons brings together our commercial partnerships and therapeutic innovation expertise to translate more cutting-edge innovations into effective treatments and diagnostics for people with cancer now. Alongside offering the full spectrum of drug discovery and clinical capabilities, Cancer Research Horizons is uniquely placed to support translational funding, licensing, collaboration and spin-out creation. The commercial partnerships team aims to bridge the gap between cutting edge academic research and industrial development of cancer therapeutics, medical technologies and diagnostics.

The Institute is part of a vibrant network of oncology research in Manchester and, in partnership with other parts of The University of Manchester and the Christie NHS Foundation Trust, is a keystone of the Manchester Cancer Research Centre (MCRC). This partnership also reaches out to key associate institutes as well as other trusts and organisations (e.g., Greater Manchester Cancer, Manchester Academic Health Sciences Centre, and Health Innovation Manchester). Work by Manchester-based researchers cross cuts all the partner organisations, creating an enabling environment to drive research impact and deliver the world-leading discoveries that change the lives of people affected by cancer. Essential to delivering this vision is identifying potentially game-changing innovations, intellectual property (IP) and opportunities for commercialisation and collaboration, and subsequently providing best road maps for translation into patient impact.

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. To facilitate the identification and translation of oncology research, we have recruited Nathalie Dhomen to a unique new role as Cancer Commercialisation and Innovation Lead for the MCRC, which links into the commercialisation support infrastructure of both The University of Manchester Innovation Factory and Cancer Research Horizons.

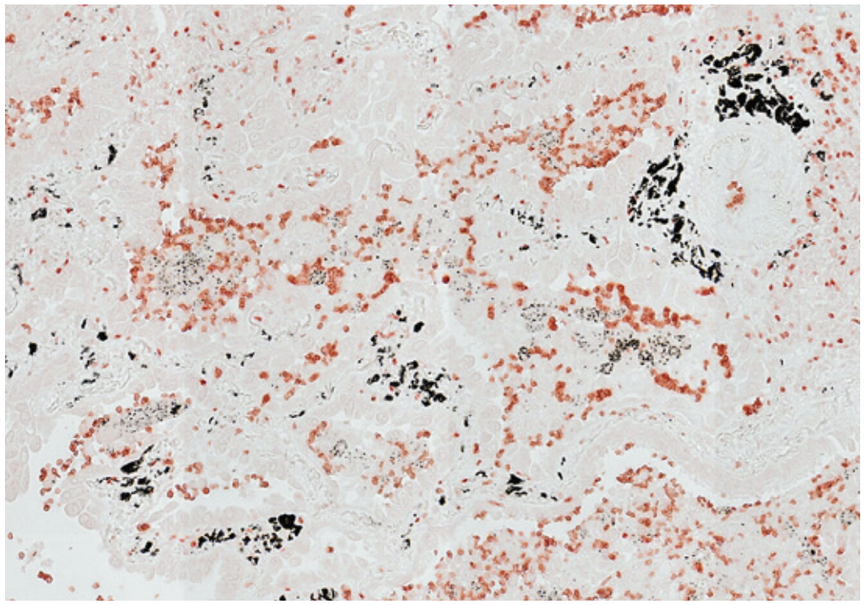
Nathalie is the first point of contact for all the research groups and clinicians working within the partner organisations including the CRUK Manchester Institute. She can support researchers and clinicians in identifying new high-value IP and innovations arising from their research and can facilitate 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.

In addition, the CRUK-PACE team, set up to understand how CRUK can *Promote an Academic Culture of Entrepreneurship* within our research community, continues to develop its programme to promote an academic culture where entrepreneurship is incentivised, enabled and rewarded. Their activities range from training-focused programmes, in partnership with the NorthByNorthwest Partners (NxNW) lean launch programme ICURe light, and the Eureka Institute's translational school, to annual innovation summits designed to connect researchers with the local entrepreneurial ecosystem, as well as the annual Cancer Research Horizons Innovation and Entrepreneurship awards ceremony. As part of this initiative, we partnered with the Alderley Park Oncology Development Programme, a national accelerator programme designed to develop and progress start-up oncology projects. Two projects from the CRUK Manchester Institute, led by Professor Richard Marais and Professor Caroline Springer respectively, successfully progressed through all stages of the programme, which resulted in two spinout companies being formed to progress both opportunities. Building on this success, CRUK has partnered with the Lean Life Science Oncology Development Programme 2, which will launch in January 2023, to continue supporting the entrepreneurial ambitions of oncology researchers both locally and nationally.

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 Santiago Zelenay's group, assets from the Cancer Biomarker Centre, and 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.

Lung large cell carcinoma, 20x whole tissue. CD56 staining (brown neuroendocrine marker) on neuroendocrine tissue, counterstained with haematoxylin.

Image supplied by Victoria Fife (Cancer Biomarker Centre)



POSTGRADUATE EDUCATION

Postgraduate Education Manager
Julie Edwards

Postgraduate Tutor
Angeliki Malliri[1]

Postgraduate Tutor
Santiago Zelenay[2]

Postgraduate Director and Chair of the Education Committee
Tim Somervaille

[1]Stepped down in 2022
[2]Appointed in 2022

The Cancer Research UK Manchester Institute offers PhD postgraduate degrees 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 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 great majority continuing in academia, industry or healthcare, and securing positions in destinations across the UK, Europe and the USA. In 2022, 100% of our graduates found positions following PhD completion: academic (32%), scientific industry (50%) and return to clinical training (18%).

In 2022, we welcomed seven new graduate students and three clinical research fellows to our PhD programme, working in a variety of fields including cancer immunology, leukaemia biology, cancer biomarkers, and translational oncology.

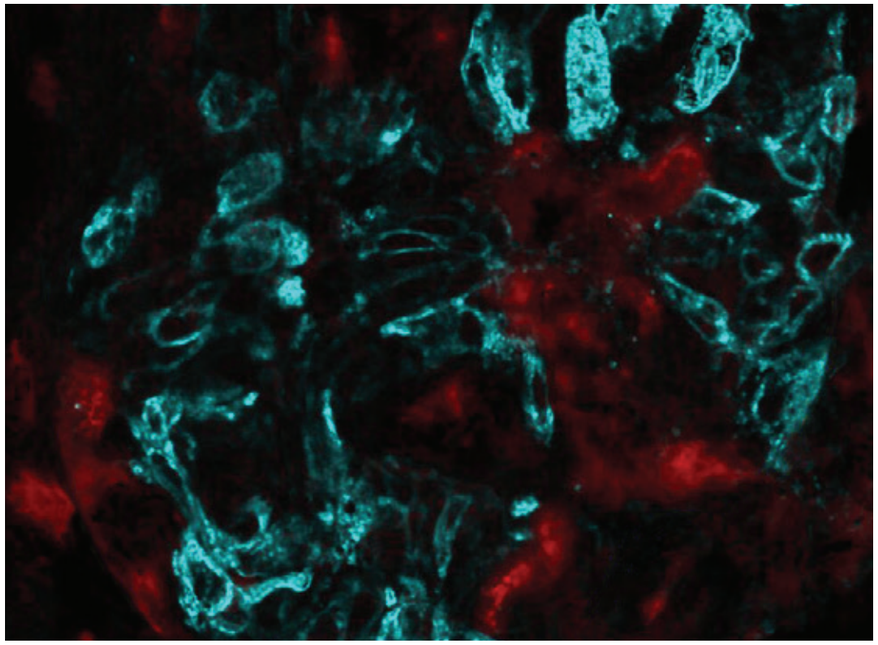
It was also particularly gratifying to see that, over the past twelve months, some of our PhD students and clinical research fellows had published first author papers in a variety of journals including *Nature Communications, Heliyon, European Urology and Cancers (Basel).* A first author review article was also published in *Clinical Cancer Research* and a comment in *Cell Stress*.

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 the student and the project can be monitored but are also critical in the development of presentation skills fundamental to 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 overleaf). 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 – similar to a personal tutor on an undergraduate programme – 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



COX-2 positive tumour cells surrounded by macrophages. COX-2 is expressed by tumour cells (blue) to promote cancer growth and progression. Tumour associated macrophages (red) mingle between tumour cells to facilitate this process.

Image supplied by Victoria Fife (Cancer Biomarker Centre)

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 a series of internal research seminars given by our scientists and PhD student attendance is also an integral part of their learning. 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 and important role in connecting and networking with colleagues across the Institute, nationally and internationally and this year we saw many of them return to in person events.

Being connected with peers and colleagues is a key component for our students' programme, not only in terms of research progress, but as a recognised way to sustain good mental health and wellbeing. A programme of in-house training events, external and internal seminars continued to be delivered in hybrid format, providing the opportunity to attend in-person or virtually, encouraging connection and engagement of students throughout home/hybrid working patterns. The Institute and Education Committee recognises the impact of recent restrictions on the mental health and wellbeing of our students and has worked hard to identify areas where additional support is necessary.

The CRUK Manchester Institute student research and activities continues to thrive, both virtually and in person, providing a continued and important basis for expanding knowledge. Our PhD students have adapted extremely well to the virtual/hybrid approach to giving their talks, PhD pre-viva seminars and PhD vivas.

STAy (Science TakeAway) is a committee group run by junior scientists and students in the Institute, with the aim of providing a forum for discussion and training related to research, communication, scientific engagement, and development of social and networking opportunities. The STAy Committee is keen to

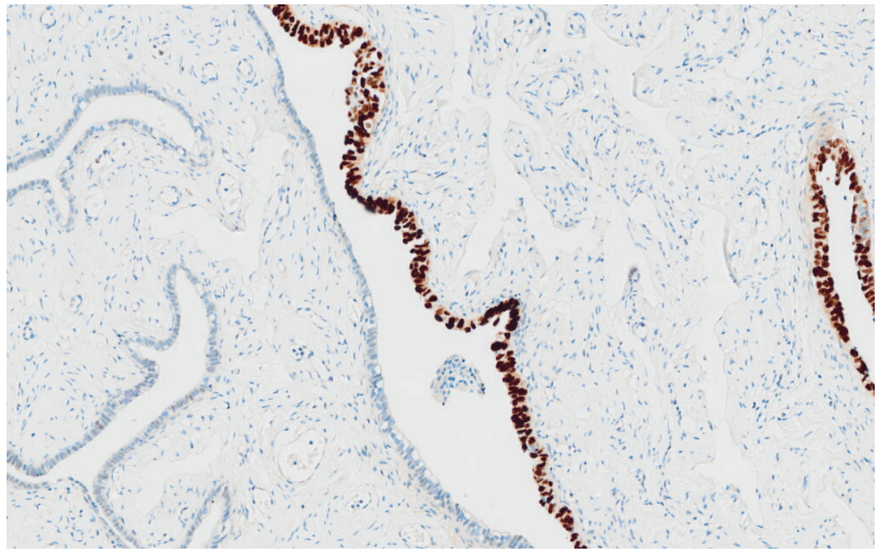
encourage networking, career progression and personal growth of early-career researchers ensuring the research community remains well connected. Activities over the last 12 months have included organising external seminar lecturers, evening welcome and social events, STAy outdoor picnics and lunchtime gatherings, and the successful ReproducibiliTea journal club that meet to discuss diverse issues, papers and ideas about improving science, reproducibility and Open Science. These activities have encouraged our students, scientific staff and postdocs to engage and connect with each other.

The CRUK Manchester Institute Colloquium took place in October at the Conference Centre in Alderley Park this year, the first time the meeting has been held in person since 2019. The event is an excellent opportunity for our new intake of PhD students to meet other established students, members of the Institute, including group leaders, postdoctoral fellows, and scientific officers. This forum communicates the latest science in the form of oral presentations given by group leaders and second year PhD students, as well as poster presentations from a range of scientists across the Institute, covering all aspects of cancer research. We congratulated clinical research fellow Melissa Frizziero from the Cancer Biomarker Centre, who was awarded the Lizzy Hitchman best poster prize for her project on preclinical models to inform treatment opportunities for patients with extra-pulmonary neuroendocrine carcinoma.

Cancer Research UK contributes towards an annual International PhD Student Cancer Conference (IPSCC) allowing high calibre students (typically in 2nd - 4th years) from 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, CRUK Cambridge Institute, CRUK Beatson Institute, Netherlands Cancer Institute (NKI), European School of Molecular Medicine, Milan (SEMM, IFOM & IFEO), Max Delbruck Centre (MDC), Berlin and the German Cancer Research Centre (DKFZ).

The International PhD Student Cancer Conference brought students together in June 2022 at the 15th annual conference hosted by students from the DKFZ Heidelberg, Germany. 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.

The Institute was represented by 19 students from years one to four. There were 31 talks in total, and four excellent talks were given by CRUK Manchester Institute students:



Human fallopian tube tissue stained for p53 showing serous tubal intraepithelial carcinoma lesions - epithelial cells transformed into pre-cancerous lesions. Unstained normal healthy epithelium visible.

Image supplied be Melanie Seaton (Genome Stability Lab, Division of Cancer Sciences, The University of Manchester) with credit to Garry Ashton (Histology Core Facility)

- Katherine Moran from Cell Plasticity and Epigenetics: "Exploring non-genetic heterogeneity and plasticity in isogenic cancer models... one cell at a time".
- Felix Heider from Systems Oncology: "Fibroblast-regulated immune clearance of pancreatic cancer cells".
- Maria Koufaki from Cancer Inflammation & Immunity: "Characterisation of a newly identified intratumoural dendritic cell population".
- Lucy Ginn from Cell Signalling: "Evaluation of RAC signalling as a therapeutic target in non-small cell lung cancer".

Prizes were awarded at the end of the conference for the best oral presentation and the top two posters from ~104 entries. We are delighted that 2nd year student Bianca Blochl, from the Cell Plasticity and Epigenetics group, won one of the best poster prizes showcasing her work on "interplay of non-genetic mechanisms and oncogene-induced transformation".

We are looking forward to joining the students at the CRUK Cambridge Institute at the 16th IPSCC in June 2023.

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 have 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, and recruitment rounds were held in person throughout 2022. We welcomed seven new PhD students and three clinical research fellows in September 2022 and January 2023.

PhD studentships and clinical fellowship funding in 2022 were awarded to the CRUK Manchester Institute core funded groups via the core CRUK funding to the Institute, Cancer Research UK Manchester Centre Clinical Training Fellowships, the Lung Cancer Centre of Excellence, biopharmaceutical group Sosei Heptares, and the University's Faculty of Biology, Medicine and Health split PhD programme collaborations between Manchester-Melbourne (Global Doctoral Research Network (GOLDEN) Dual Award).

Our students benefit from access to advanced state-of-the-art facilities, including advanced imaging, mass spectrometry, flow cytometry, histology and next generation sequencing. CRUK Manchester Institute research groups offer PhD studentships and projects covering a wide breadth of cancer research, currently based over two sites: Alderley Park in Cheshire and the Oglesby Cancer Research Building in Manchester.

Education Committee 2022

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
Angeliki Malliri, Postgraduate Tutor[2]
Santiago Zelenay, Postgraduate Tutor[1]
Julie Edwards, Postgraduate Manager
Carlos Lopez-Garcia
Claus Jørgensen
Elaine Kilgour[2]
Georges Lacaud
Amaya Virós
Caroline Wilkinson
Mark Williams[1]

Student Representatives

Melissa Frizziero[2]
Victoria Fife
Seung Lee[1]

[1]Joined in 2022
[2]Left in 2022

THESES

Luke Chisholm
Molecular Oncology

The role of UVR-induced DNA damage in melanomagenesis

Zaki Fadlullah Wilmot
Stem Cell Biology

Deciphering blood cell development one cell at a time

Pablo Garcia Martinez
Molecular Oncology

Molecular analysis of poor prognosis melanoma, from UVR exposure to visceral metastasis

Konstantinos Georgiadis
Systems Oncology/ Cancer Biomarker Centre

The phenotypic and genetic landscape of circulating tumour cells in pancreatic ductal adenocarcinoma

Christian Bromley
Cancer Inflammation & Immunity

The dual role of inflammation in cancer progression and immunotherapy response

Callum Hall
Tumour Suppressors

p53 Mutations in small cell lung cancer

Alexander Du Feu
Prostate Oncobiology

Investigating the biology underpinning castration resistance in LY6D+ prostate epithelia

Charlotte Bell
Cancer Inflammation & Immunity

Manipulating the dark side of chemotherapy induced inflammation to unleash anti-cancer immunity

Francesco Camera
Leukaemia Biology

An investigation of IRX3 in acute myeloid leukaemia

Zoe Lee
Cell Division

Regulation of protein phosphatase 1 by phosphorylation

Sarah Craig
Skin Cancer & Ageing

Sun damage and skin cancer: the impact of epidermal solar ageing on skin cancer behaviour and chemoprevention

Alicia Conway
Cancer Biomarker Centre

cfDNA multi-modal profiling to detect tissue of origin and direct therapy in cancer of unknown primary: Taking the 'U' out of 'CUP'

Hannah Reed
Cell Signalling

The role of Rac1 activator STEF/Tiam2 in non-small cell lung cancer

THESES (CONTINUED)

Zena Salih
Molecular Oncology

Personalising immunotherapy in early and advanced stage melanoma

Ewan Selkirk
Stem Cell Biology

Long non-coding RNAs in AML

Felix Heider
Systems Oncology

Regulators of fibroblast function in pancreatic cancer

Amelia Jones
Cell Division

The role of phosphorylation in the regulation of protein phosphatase 2A

Jack Ashton
Translational Oncogenomics

Mechanistic studies of hypoxia as a driver of genomic instability in prostate cancer

Shilpa Gurung
Skin Cancer & Ageing

The young and aged cutaneous adipocyte lipid cues that shape melanoma metabolism, metastatic fitness and tropism

Ryan Guilbert
Cell Signalling

The role of RAC1 signalling and cell-cell adhesion in the survival of SCLC cells

Alexandru Suvac
Translational Oncogenomics

Studies in genetic instability under hypoxia in prostate cancer

Alessia Catozzi
Cancer Biomarker Centre

Characterisation of heterogeneity in neurogenic transcription factor expression in small cell lung cancer

Katherine Moran
Cell Plasticity & Epigenetics

Exploring non-genetic information, cell plasticity and heterogeneity in isogenic cancer models

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 able to get out and meet people face to face.

An important day in our calendar and a galvanising start to the year, World Cancer Day is held annually 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.

We all have a role to play in reducing the global impact of cancer and our researchers used World Cancer Day as a platform to discuss the impact of inequalities on global health. The Institute's STAy (Science Takeaway) group – comprising postdocs, PhD students and scientific officers – commemorated the occasion by hosting 'Research with a Global Outlook', highlighting the disparity in cancer treatment for patients in low and middle-income countries. Organised by PhD student Cath Felton, they discussed how global inequalities impact cancer research. Keynote speaker David Wedge from The University of Manchester explored his work on understudied populations, in particular the genomics of breast cancer in Nigerian women, and sparked conversations about the need to improve racial and ethnic representation in data if we are to address differences in presentation, progression, and overall biology of cancer in these groups. STAy have now been motivated to explore this theme further and include it in their programme for next year.

Images, top: Institute scientists amazed children at The Barlow RC High School. Left to right: Scientific Officer Joanne Kelly, PhD student Alexandru Suvac, Head of Biological Mass Spectrometry facility Duncan Smith, and PhD student Mihaela Ficu; bottom: The team of scientists engaging pupils at Ladybarn Primary School. Left to right: PhD student Bradley Revell, postdoc Bettina Wingelhofer and Research Associate Ana Vitlic.

Helping to inspire the next generation of cancer researchers is an important 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.

During British Science Week, a team of researchers from the CRUK Manchester Institute and The University of Manchester's Division of Cancer Sciences visited three local primary schools. Over three days, they organised fun and interactive sessions for the school children, giving them an insight into the work conducted here and enthusing them about research and jobs in science. The young students were inspired by our researchers' personal journeys into cancer research and enjoyed getting hands on extracting DNA from strawberries. The feedback from the schools was overwhelmingly positive and notably commented that pupils could relate to the researchers learning paths and made it seem a realistic achievement.

On the back of this positive experience, several of our researchers then participated in 'I'm a Scientist – Get me out of here!', a free online event where school students can interact with scientists. Students challenge the scientists over intense, fast paced online live chats where they ask any questions they want and get to vote for their favourite scientist. This activity reaches out to students from underserved schools who otherwise wouldn't get to meet people working in STEM. Our researchers answered many questions, noting that the students were keen to ask and learn all they could about cancer research, as well as gathering advice for their own career and education journeys. Special mention goes to postdoc Tim Budden, from the Skin Cancer and Ageing group, who was voted 'Scientist of the Week' in week 2.

Tim Budden wins students' choice 'Scientist of the Week'.

Carrying on with the theme, in a joint activity with the Cancer Research UK Beatson Institute in Glasgow, we were delighted to invite a number of schools to a 'work experience week' organised across the two Institutes. Researchers from both Institutes delivered six virtual sessions to schools across the Greater Manchester and Glasgow areas, as well as other parts of the UK. We were also pleased to be able to host site visits for school students for the first time since 2020. Manchester Cancer Research Centre and CRUK MI scientists welcomed to the Oglesby Building four schools from across Greater Manchester to a 'taster day', designed to bridge the gap between A-level studies and science as a career researcher. The students enjoyed a science-packed day learning about cancer research in a modern facility.

Fundraising for Cancer Research UK is fundamental to our work, so we greatly appreciate the efforts and donations to the charity.

The Institute's Chief Operating Officer, Caroline Wilkinson, visited the CRUK Crewe and Nantwich Fundraising Committee to update them on the Institute's progress. Caroline has been attending the committee's annual general meeting for several years and was delighted to be able to meet the group again in person this year. They carry out an extraordinary amount of work each year and have raised huge sums of money for cancer research over many years, so it was a perfect opportunity to thank them and explain how their funding is making an impact.

When Cancer Research UK welcomed supporters to an in-person event at the Science and Industry Museum in Manchester earlier this year, our Institute scientists jumped at the chance to get involved. It was a wonderful opportunity to meet the people who have supported our work for many years – including through the difficult times of the pandemic – and to hear about their experiences. PhD student Kirsty Tinsley, led interactive demonstrations at the event, explaining some of the science behind our research, while Andrew Porter, our Research Integrity and Training Adviser, shared some recent scientific achievements as well as the exciting plans for the new Paterson Building.

We recognise it is important to have honest conversations about the experience of a cancer diagnosis and treatment to the public. So, we were excited when in October 2021, the Science Museum Group in partnership with CRUK launched 'Cancer Revolution: Science, Innovation and Hope' at the Science and Industry Museum in Manchester. This ground-breaking exhibition was developed with people living with cancer and featured research from the CRUK Manchester Institute. We were proud that the Science & Industry Museum won Best Exhibition at the 2022 Manchester Culture Awards.

Looking ahead to 2023, we are excited about welcoming more schools, CRUK supporters and fundraisers into our new Paterson Building and showcase the outstanding science being undertaken at the CRUK Manchester Institute.

Images, bottom left: PhD student Parsa Parhady shows an A-level student how to use a pipette, bottom centre: Head of Flow Cytometry Toni Banyard explains fluorescence activated cell sorting (FACS) analysis to the students, bottom right: Iqra Choudhry, CRUK Research Engagement Manager for North of England (third in on the right), collects the Best Exhibition Award on behalf of CRUK.

ACKNOWLEDGEMENT FOR FUNDING FOR THE CANCER RESEARCH UK MANCHESTER INSTITUTE

The total funding of the CRUK Manchester Institute for 2022 was £23.8m. The major source of this funding was awarded by Cancer Research UK (CRUK) via a core grant of £11.6m plus additional strategic funding of £2.6m. This funding enables the various scientific groups and service units within the Institute to carry out their research.

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

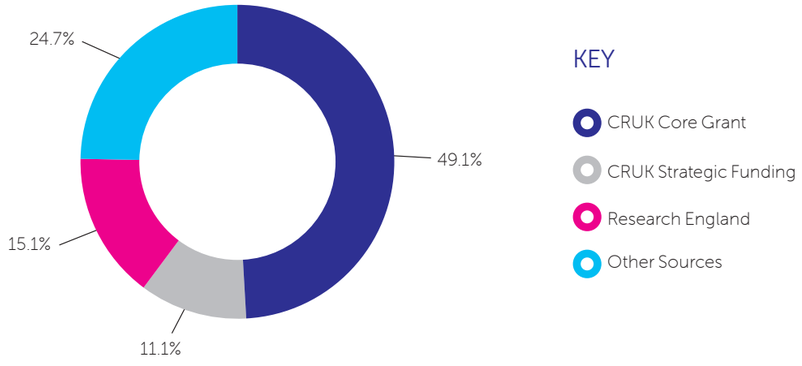
The balance of the Institute's funding is received from a number of additional sources. The research carried out through these additional projects enhances and supports the research undertaken by the core funding.

These sources are as follows:

- Amgen
- Angle Inc
- Astex Pharmaceuticals
- Astra Zeneca
- Bioven
- Bloodwise
- Boehringer Ingelheim
- Carrick Therapeutics
- CellCentric
- Christie Hospital NHS Foundation Trust
- Clearbridge Biomedicals
- CRT Pioneer Fund
- David & Ruth Lewis Trust
- Euclises Pharmaceuticals Inc
- European Commission
- European Organisation for Cancer Research and Treatment of Cancer
- European Research Council
- Fondation ARC pour la Recherche sur le Cancer
- GlaxoSmithKline
- Harry J Lloyd Charitable Trust
- Institut de Cancerlogie Gustave Roussy
- Kay Kendall Leukaemia Fund
- Leo Pharma Foundation
- Medical Research Council
- Medimmune LLC
- Menarini Biomarkers Singapore
- Merck
- Moulton Charitable Trust
- My-T Bio Ltd
- National Institute of Health Research
- Neuroendocrine Cancer UK
- Ono Pharmaceuticals
- Pancreatic Cancer Research Fund
- Perfusion Biotech
- Pickering Leukaemia Research
- Prostate Cancer UK
- Rosetrees Trust
- 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 2022



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.

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.

LOOKING TO THE FUTURE: THE PATERSON REDEVELOPMENT PROJECT PROGRESS

As we reach the end of the year, we look forward to 2023 and the completion of our new building, which replaces the old Paterson building that was damaged by a significant fire in 2017.

Ten storeys of glass-fronted building will give superb views across Greater Manchester from inside, while creating a wonderful variety of skyscape reflections from outside.

In April 2017, a devastating fire resulted in the CRUK Manchester Institute having to relocate 14 miles to temporary premises at Alderley Park. While we have made this a great home for our scientists and support staff - enabling us to continue our fantastic research - we are eagerly awaiting the return to our original site.

The bridge will link us directly to the Christie NHS Foundation Trust, enabling clinical samples to reach our scientists more efficiently.

The Paterson Redevelopment Project (PRP) has been led and managed by the three partners – The Christie NHS Foundation Trust, Cancer Research UK and The University of Manchester. Together, they created an ambitious vision of the future of cancer research in Manchester and planned to build a brand new, globally leading cancer research facility.

Being in the new facility will not only reunite us with our colleagues, but also fellow researchers in The University of Manchester's Division of Cancer Sciences - based in the Oglesby Cancer Research Building - and importantly, our clinical colleagues at the Christie NHS Foundation Trust.

At over 25,000m^2 and ten storeys, this new comprehensive cancer facility will be more than twice the size of the building it replaces. It will house 300 scientists, 400 clinicians and operational staff that will enable us to truly practise 'team science'.

Left: Work continues on the striking entrance that will look up through three storeys. Right: Open plan space to locate all Operations teams together to streamline support of the Institute. This area will be home to HR, Scientific Operations, and Finance.

Our new building will bring together on one site the largest concentration of researchers, clinicians and allied healthcare professionals in Europe, which will foster more powerful collaboration between these specialists. As a result, joint research programmes will be stimulated that tackle some of the most important and hardest challenges in cancer detection and care, helping to accelerate progress for cancer patients in Manchester and across the world.

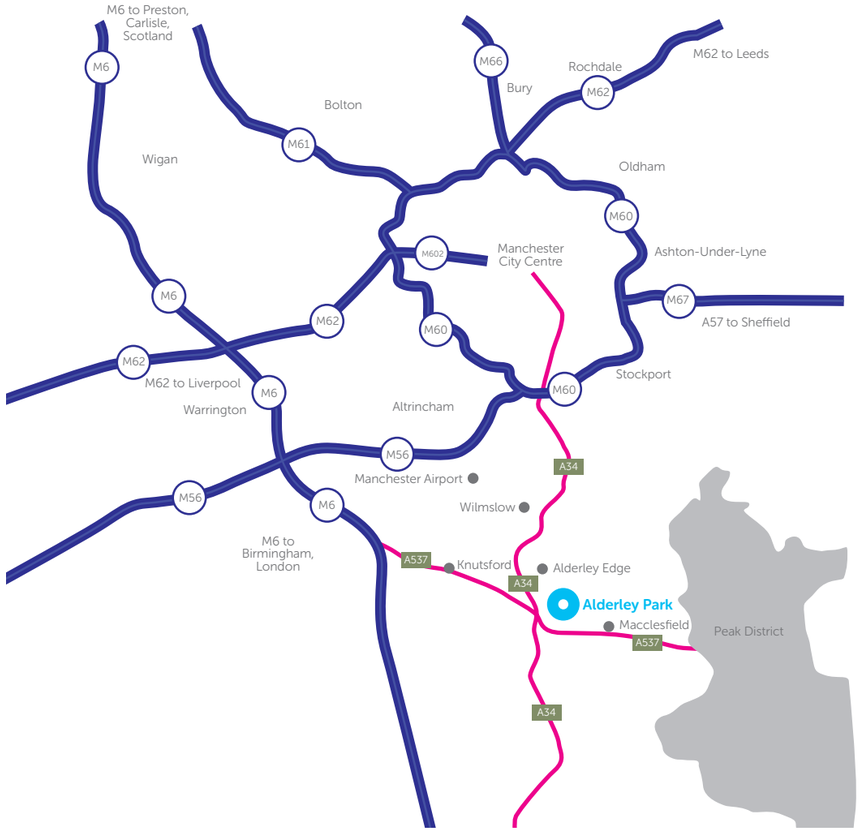
The new building will be filled with state-of-the-art equipment and with the research it supports will attract and retain the best talent from around the world, which will help deliver solutions to cancer more quickly. And as part of the project to build the new Paterson Building, we have had the opportunity not only to create inspirational laboratory spaces suitable for the developing research requirement but also to create motivating spaces for office areas and collaboration, making it a superb place to work.

We are pleased to report that construction is progressing apace, and we are on track to move in and start our first experiments in the first half of 2023. But we recognise that we would not be here without the generous gifts from donors and funders around the world, so we would like to express our enormous thanks for their crucial support, it really is making a difference. And going on in the background has been an incredible amount of hard work by several key members of staff in the planning of our laboratories and all our facilities. So, we thank them for their hard work as well as the patience and resilience of all our staff over the past six years. We have been through a lot together, but we are now close to returning home and to our new building. We are excited about the future of cancer research in Manchester.

Clinical colleagues Mark Williams and Tim Somervaille enjoy a preview of their new working space on the 5th floor. Both focus on leukaemia, so locating their research groups together, along with groups with similar interests, will facilitate the sharing of ideas and development of rich collaboration.

Head of Biological Resources Unit Experimental Services Lisa Doar visits her new facility. Lisa and colleagues have helped with the detailed planning of our animal facility, which will enable us to operate at the highest standards of animal and staff welfare.

CONTACT DETAILS



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Cancer Research UK
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www.cruk.org

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