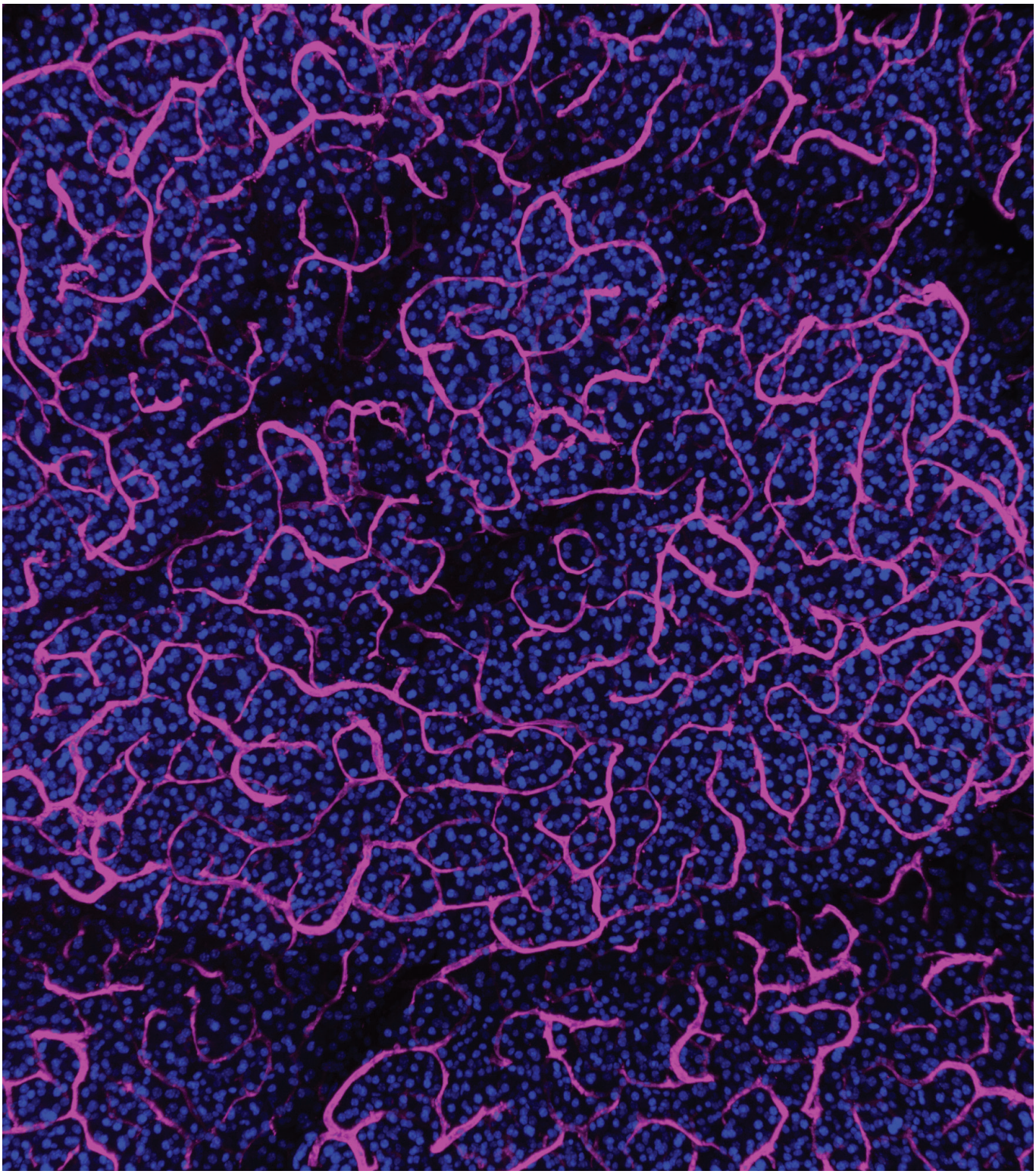
CANCER RESEARCH UK | Scotland Institute

SCIENTIFIC REPORT 2024



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COVER IMAGE

The intricate vascular maze of the pancreas (blue: cell nuclei; pink: blood vessels).

Image credit: Ximena Raffo Iraolagoitia

SCIENTIFIC REPORT 2024

SCOTLAND INSTITUTE

University of Glasgow

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Cancer Research UK Scotland Institute *Image by Laura Ashman*

DIRECTOR'S INTRODUCTION

Director of the Cancer Research UK Scotland Institute

Professor Owen Sansom

FRSE, FMedSci, FRCPS(Glasg)

There were many exciting and important developments at the CRUK Scotland Institute this year. Through joint funding with the University of Glasgow, we made two key strategic appointments. Professor Srikala Raghavan (Chair of Epithelial Immunometabolism) started in October, further strengthening our immunology theme and Professor Ram DasGupta (Chair of Liver Cancer) will join in January 2025, bringing considerable computational and single cell biology expertise to the liver theme. We also now jointly fund Dr Ke Yuan, who brings expertise in artificial intelligence (AI) and machine learning and is working with Professor Crispin Miller to develop our AI/deep learning strategy.

We said farewell to two of our group leaders. Dr Saverio Tardito left the Institute in September to join the Medical University of Vienna as an assistant professor, while in November Professor Sara Zanivan took up an exciting position at the MD Anderson Cancer Center in Texas. We thank both for their contributions and wish them all the best for the future. In March, we held a symposium to celebrate the work of Professors Laura Machesky and Robert Insall while at the Institute. This was a fantastic opportunity to hear about their important contributions to our understanding of metastasis and welcome back some their former postdocs and students.

We published our scientific strategy (see page 6) and developed structures and processes for its delivery, including a Scientific Strategy Group, reporting six-monthly to our Board of Directors, and working groups focused on delivering on specific topics. Collective responsibility for strategy is enabled by the involvement of all our faculty in at least one of these working groups, which also include early career researchers.

We congratulated Professor Martin Bushell and his group on a highly successful QQR in March with a score Forefront/Outstanding and both Drs Leo Carlin and David Lewis and their groups on their successful promotion to Senior Staff Scientist in April. We also held one-year check-in reviews with our new research fellows, Drs Zoi Diamantopoulou, Xiao Fu and Kendle Maslowski, who have all settled in well and in June heard that Zoi's UKRI Future Leaders Fellowship application 'Unleashing the power of the circadian rhythm to tackle metastasis' had been successful. We also congratulated final year PhD students Jasmine Peters and Bianca Bochl who shared this year's John Paul Career Award.

Importantly, we made excellent progress in targeting additional external funding to support our key research areas and equipment needs. Our CRC-STARS proposal to CRUK 'Colorectal Cancer - Stratification of Therapies through Adaptive Responses' led by me and Professor Simon Leedham (Oxford) was funded. This aims to use tissue samples and disease positioned models to interrogate adaptive CRC therapy response and cellular plasticity and inform the design of more effective treatment strategies. The second tranche of MRC funding for the mouse data platform was released and Professor Crispin Miller and postdoc Dr Holly Hall were awarded a NC3Rs computational infrastructure grant, while Professor Jim Norman was awarded an MRC Programme Grant and Professor Ram DasGupta a Royal Society Wolfson Fellowship. Our joint application to the MRC National Mouse Genetics Network Business Engagement Fund with Oxford Drug Design, a biotechnology company with core expertise in AI drug discovery, was successful, as were equipment to the MRC for a MALDI matrix sprayer system, the Beatson Endowment for a Lunaphore spatial biology platform and the University of Glasgow and Beatson Cancer Charity for a Stellaromics 3D spatial genomics platform. Gratifyingly, many of our early career researchers were also successful with smaller grant applications and in total were awarded ~£0.57 million.

We worked closely with University of Glasgow leadership to address the requirements for an upgrade to our BRU/BSU and capitalise on the opportunity to expand our spatial biology and computational biology capabilities. This has resulted in the Matrix building concept, which will provide world-leading facilities in preclinical modelling, imaging and computational and spatial biology (at an estimated cost of £45 million).

We remain committed to promoting equity, diversity and inclusion (EDI) within our community. This year, Sharon Gorman, our Head of People and Culture begun reviewing our EDI action plan and comparing it with best practice across the sector. Driven by PhD student Sarah Williams, we participated for the first time in CRUK's Black Leaders in Cancer PhD studentships call and were pleased by the number of high-quality applications we received. Interviews will be held in early 2025. To engender a great sense of community and inclusivity, we participated in a Pride Picnic and events focused on career development in academia, neurodiversity in the workplace and raising awareness of Ramadan and Diwali. We also held our 2nd Betty MacGregor Memorial Lecture celebrating women in science, given by Professor Ruth Plummer (Newcastle), whose work focuses on early clinical trials and who was central to the introduction of PARP inhibitors. We had a very well received Patient Public Involvement panel discussion at our Institute Retreat and now have a PPI representative on our Board of Directors. In addition, Dr Catherine Winchester was promoted to Head of the Research Integrity Service and has continued to engage with the research integrity community, acting as an advisor to UKCORI and speaking at conferences in Warwick and Athens.

We continued to raise the international profile of the Institute. Our 25th Anniversary Beatson International Cancer Conference 'Mouse Models of Cancer: Cages to Clinic' in July was an outstanding success and saw the inception of The Sir George Beatson Lecture, which was given by Dr Allan Balmain (UCSF), a world leader in mouse models and former Beatson (CRUK Scotland Institute) group leader. Driven by postdoc Dr Susanti Susanti, we also hosted a visit by the Indonesian Ambassador to the UK and signed a memorandum of understanding with the Institute and the Indonesian Ministry of Health. This will allow funding for studentships and identify key projects of interest in mesothelioma and early onset bowel cancer.

OUR VISION AND STRATEGY: APPLYING CANCER DISCOVERY TO PATIENT BENEFIT

At the CRUK Scotland Institute, our overarching vision is to make pioneering discoveries that increase our understanding of cancer; build collaborative, multidisciplinary teams that drive progress towards clinical translation; and train the next generation of diverse and leading cancer researchers.

By understanding the fundamental mechanisms that drive cancer, both at early and late stages of the disease, we aim to uncover new preventative and therapeutic approaches for the benefit of patients. To do this, we are addressing three fundamental cancer challenges:

1) Biology of early disease (How cancers start): How can we detect cancer earlier, identify factors leading to poor prognosis ('bad actors') and design preventative strategies?

2) Energetic needs and metabolism (How cancers grow): What are the novel therapeutic vulnerabilities associated with increased energetic stress in tumours cells; either alone or following chemotherapy?

3) Metastasis and microenvironment (How cancers spread): What are the therapeutic vulnerabilities of metastasising cancers and disseminated disease, and what is the impact of the immune system on metastasis?

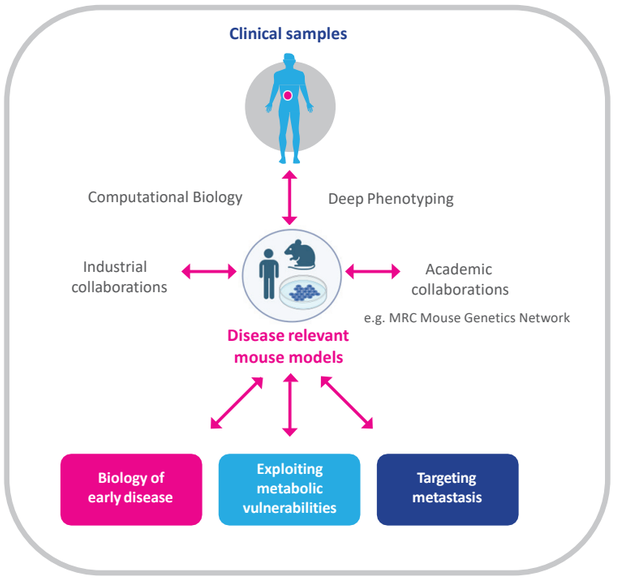
At the heart of our strategy is the generation and use of an unparalleled suite of complex *in vivo* and organoid models that accurately recapitulate critical events in the human disease - such as tumour initiation, growth and metastasis (Figure 1). To achieve this effectively, we have put particular emphasis on ensuring that our in vivo models are extensively and rigorously benchmarked against the appropriate human cancers, their pathology, and co-morbidities – something we term 'disease positioning'. The recent explosion in technologies (including single cell RNA sequencing, spatial transcriptomics and metabolomics, multiplexed imaging and computational biology) to probe tissue deeper than ever before, allows our in vivo models to be comprehensively disease positioned and supports an approach allowing close integration of our mechanistic biology with human disease cohorts (for forward and back translation). In future, a greater understanding of the impact of host physiology could also revolutionise our approach to treating and detecting cancer. Factors such as diet, age, temperature, obesity and microbiome can have profound effects on the initiation and progression of cancer. We believe that by studying these processes in our disease relevant models, we can better understand how they affect tumour biology from a whole-body perspective.

We are focusing our efforts on key tumour types to translate our targeted observations, some of which are defined by CRUK as cancers of unmet need (liver, pancreas, lung), are specifically relevant to our local population (mesothelioma) and/or are high contributors to cancer-related death (colorectal). We are focusing on liver and lung as sites of metastasis and on insights that will arise from cross-comparing primary and metastatic growth. These studies are supported by our excellent technology platforms, which we will need to maintain and invest in to remain state-of-the-art. With the tumour specific focus of CRUK Scotland Centre, we have excellent clinical support to be able to translate our finding from our models into the clinic.

Partnership is a key objective of our strategy, and we believe we are the world's foremost partner of choice for models, imaging and energetic stress collaborations for both academia and industry. Therefore, over the next five years, we aim to extend our research into cancer as a whole-body disease in partnership with the CANCAN (lead Eileen White, co-investigator David Lewis) and OPTIMISTIC Cancer Grand Challenge teams (lead Wendy Garrett) to drive forward our exploration of cancer cachexia and the microbiome, respectively. These programmes of work will allow us to investigate inter-organ communication and the whole organism in both early and late disease. In collaboration with the MRC National Mouse Genetics Network, we are also planning to make a step-change in 'humanisation', phenotyping and therapeutic targeting in cancer models. We are aiming for new alliances with industry in the energetic stress, metastatic targeting and disease-positioning space.

To deliver this exciting and highly relevant strategy, we have defined some specific objectives in areas where we feel we are ideally placed to make a clear impact in the next five years and where we will aim target our efforts and resources:

1. Becoming a centre of excellence for liver cancer and metastasis
2. Making a step change in cancer models
3. Targeting protein turnover to block initiation and progression
4. Targeting energetic stress and tumour microenvironment following radiotherapy
5. Determining the impact of mitochondrial biology in initiation and progression
6. Building a greater understanding of cancer immunology
7. Revolutionising computational biology



RESEARCH HIGHLIGHTS

Copland *et al.*, EMBO Mol Med 2024: Kendle Maslowski's group showed for the first time that *Salmonella* (bacterial) cancer therapy metabolically disrupts tumours at the cost of T cell immunity.

Dearlove *et al.*, Elife 2024: Danny Huang's lab, in collaboration with Martin Bushell, discovered a novel nucleotide ubiquitylation activity of the DTX3L E3 ligase.

Fetit *et al.*, Cancer Research Communications 2024: In the immune/inflammation space, Colin Steele's team and working with many CRUK SI groups characterised neutrophil subtypes in cancer using single cell RNA sequencing and demonstrated the importance of the IL-1 /CXCR2 axis in the generation of metastasis specific neutrophils

Fey *et al.*, Cancer Res 2024: Owen Sansom's lab demonstrated the repressive role of wild-type KRAS during pancreatic tumorigenesis, highlighting the impact of wild-type KRAS on both tumour progression and therapeutic response in pancreatic cancer.

Jans *et al.*, Nature 2024: Johan Vande Voorde and David Sumpton contributed to an important paper from Lars Vereecke's lab in Ghent describing how the oncogenic potential of *E. coli* depends on bacterial adhesion to host epithelial cells, which leads to DNA damage and colorectal cancer development.

Kiourtis *et al.*, Nature Cell Biol 2024: Tom Bird's lab and several other Institute groups demonstrated that senescence induced in the liver can systemically spread to other tissues, which could explain toxicities in those tissues.

Loi *et al.*, Cancer Discovery 2024: As part of the SpecifiCancer Grand Challenge, the Sansom lab working with Karen Cichowski (Harvard) discovered novel epigenetic combinations that can cause tumour regression of KRAS mutant colorectal cancer, a particularly hard to treat group of CRCs.

Lukoszek *et al.*, Cell Reports 2024: Vicky Cowling's group, in collaboration with Ed Roberts, used mass spec to describe an RNA cap methylation control mechanism whereby CK2 controls CMTR1, enhancing co-transcriptional capping during critical transcriptional bursts, such as cell cycle progression or immune defence.

Mahmood *et al.* Nature Cancer 2024: Payam Gammage and Ed Roberts described how tumour mitochondrial DNA mutations drive aerobic glycolysis to enhance checkpoint blockade.

Malla *et al.*, Nature Genetics. 2024: Work from Philip Dunne (Belfast) and Owen Sansom's group used pathway level subtyping to identify a slow-cycling biological phenotype associated with poor clinical outcomes. This study used computational tools that were originally designed to allow better cross-comparison of mouse and human pathway data. On the back of this, there was a large release of our CRC models data (over 1000 samples).

Malviya *et al.*, Clinical Cancer Research 2024: David Lewis and colleagues used multiplexed PET radiotracer imaging to investigate the impact of tumour intrinsic and extrinsic factors on PET imaging signatures, and established the feasibility of non-invasive molecular stratification using multiplex radiotracer PET.

Müller *et al.*, Nature, in press: Tom Bird and Crispin Miller developed and integrated mouse models of liver cancer with human samples to generate a new subtyping for hepatocellular carcinoma (HCC) and then identified subtype selective novel therapeutic regimes.

Najumudeen *et al.*, Nature Communications 2024: A study from Owen Sansom's lab described how KRAS allelic imbalance drives tumour initiation yet suppresses metastasis *in vivo*.

Vringer *et al.*, EMBO Journal 2024: Stephen Tait's lab and others showed mitochondrial outer membrane integrity regulation of a ubiquitin-dependent and NF-κB-mediated inflammatory response.

Xavier *et al.*, Life Sci Alliance 2024: Using super-resolution microscopy, Tom MacVicar's group and others established a new link between cell proliferation and mitochondrial nucleic acid homeostasis.

BACKGROUND

In 1890, Sir George Thomas Beatson, a pioneer in the field of oncology born in Campbelltown on the West Coast of Scotland, was appointed as consulting surgeon at the newly opened cancer hospital in Glasgow. Beatson soon became head of the Institution, and in 1912, established a research department in the hospital.

This department became independent from the hospital in 1967 when The Beatson Institute for Cancer Research was founded by the then Director, Dr John Paul. Dr Paul also raised sufficient funds to move the Institute in 1976 to our present location on the Garscube Estate in Glasgow.

Prof John Wyke became Director in 1987 and worked to develop links between the Beatson Institute and the University of Glasgow – specifically the departments of Medical and Radiation Oncology. In 1990, Glasgow University researchers moved to adjacent refitted accommodation. More recently, other teams with university affiliations have moved here to share laboratory facilities with us and, in 2013, to the adjoining Wolfson Wohl Cancer Research Centre. The resulting School of Cancer Sciences provides a cutting-edge research environment situated in the beautiful, leafy green Garscube Estate on the north-western edge of Glasgow.

In September 2023, the Institute changed its name to the Cancer Research UK Scotland Institute in recognition of its position as a national centre of excellence and to enable global wider recognition, following Cancer Research UK's announcement of their biggest ever investment in Scotland which will be awarded to the Institute over the course of 7 years.

Sir George Beatson
1848 - 1933

Cancer Research UK
Scotland Institute

CANCER RESEARCH UK SCOTLAND INSTITUTE

RESEARCH GROUPS

MODELS OF ADVANCED PROSTATE CANCER

Group Leader
Imran Ahmad

Professor of Urological Oncology (CRUK Scotland Institute/University of Glasgow)
Consultant Urological Surgeon (NHS Greater Glasgow & Clyde)

Research Scientist
Richa Vasan

Graduate Students
Poppy Brown (PhD)[1]
Zhangdong Jiang (PhD)[2]

[1]Funded by The Beatson Cancer Charity
[2]Funded by the China Scholarship Council

Prostate cancer is a leading cause of cancer mortality in men in the western world. Identifying and understanding the pathways that drive advanced and treatment-resistant prostate cancer will provide important information that will allow prognostication and individualised patient treatments.

Our current research interest lies in understanding the mechanisms of treatment resistance in advanced prostate cancer. Work in our lab together with the Leung group uses state-of-the-art *in vivo* models in conjunction with patient samples to interrogate the disease processes in advanced and treatment-resistant prostate cancer. This work will help to provide information on drivers of prostate cancer progression and to identify novel biomarkers of disease and/or drug targets to treat the disease.

As an Honorary Consultant Urological Surgeon based at the Queen Elizabeth University Hospital in Glasgow, I have one of the highest-volume robotic prostatectomy practices in the UK for patients with aggressive and locally advanced prostate cancer, allowing me to keep my translational research clinically relevant.

Sleeping Beauty screen reveals Pparγ activation in metastatic prostate cancer

Using a murine forward mutagenesis screen (Sleeping Beauty) in a PtenNull background, we were able to identify the gene peroxisome proliferator-activated receptor gamma (Pparγ, which encodes a ligand-activated transcription factor), as a promoter of metastatic prostate cancer. PPARγ is a critical regulator of fatty acid and glucose metabolism, influencing lipid uptake and adipogenesis. In our model, upregulation of PPARγ was associated with an activation of lipid signalling pathways, including upregulation of lipid synthesis enzymes (fatty acid synthase (FASN), acetyl-CoA carboxylase (ACC) and ATP citrate lyase (ACLY)), resulting in aggressive prostate cancer.

As a proof of principle, we were able to demonstrate that inhibition of PPARγ suppressed tumour growth *in vivo*, with downregulation of the lipid synthesis programme. We showed that elevated levels of PPARγ strongly correlated with elevation of FASN in human prostate cancer and that high levels of PPARγ/FASN and PI3K/pAKT pathway activation conferred a poor prognosis, with these patients succumbing to their disease up to five years earlier.

Our data suggests that prostate cancer patients could be stratified in terms of PPARγ/FASN and PTEN levels to identify patients with aggressive prostate cancer who may respond favourably to PPARγ/FASN inhibition (low PTEN/high pAKT expression); a finding that has potential to guide the design of future clinical trials. Ongoing research by our group has demonstrated that this lipid synthesis phenotype may be driven through alterations in mitochondrial function and AKT3 activations. Our new graduate student, Zhangdong Jiang, will work closely with the MacVicar lab to look at the role of mitochondrial metabolism in the PPARγ mutant tumours.

In addition, to our knowledge, we were the first to demonstrate the strength of the Sleeping Beauty transposon model system in successfully determining low-frequency somatic mutations that may drive prostate tumorigenesis. We are further investigating and validating other novel and clinically relevant 'hits' from this screen.

Identification and validation of new therapeutic targets in castrate-resistant prostate cancer

Androgen receptor aside, current treatment for advanced prostate cancer remains non-targeted. The development of targeted therapies has been hampered by a paucity of genes and pathways identified to be responsible for prostate cancer progression.

We aim to identify novel genes and pathways in castrate- and enzalutamide-resistant prostate cancer (CRPC and ERPC, respectively). We are using an unbiased insertional transposon mutagenesis screen (PiggyBac) and then validating the top genes of interest in patient-derived samples. Validating these genes in mice and humans will allow us to discover new pathways that can be targeted in patients with CRPC and ERPC.

Using cross-species oncogenomics, we will overlay identified genes with those from human sequencing projects, allowing better stratification of the human somatic mutational landscape into 'driver' and 'passenger' events. Once validated, candidate genes will provide insight into the biology, as well as offering potential diagnostic, prognostic and therapeutic targets in advanced disease, and offering insight into the mechanisms of CRPC and ERPC. Richa Vasan and Poppy Brown in the group are currently working on validating targets from this screen in the Wnt and immune landscapes respectively.

Role of Arid1a in prostate cancer

ARID1A was also identified as a potential driver in prostate cancer by the Sleeping Beauty screen. ARID1A is part of the BAF complex, and functions as a key regulator controlling DNA accessibility and organisation by chromatin remodelling. The BAF complex itself is highly mutated in metastatic prostate cancer. Including mRNA alterations, the BAF complex is mutated in 60–70% of metastatic prostate cancer cases (Figure 4). The potential for therapeutically targeting the BAF complex in prostate cancer is reviewed in our recent publication. Our former graduate student Andy Hartley has investigated the role of ARID1a loss in driving prostate cancer using genetically engineered mouse models, and successfully defended his thesis in 2023.

Role of MBPTS2 in prostate cancer

Membrane-bound transcription factor site-2 protease (*Mbtps2*), which was also identified from our Sleeping Beauty screen and demonstrated to be associated with metastatic prostate cancer *in vivo*. Regulated intramembrane proteolysis (RIP) plays an integral role in maintaining multiple cellular pathways. The most well described RIP pathway is carried out by serine proteases, S1P (site-1 protease) and MBTPS2. The sequential cleavage of membrane spanning proteins results in the release of mature N-terminal fragments that can shuttle to the nucleus and function as transcription factors. Among reported S1P and MBTPS2 targets are the sterol regulatory element binding proteins (SREBPs) and the activating transcription factor 6 (ATF6).

Our group has been working on characterising its role in cholesterol uptake and synthesis along with regulation of fatty acid synthesis in metastatic prostate cancer.

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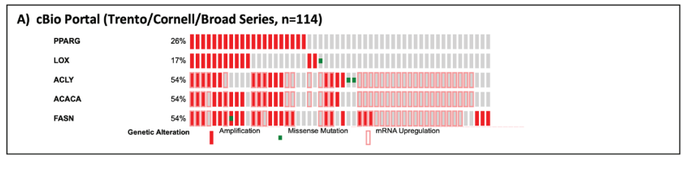


Figure 1. Data from cBio portal (www. cbioportal.org) demonstrating PPARγ gene amplification or its upregulated mRNA expression in 26% of clinical castrate-resistant prostate cancer specimens, with upregulation of one or more of the lipid synthesis genes (FASN, ACC, ACLY)

Figure 2.

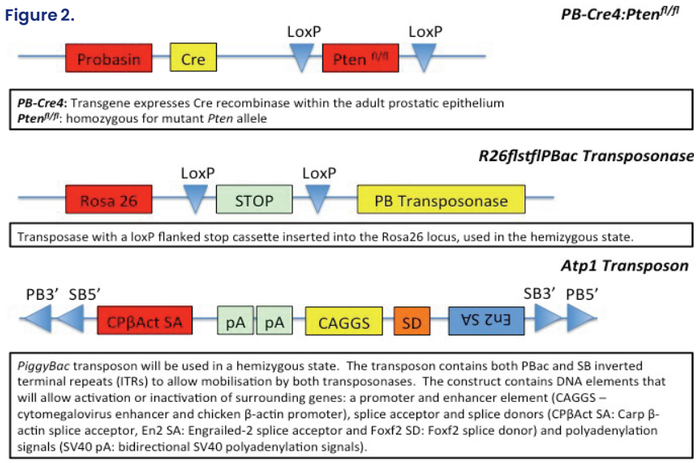


Figure 3.

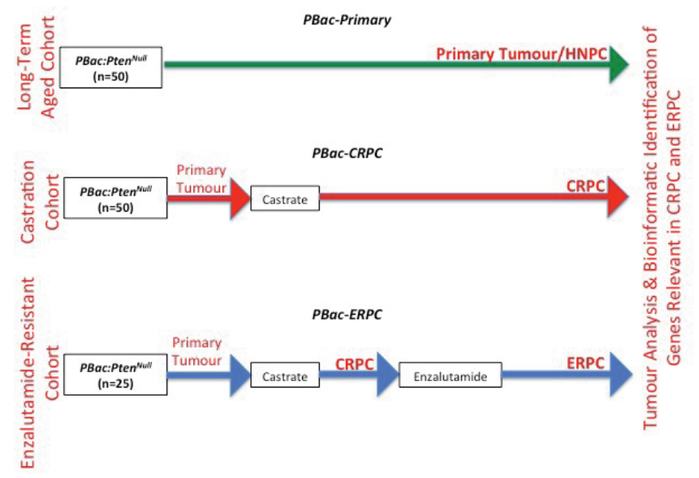
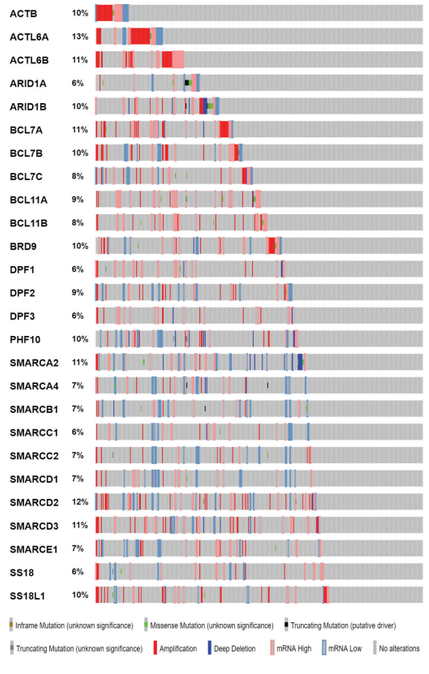


Figure 2. Genetic modifications of the PiggyBac mice.

Figure 3. Experimental design for the ageing, castration and enzalutamide-treatment of the PiggyBac (PBac) mice.

Figure 4. Mutations in the BAF complex in metastatic prostate cancer



LIVER CANCER FOLLOWING DISEASE AND ATTEMPTED REGENERATION

Group Leader
Tom Bird

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Honorary Consultant Hepatologist
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George Skalka
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Megan Quince
Danis Thomas
Kyi Lai Yin Swe
Ciara Cullen

THE UNIVERSITY of EDINBURGH

Liver cancer is now the third most common cause of cancer-related death worldwide; with a trebling in incidence in the UK in the last 25 years. This is driven by underlying liver diseases, including those related to obesity and alcohol consumption. Our group works at the interface of clinical care and the development of preclinical models to study liver biology. Understanding how, within an individual, liver cancer forms and evolves will allow us to target that tumour with precision medicine. We want to be part of improving outcomes for these patients, both in Scotland and across the globe.

Hepatocytes are the key target for regenerative therapy for patients with liver disease and are the source of most liver cancers (specifically hepatocellular carcinoma - HCC). These cells show immense regenerative capacity but are also prone to mutations during chronic disease and aging, leading to dysregulated regeneration and cancer formation. A range of specific oncogenic driver mutations have now been identified in HCC. Understanding why, in only some instances, these mutations and epigenetic perturbation leads to cancer is central to precision prevention strategies for liver cancer development and may aid the early detection of disease. Similarly, understanding how specific forms of the disease are sustained and resist therapy may provide unique therapeutic strategies which could be applied to precision medicine in HCC.

Current pharmacological therapy for HCC is only minimally effective, and no therapy is currently directed to specific molecular forms of the disease. We have developed, and continue to expand, a suite of genetically engineered mouse models (GEMMs) of HCC (Figure 1). The GEMMs are designed using the genetic blueprint of different human HCCs. The aim of our lab is to use the GEMMs and allied model systems to understand HCC disease biology and guide human clinical trials to target specific therapies to specific subtypes of HCC.

Transformation of regenerative cells into malignancy – prevention and therapy

We use GEMMs of HCC to track the expansion of the carcinogenic hepatocyte clones as they progress from single cells, into large tumour nodules and spread to distant sites over months. Using the Institute's advanced facilities, we can track and characterise tumours as they develop using preclinical imaging and molecular analysis. We study how these tumours evolve as they grow and have identified specific pathways that can be targeted to aid removal of early cancer cells or kill specific types of cancer in models of late-stage disease (Figure 2). We are aiming to understand whether specific forms of background liver disease, e.g. hepatic steatosis, can be targeted directly and how they impinge upon potential prevention strategies.

We collaborate widely to explore tumour biology using our models. We are dissecting the range of models as part of the CRUK HUNTER Consortium. The consortium has created a network for HCC research and aims to develop HCC therapies through improved understanding of immune interactions with this cancer. We are also working with a number of industrial pharmaceutical partners to explore drug repurposing and novel drug development.

Ongoing work targeting cancer is examining combinations of therapies to target growth in HCC. As β-catenin mutations drive proliferation and are emerging also as a resistance pathway to immune checkpoint therapies, we are investigating how the blockade of β-catenin can affect both growth and sensitisation to immunotherapy in this disease subtype. Ongoing work has shown that interactions between immune populations could inhibit successful immune checkpoint anti-cancer therapy in preclinical models of HCC and a clinical trial is underway in patients to explore promising drug combinations uncovered in our models. Additionally, we are examining repurposing existing anticancer therapies for subtype specific treatment in HCC and working with new therapies in HCC trials. We have shown that different types of HCC responded differently to therapy and that specific therapies identified in this way could be highly effective both prolonging survival and eradicating tumours. Our aim is to be able to take these therapies into further clinical trials, targeting specific therapies to specific tumours for precision medicine in liver cancer.

Early detection and stratification of hepatocellular carcinoma

Deaths from liver cancer are likely to continue to increase until we can identify people at risk of liver disease and HCC, prevent their disease and provide effective rescue therapies for those detected with later stage disease. Using large patient cohorts, we are studying how we can improve the use of serum biomarkers to identify patients at risk of liver cancer. This includes work within the CRUK Scotland Centre and a CRUK programme grant, together with the Zanivan lab, collaborating across the UK to uncover novel biomarkers. We hope to provide a rationale for potential inclusion of these biomarkers in routine NHS practice. We already collaborate with experts in public health and statistics to gather and analyse additional data collected from across Scotland with the aim of making screening tests more accurate.

Allied to this we are developing, through support from a Sir Jules Thorn Trust Award, metabolic biomarkers for the detection of subtypes of HCC which we hope to use from the blood and in concert with advanced PET-based imaging to detect, treat and monitor treatment responses in patients The aim is that through catching and treating these cancers early and through combining understanding of individual tumour biology we can provide better opportunities and outcomes for patients with liver cancer.

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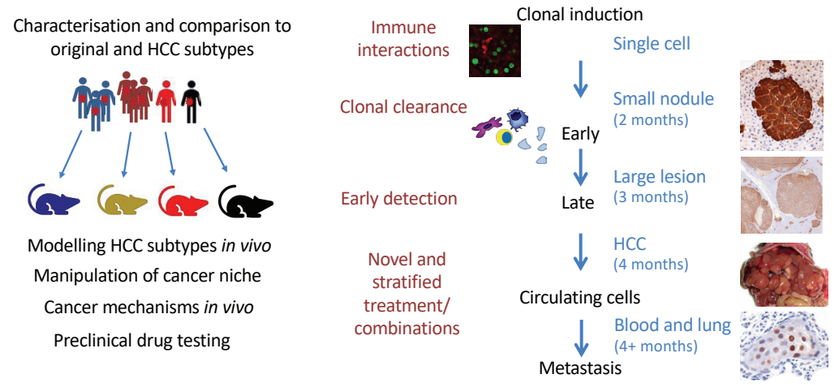

Figure 1. Human HCCs can be grouped into different functional and genetic subclasses. We are mimicking the genetic alterations in human HCC subclasses using *in vivo* models in the mouse. Our strategy is to induce clonal hepatocytes and then follow the clones as they develop into metastatic HCC. We aim to dissect and then target the vulnerable mechanisms critical for tumour growth and survival. We focus on stratified therapy for advanced HCC and precision disease prevention taking advantage of senescence in early clones to remove these premalignant cells.

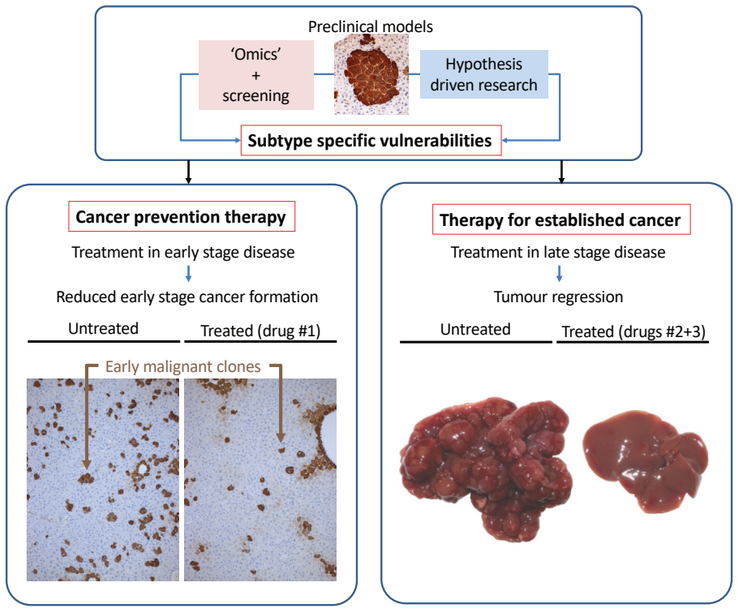

Figure 2. Cancer prevention in preclinical models by targeting early tumour clones. We are able to explore specific vulnerability of individual liver cancer subtypes. We have identified pathways which are specifically activated in early disease. When we apply therapies to early disease, we can reduce the numbers of cancer clones that become established and improve survival in our multifocal cancer models. Alternatively, using drug screening approaches, we have identified a class of compounds already in clinical use in other forms of cancer which synergise with current HCC therapy to promote highly effective tumour regression in one specific subtype of our models representing approximately 1 in 3 liver tumours.

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IN VIVO CANCER BIOLOGY

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Placement year student (Cardiff University)
Louis O'Sullivan

[1]Prostate Cancer Research
[2]The Urology Foundation/The John Black Charitable Foundation
[3]MRC National Mouse Genetic Network
[4]Cancer Research UK Radiation Research Network Project Seed Funding Award
[5]Pancreatic Cancer UK

Our lab uses preclinical models to study cancer, interrogating the role of cancer-related pathways within a biological context. By validating *in vitro* discoveries in physiologically relevant models, we hope to expedite novel therapeutic approaches to the clinic. Specific projects in the lab focus on how the RUNX/CBFβ transcriptional complex and the BCL-2 family of apoptotic regulators contribute to tumour progression, metastasis and recurrence in breast, prostate, pancreatic and other cancers. The group also co-leads the MRC NMGN *Cancer Cluster* using complex state-of-the-art mouse models to improve the understanding and treatment of cancer.

Deciphering the role of the RUNX/CBFβ transcriptional complex in breast cancer.

Mutation of the genes *RUNX1* and *CBFB* are common driver events in breast cancer. As transcription factors, the RUNX/CBFβ complex is involved in the regulation of many cellular and developmental pathways, and we are using genetically engineered mouse models to help unravel the elaborate and dichotomous role of the *RUNX/CBFB* genes in breast cancer. Amy Lawlor, Dale Watt and Dylan Fowler have been investigating the impact of RUNX/CBFβ complex alterations on mammary tumours and their tumour microenvironment. Amy is using her background in immunology to understand how RUNX/CBFβ activity within the mammary tumour epithelium orchestrates the immune microenvironment. In parallel with Amy's work studying regulation of tumour epithelium/immune interactions, Dale Watt, Laura Galbraith and masters student Dylan Fowler, have been probing the role of this complex in cancer associated fibroblasts, another important component of the breast tumour microenvironment. Together these studies aim to deconvolute the interlaced role of the RUNX/CBFβ complex throughout the breast cancer ecosystem.

Investigating the role of aberrant apoptosis in tumour development and targeting the BCL-2 family as an approach to reinstate apoptosis and improve cancer therapy.

Evasion of cell death is a hallmark of cancer that can enable tumour initiation, growth, metastasis and resistance to therapy. The most frequently altered cell death pathway in tumour development is intrinsic/mitochondrial apoptosis where elevation in pro-survival BCL-2 family proteins is a common event. Kirsteen Campbell leads several projects focussed on the BCL-2 family in solid cancers. Triple negative breast cancer is a particularly aggressive subtype of breast cancer that currently lacks targeted treatment options. Utilising our newly developed genetically engineered mouse models of triple negative breast cancer (Figure 1), Matthew Winder and Louis O'Sullivan have been investigating the role of aberrant apoptosis throughout the tumorigenic process and have uncovered important roles for distinct BCL-2 family proteins at different stages. Using these novel models, and strongly supported by expertise in the Transgenic Models of Cancer Lab, they are testing the impact of specific targeting of pro-survival BCL-2 family proteins in breast cancer prevention and treatment.

In Prostate Cancer Research-funded work, Laura Martinez-Escardo and masters student Julia Chong have been investigating the dependence of prostate cancer on pro-survival BCL-2 family members MCL-1 and BCL-XL. Together with Hing Leung and John Le Quesne's team, they are using multiplex immunocytochemistry to characterise BCL-2 family protein expression in a prostate cancer tissue microarray (Figure 2). Through a combination of *in vivo* and *in vitro* approaches they have identified vulnerabilities in the BCL-2 family that have the potential to be harnessed to improve prostate cancer treatment outcomes. Laura was recently awarded an innovation and research award from The Urology Foundation/The John Black Charitable Foundation which will allow her to further investigate how these findings can be translated into new treatment approaches.

Pancreatic cancer is a difficult to treat cancer with devastatingly poor prognosis and we have identified that pancreatic cancers with high levels of MCL-1 and BCL-XL have worst outcomes. Yasmin Hunter has therefore been targeting the BCL-2 family in pancreatic cancer patient-derived cell lines to identify patient sub-groups that might respond to novel therapeutic treatments, including combinations with radiotherapy. Unifying our interests in targeting the BCL-2 family for therapeutic gain in breast, prostate and pancreatic cancer, Nimrit Kaur, together with Mark Jackson in Anthony Chalmer's team, has been investigating radiosensitisation through targeting the BCL-2 family across a large panel of cancer cell lines. This has extended our interests to glioblastoma and lung cancer. Through correlating radiosensitisation with genomic, transcriptomic and proteomic data they aim to identify patient sub-groups who will benefit from these new treatment combinations.

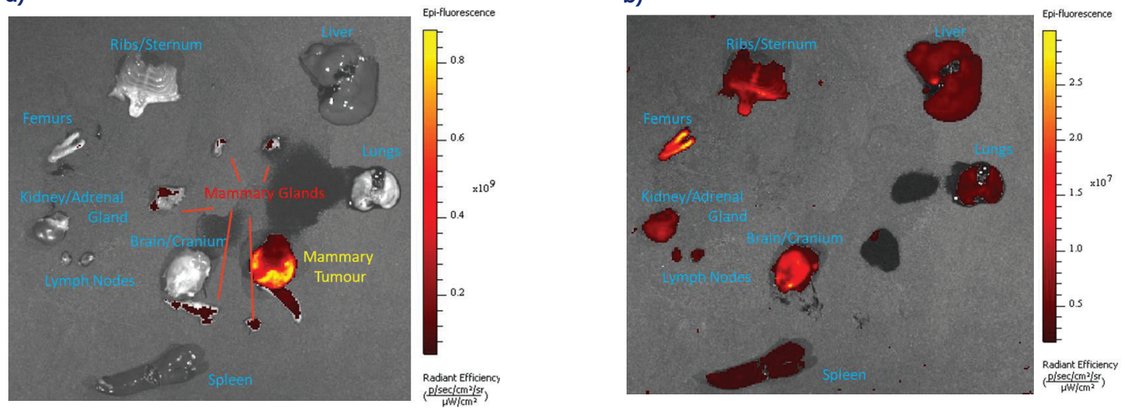

Figure 1. New genetic mouse models of breast cancer to study tumour growth and metastasis Incorporation of a fluorescent reporter gene into a novel mouse model that mimics common genetic events in breast cancer allows visualisation of tumour growth in (A) primary mammary tumour and (B) bone metastases, observed through increased signal when tissues are imaged ex vivo using an IVIS Spectrum Imaging System.

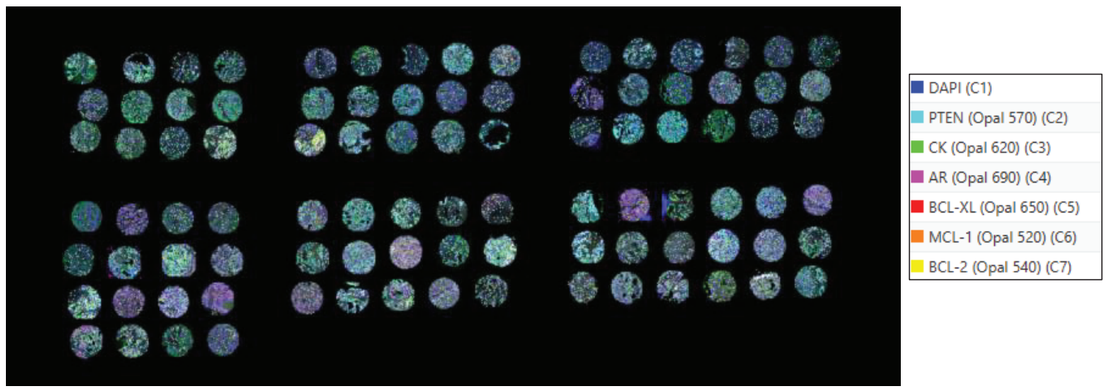

Figure 2. Multiplex immunocytochemistry to investigate expression patterns of BCL-2 family proteins in prostate cancer samples. Prostate cancer tissue microarrays analysed using multiplex immunocytochemistry against multiple BCL-2 family proteins to characterise the cellular and spatial relationships of BCL-2 family proteins.

MRC National Mouse Genetic Network (NMGN) Cancer Cluster

The lab co-leads the *Cancer Cluster* as part of the MRC National Mouse Genetic Network (NMGN) (https://nmgn.mrc.ukri.org/clusters/cancer/). With colleagues in Glasgow, Belfast, London and Oxford we are using state-of-the-art technologies such as spatial phenotyping to study cancer-host interactions and position mouse models that recapitulate the human disease. Working with the Mary Lyon Centre at Harwell, Dale Watt is developing novel mouse models which will mirror tumour evolution more accurately, and through robust patient-relevant mouse models, assess responses to novel therapies with improved predictability. The Network is also proactive in training of the next generation of *in vivo* scientists and in November, Amy from the lab joined early career researchers from across the NMGN in Harwell for a residential training course in multiple aspects of mouse genetics.

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A feature of most tumours is that they become less organised as they progress. Changes in normal tissue organisation is therefore a strong predictor of poor outcome. Our laboratory studies the molecular mechanisms of how cells organise to form tissues, and how this goes awry during tumour formation. We aim to understand this process such that we can identify new drugs for therapy in cancer.

Our group extensively utilises 3-dimensional culture to understand how collections of cells work together in a tissue-like structure. We examine this through the lens of two molecular pathways that contribute to cell polarisation and metastasis: 1) phosphoinositide signalling, including the kinases, phosphatases, and GTPases that regulate their production, and 2) the apical membrane and metastasis-associated glycoprotein, Podocalyxin.

Using 3-Dimensional (3D) culture to study collective behaviours

Traditionally, cell movement has been studied using single cells grown on glass or plastic. Tumours are collections of many, not singular, cells. Dissecting how collective cell invasion is regulated requires developing methods to allow for 3D 'mini-tumours' (organoids) to be grown, imaged and analysed *ex vivo*. Analysis methods for studying collective invasion have lagged far behind that of single cell analyses, primarily because of a lack of quantitative tools to do so. Our group has developed methods to overcome such limitations. Through an Industrial Partnership with Essen Bioscience, we have developed image analysis tools to automate this process and provide bioinformatics solutions to studying 3D cultures via live imaging (Freckmann *et al*., 2022, *Nat Commun*). This allows us to scale such analysis to parallel genetic perturbations, to make functional genomic screening in 3D culture possible (Sandilands *et al*., 2023, *J Cell Biol*).

ARF GTPase circuits controlling cell invasion

The ARFome is a network of five GTPases, multiple regulatory proteins (GEFs, GAPs) and effectors that are involved in lipid signalling, cytoskeletal organisation and membrane trafficking. They form a highly overlapping network and are thought to share many of the same binding partners. This makes untangling specific functions for each GTPase difficult. We have performed a functional genomic screen to systematically interrogate each member of the ARFome's influence on prostate cancer cell invasion (Sandilands *et al*., 2023, *J Cell Biol*).

In collaboration with the Blyth, Leung and Zanivan groups, we are interrogating their function in metastasis. We find that many ARFome family members assumed as redundant have highly divergent and sometimes opposing roles in invasion and show that there is specificity of signalling between family members. We identified that the ARF6 GTPase is a vulnerability in PTEN-null ovarian cancers, by regulating the membrane transport of active integrin cargoes required for invasive behaviours into the extracellular matrix (Nikolatou *et al*., 2023, *EMBO J*). In contrast in prostate cancer cells, we found that the ARF3 GTPase regulate cell-to-cell adhesion and metastasis by controlling the membrane transport of the cell adhesion regulator N-cadherin (Sandilands *et al*., 2023, *J Cell Biol*). These studies identify that the ARF GTPases may be targets for future therapeutic inhibition studies to control cell movement in cancer.

Podocalyxin function in collective cancer cell invasion

Podocalyxin is mutated in some families with congenital prostate cancer. Additionally, amplification of Podocalyxin expression is a predictor of poor outcome in several cancer types. We are characterising the molecular mechanisms by which Podocalyxin promotes collective cell invasion.

In collaboration with the Zanivan group, we are using in-depth quantitative mass spectrometry to identify the interacting partners of Podocalyxin ('Podxl interactome') that control its pro-invasive function. Additionally, we are mapping the proteomic changes required during cancer progression to promote Podocalyxin function. Furthermore, we have used our functional genomic approach to systematically evaluate each member of the Podxl interactome for its role in invasion from spheroids. In collaboration with the Blyth and Leung groups, we identified a molecular mechanism of how Podocalyxin controls prostate cancer metastasis and tumour growth *in vivo* (Roman-Fernandez *et al*., 2023, Sci Adv). In collaboration with the Sansom laboratory, we are extending these studies to colorectal cancer, where elevated expression of Podocalyxin is associated with very poor outcome. Our current aim is for a rigorous dissection of the exact cooperating protein modules that promote Podxl-driven invasion. Our future aim is to understand which of these *in vitro* modulators of invasion are consistently altered in cancer patients, such that they may be potential therapeutic targets in the clinic in the future.

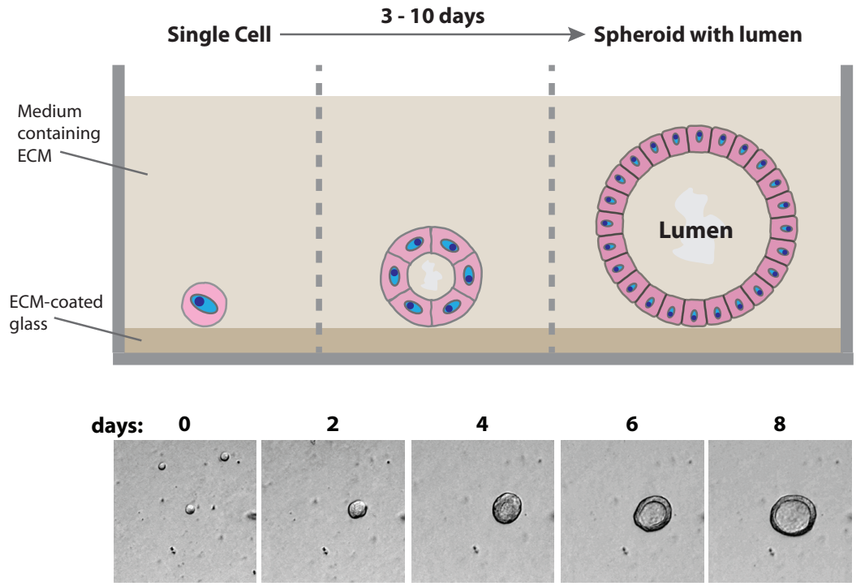

Figure 1. By culturing cells on glass-bottomed chambers coated with extracellular matrix (ECM), we direct the self-assembly of single cells into multicellular spheroid structures with a single, central lumen. This process occurs over 10 days, allowing us to study the dynamics of tissue formation.

Phosphoinositide signalling in cell polarity and metastasis.

A major new direction of the laboratory is to understand how a particular class of membrane-associated lipids, phosphatidyl-inositol phosphates (PIPs), contribute to tissue formation and its alteration during metastasis. We previously discovered pathways for how these lipids control the ability of cells to assemble into tissues. In collaboration with Owen Sansom's lab, we are examining how these lipids control the disruption to tissue organisation and overgrowth that occurs during colorectal cancer progression. We are asking how changes in PIP signalling control the tissue microenvironment to allow cancer progression.

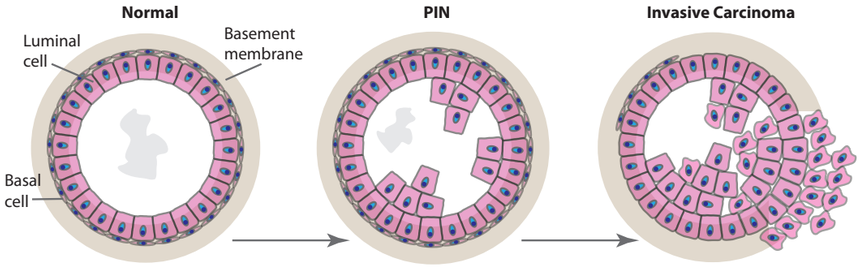

Figure 2. 3D cultures of cells to form cysts (also called spheroids or organoids) also allows us to model the loss of normal tissue architecture that occurs in cancer. For example, the progressive disrupted organisation of Normal, to Prostatic Intraepithelial Neoplasia (PIN), to Invasive Carcinoma typifies prostate cancer progression.

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RNA AND TRANSLATIONAL CONTROL IN CANCER

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The dysregulation of protein synthesis is an emerging hallmark of cancer, where altered translation is essential for the induction of oncogenic gene programmes. Distinct programmes of gene expression drive tumour growth and create the supportive microenvironment in which it flourishes. Our research aims to understand how components of the translation machinery are required to increase the rate of translation of key oncogenic mRNAs and how best we can target these pharmacologically.

Understanding the essential role of eIF4A1 for the translation of oncogenic mRNAs and how this can be targeted

Translation initiation is a major determinant of protein production and requires precise regulation to drive translation of selected mRNAs. Eukaryotic translation initiation factor (eIF) 4A1 is a DEAD-box RNA helicase and catalyses at least two major reactions during translation initiation: mRNA loading onto the 40S ribosomal subunit and translocation of the initiating ribosome along the 5′ untranslated region (UTR) to the start site. While not all eIF4A1-dependent mRNAs require these activities to the same degrees, dysregulated eIF4A1 activity is at the root of oncogenic translational programmes, reflected by a strong therapeutic interest in targeting eIF4A1 in cancer. Currently, a range of chemically diverse eIF4A1-inhibitors have been described, including hippuristanol and eFT226, which is the first-in-class eIF4A1-inhibitor to have entered clinical trials. Despite this, it is still only incompletely understood how eIF4A1-dependent mRNAs, such as oncogene mRNAs, recruit and activate specific eIF4A1 functions, and how eIF4A1 inhibitors perturb these mechanisms to inactivate eIF4A1-dependent oncogene translation. Also, of critical importance, all current eIF4A inhibitors target both eIF4A1 and its paralogue eIF4A2, which share roughly 90% identity at the amino acid level. Yet eIF4A2 has a distinct role from eIF4A1, in that it can act as a translational repressor in conjunction with the CCR4-NOT complex. Hence, to uncover eIF4A1's full therapeutic potential, we need to both better understand its role and basic mechanism of function in cancer and also understand the consequences of eIF4A inhibition on the distinct functions of eIF4A1 and eIF4A2.

Data from the Sansom lab show that loss of either eIF4A1 or eIF4A2, but not both, in the intestine of wild-type mice is partially tolerated. However, in colorectal cancer (CRC) models, loss of eIF4A1 leads to reduced proliferation and increased survival, but the loss of eIF4A2 accelerates tumourigenesis and leads to decreased survival. This loss of eIF4A1 in the CRC models phenocopies the loss of Myc. We therefore hypothesised that eIF4A1 is required to support the translational landscape following loss of *Apc* and that that oncogenic Wnt signalling requires both the upregulation of Myc's transcriptional targets and the eIF4A1-dependent translation of these mRNAs. To test this, we first carried out RNA-Seq on the small intestines from either wild-type (WT), *Apc*$^{-/-}$ (APC) or *Apc*$^{-/-}$ *Kras*^{G12D} (AK) mice, following loss of either eIF4A1 or eIF4A2. Interestingly, this showed a collapse of the Myc driven transcriptional signature, specifically in the oncogenic setting following loss of eIF4A1, but not eIF4A2 (Fig 1A). This suggests that eIF4A1 is required to support the translation of these down-stream targets of Myc. To test this, we performed ribosome-profiling on AK mice, following loss of eIF4A1. Indeed, this showed that these same mRNAs were translationally repressed following loss of eIF4A1, in that their ribosome occupancy decreased significantly more than their total mRNA abundance (Fig 1B). This suggests that oncogenic Wnt signalling and hyper-proliferation is dependent, not only on the transcriptional activity of Myc but the activity of 4A1 to support the translation of its targets. To test this, we tried to rescue the hyper-proliferation phenotype by over-expressing a Myc transgene that is not dependent on eIF4A1 for its translation. As this did not rescue the phenotype (Fig 1C), this supports our model that eIF4A1 is required to support the translational landscape following loss of *Apc* in a manner analogous to the role played by Myc at the transcriptional level (Fig 1D). This interdependency of Myc and eIF4A1 to drive hyperproliferation could represent a new axis to target Myc-driven cancers, where targeting Myc directly has proved difficult.

To understand how pharmacological inhibition of eIF4A1 compares to its genetic loss we performed ribosome-profiling in MCF7 cells following treatment with the eIF4A inhibitors hippuristanol and eFT226 **(Fig. 2A)**. These two compounds show distinct modes of action in vitro. Namely, eFT226 follows a gain of function mechanism by inducing and stabilising RNA-binding, which leads to increased RNA unwinding activity **(Fig. 2B)**, while in contrast hippuristanol results in a loss of function of eIF4A1 by inducing dissociation of RNAs off eIF4A1, which leads to inhibition of eIF4A1's RNA unwinding activity **(Fig. 2B)**. Thus, we hypothesised that the compounds also show distinct modes of action in cells which should reveal a wide spectrum of mRNA targets that depend on eIF4A1 activity for their translation. Indeed, by ribosome profiling we identified 241 mRNAs that were sensitive to both compounds and, most interestingly, also two groups of 2,092 and 100 mRNAs that were only sensitive to eFT226 or hippuristanol **(Fig. 2C)**. As eFT226 and hippuristanol affect eIF4A1 activity distinctly, we hypothesised that the compounds inhibit distinct functions of eIF4A1, that are specifically required by these compound-specific mRNAs for their translation.

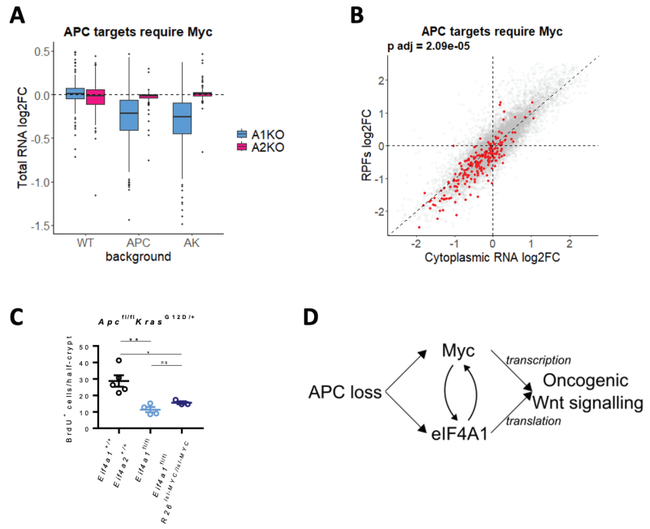

Figure 1. (A) Boxplot depicting changes in mRNA abundance of all Myc-targets (downstream of APC loss), within the small intestines of *Eif4a1*$^{fl/fl}$ (A1KO) or *Eif4a2*$^{fl/fl}$ (A2KO) mice in the stated genetic background. Loss of eIF4A1 but not eIF4A2 leads to the downregulation of the abundance of Myc-dependent mRNAs, but only in the presence of oncogenic signalling. **(B)** Scatter plot comparing the log2FC in ribosomal occupancy (RPFs) and cytoplasmic RNA from ribosome profiling analysis, following loss of eIF4A1 in AK small intestines. Myc-targets (downstream of APC loss) are coloured in red and are statistically enriched below the line of x=y (adjusted p-value = 0.2^{-5}), therefore showing translational downregulation of c-Myc target mRNAs. **(C)** BrdU incorporation in small intestine of AK mice with the additional stated genetic alterations. **(D)** Model depicting the interdependency of Myc and eIF4A1 to support the transcriptional and translation landscapes of oncogenic signalling following loss of *Apc* in colorectal cancer.

In contrast to eFT226, the exact molecular and structural mode of action of hippuristanol is unclear. To understand the mechanism of translational repression inferred by hippuristanol better, we turned to structural approaches, which suggested that hippuristanol binding to eIF4A1 interferes with RNA-binding and indicated a conformation of the eIF4A1-hippuristanol complex that favours eIF4G binding. Supporting this, we find that mRNAs that preferentially associate with eIF4G in cells, are those mRNAs that are most translationally repressed by hippuristanol. This suggests that eIF4A inhibition with hippuristanol specifically inactivates the eIF4F complex and its associated functions. Utilising bioinformatics-approaches we have investigated the mRNA features that may drive these distinct mRNA sensitivities towards the compounds. These data reveal that the mRNA features, that render their translation sensitive to hippuristanol, are also the features that characterise mRNA strongly associated with eIF4G (Fig. 2E). Further, the data highlights that AG-rich sequences are linked to eFT226-sensitivity, while C/GC-rich motifs are associated with hippuristanol-sensitivity. We are currently validating the relationship between these features and associated eIF4A1 functions.

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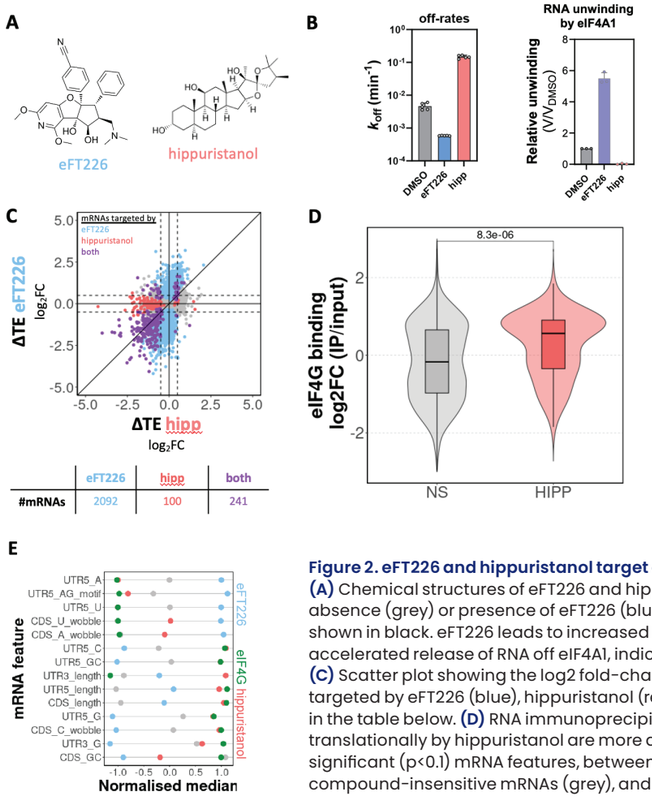

Figure 2. eFT226 and hippuristanol target distinct eIF4A1-dependent mRNAs.
(A) Chemical structures of eFT226 and hippuristanol. **(B)** RNA release of eIF4A1 bound to RNA in the absence (grey) or presence of eFT226 (blue) and hippuristanol (red). RNA in the absence of protein is shown in black. eFT226 leads to increased binding of eIF4A1 to RNA, while hippuristanol induces accelerated release of RNA off eIF4A1, indicated by the increased and decreased signals, respectively. **(C)** Scatter plot showing the log2 fold-changes (log2FC) in translational efficiency (TE) of mRNAs targeted by eFT226 (blue), hippuristanol (red) or both (purple). Numbers of identified mRNAs is given in the table below. **(D)** RNA immunoprecipitation (RIP) of eIF4G shows that mRNAs inhibited translationally by hippuristanol are more associated with eIF4G. **(E)** Normalised means of statistically significant ($p<0.1$) mRNA features, between eFT226- (blue), hippuristanol-sensitive (red) mRNAs, compound-insensitive mRNAs (grey), and mRNAs enriched for eIF4G-binding (green).

BIOLOGY OF THERAPEUTICS

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Translating therapeutics from the bench to the bedside has proven a challenge. Focusing on cancer and rare genetic diseases, my laboratory explores the 'biology of therapeutics': why do some therapies make the successful leap from pre-clinical models to clinical success while others fail? We use Drosophila, mouse, human, and chemputer based tools to explore these questions, focusing on genetically complex models and building on our experience in bringing therapies to the clinics.

Our laboratory uses Drosophila along with a variety of complementary tools to explore why some therapies succeed and others fail. We then use this information to develop network- and whole animal- based candidate therapies. We recently tested these ideas in an experimental fly-to-bedside clinical trial and are using this information to build a new generation of lead therapeutic compounds for cancer and rare genetic diseases.

Colorectal cancer: A key unmet need in the cancer field is effective, durable treatments for solid tumours. A particular challenge is tumours with oncogenic RAS isoforms, contributing to ~30% of all solid tumours and perhaps 30,000 cancer deaths annually in the UK alone. *KRAS* mutations are associated with poor patient outcome, and RAS pathway inhibitors have proven ineffective for most solid tumours.

In an experimental fly-to-bedside clinical trial (*NCT02363647*), we generated a 9-hit 'personalised fly avatar' for an advanced, KRAS-mutant, treatment-resistant colon adenocarcinoma patient (Figure 1). Screening identified a trametinib–zoledronate combination that rescued avatar viability and produced a strong partial response in the patient lasting over 11 months (Figure 1). In a broader set of patient-based 'personalised fly avatar' lines, we have found that increasing genetic complexity drives multiple mechanisms of drug resistance, notably through upregulation of detoxification pathways when specific cancer genes co-occur. Inhibiting these detox networks reinstated drug activity and restored tumour shrinkage. We are pursuing multi-drug and medicinal chemistry strategies to bypass these resistance networks in flies and mouse/human organoids. These efforts help clarify how complex mutation profiles can directly influence drug response in colon cancer.

Adenoid cystic carcinoma: Adenoid Cystic Carcinoma (ACC) is the most common malignant tumour of the minor salivary glands and the second most common of the major salivary glands. Unfortunately, once disseminated there are currently no effective therapies.

As part of our fly-to-bedside clinical trial, we reported treatment of an ACC patient presenting with treatment-resistant metastatic disease (Figure 1). We used a bespoke 5-hit 'personalised fly ACC avatar' along with our robotics-based approach to identify the novel three-drug combination tofacitinib-vorinostat-pindolol: the patient displayed partial response for ~year on treatment, with tumour burden reduced by 49% across all lung and bone marker lesions (Figure 1). Constructing an expanding set of fly ACC avatars plus a new murine model, we now have evidence for a drug that can reduce tumours in a broad palette of preclinical models. We are now working to bring this candidate lead into clinical trials, while expanding our understanding of ACC networks through spatial omics.

RASopathies: Rasopathies are a family of rare Mendelian diseases characterised by mutations that activate RAS pathway signalling. There are currently no treatments approved for RASopathies, a common situation for inherited diseases. Further, accruing sufficient Rasopathy—or other rare disease—patients for clinical trials is challenging and, ideally, a trial would test a single drug that works across a broad cross-section of patients.

To compare different RASopathy isoforms, we collaborated with Bruce Gelb's laboratory to develop 29 Drosophila models that express human RASopathy isoforms including *PTPN11*, *KRAS*, *HRAS*, *BRAF*, *RAF1*, and *MEK1*. Different isoforms showed distinct phenotypes, distinct levels of RAS activity, and distinct response to drugs. We identified a promising, clinics-relevant lead that can act broadly; this compound has also shown promise in a moue RASopathy model.

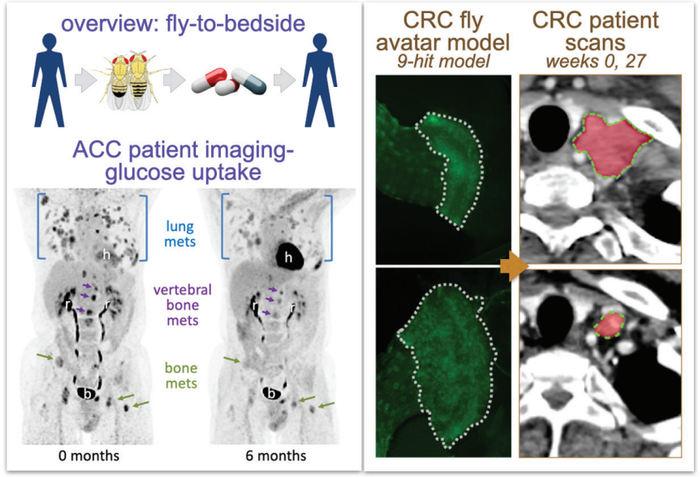

Figure 1. Our fly-to-bedside clinical trial, which led to successful treatment of adenoid cystic and colorectal cancer patients.

We are currently expanding our RASopathy efforts to neurofibromas in NF1 patients, both to understand the unique aspects of the disease and to identify promising new therapeutic approaches. Our genetic screening studies have identified new candidate targets to promote neurofibromas. These targets also represent potential therapeutic targets, a point we are exploring.

Drug development: Despite exciting new advances, targeted therapies are effective in less than 30% of solid tumours. A particularly vexing problem is the identification of an effective and durable drug for RAS-mutant solid tumours. One approach is 'polypharmacology': single agents that target multiple points along a disease network to optimise efficacy and minimise liabilities including toxicity. Polypharmacology is challenging, and several laboratories including my own are working to bridge this chemistry gap.

For example, we have established a 'drug evolution' platform designed to attack whole body disease networks through 'rational polypharmacology' by combining fly genetics with medicinal and computational chemistry (Figure 2). The results can be striking when tested in standard mammalian models. To date we have used our platform to evolve lead compounds for specific types of thyroid, lung, and colorectal cancers, as well as RASopathies. We are currently working with Lee Cronin's laboratory to further advance this technology through advanced automation, merging chemical evolution and 'chemputer' technologies. Our efforts have yielded interesting new 'network-targeted' therapeutic leads for colorectal cancer that are designed to act in the context of the whole body. We are now extending this unique platform to other diseases as well as to create unique chemical genetic tools to explore complex biology.

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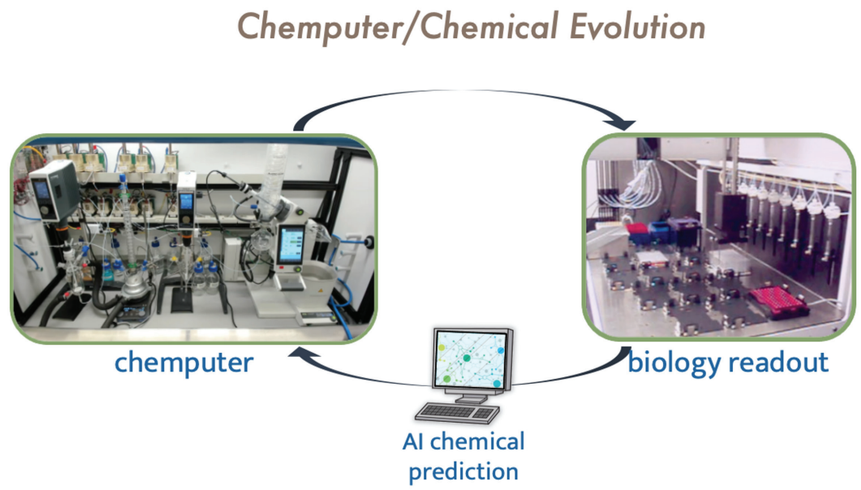

Figure 2. We are working with the Cronin lab to develop a closed-loop system designed to build chemical tools and therapeutic leads.

LEUKOCYTE DYNAMICS

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The immune system can exert both anti- and pro-tumour activity, therefore, understanding the role of immune cells in the cancer microenvironment is of critical importance. Our lab uses cutting-edge light microscopy and other techniques to investigate the spatiotemporal dynamics of immune cells in cancer.

The immune system has been implicated in almost every stage of cancer development, from initiation and growth, to dormancy, invasion, and metastasis. As the immune system co-evolved with microbes to protect against infection and as cancer cells are mutated host cells, the role of immunity in cancer is complicated. Even though immune cells can kill cancer cells and stabilise the primary tumour to help prevent its spread, they can also suppress anti-cancer immunity and benefit tumour growth and dissemination. The immune compartment of cancer is composed of the resident immune cells of the tissue and leukocytes that infiltrate from the circulation. The development of the cancer immune environment is inherently dynamic, and the processes that regulate immune cell recruitment and function are not well understood. Recent success in directing and strengthening the immune system's anti-cancer functions (e.g. immune checkpoint inhibition and CAR-T cells) highlight the potential for new therapies that can come from a better understanding of how immune cells are (dys) regulated. However, these strategies do not work for all cancers or all patients.

Specialised vasculature and leukocyte dynamics

Our group has a particular interest in the lung and the liver, both as sites of primary tumour development and as targets of metastasis. The extensive capillary network of the lung is unusual in several ways. Alveolar capillaries are of exceptionally small diameter (~5µm) and are in such close proximity to external mucosa which they share a basement membrane with the epithelium. In contrast to other organs, pulmonary capillaries are thought to be a major site of leukocyte extravasation, with markedly different mechanisms to the general paradigm of leukocyte recruitment.

Tumours in the lungs and liver interact with the vasculature in markedly different ways to those in other organs. For example, some tumours grow into and co-opt the existing microvasculature whereas others replace or push the vasculature and other tissue structures out of the way, generating their own neovasculature. This affects the way that immune cells access the different tumours. The liver is also a highly specialised immune environment consisting of a network of specialised blood vessels with a huge surface area. The liver's importance in homeostasis makes particular requirements for the way that immunity must function in this organ. Localisation and regulation of leukocytes within the pulmonary capillaries and liver sinusoids is not fully described or well understood.

The work of several groups has suggested that neutrophils are important in onco-immunology, and a high neutrophil-to-lymphocyte ratio is associated with poorer prognosis in many advanced cancers. Myeloid cells in general are crucial in many anti-microbial and tissue damage reactions and play a key role in initiating the host immune response to infection. Emerging data suggest that they are exquisitely sensitive to their microenvironment. In addition to potent effector mechanisms, including phagocytosis, degranulation and the recently described process of NETosis, neutrophils can contribute to the inflammatory milieu in a number of ways. Neutrophils can produce and consume chemokines, cytokines and growth factors and can modify the extracellular matrix. Additionally, the accumulation of apoptotic neutrophils and their subsequent clearance by macrophages is thought to directly contribute to anti-inflammatory programmes at the end of acute inflammatory responses. **Taken together, these features mean neutrophils have the potential to both antagonise and promote tumours depending on context** (McFarlane *et al.*, 2021, *J.Clin.Invest.*), and recent work has demonstrated that neutrophils actually benefit cancer spread in the process of lung and liver metastasis. Because of this diversity of actions and importance in the host defence, we need more mechanistic detail in order to interact with neutrophils in a way that would inhibit cancer but not leave the patient at risk of serious infection. Myeloid cells can be regulated by – and can regulate the function of – other immune cells, so an important goal is to look at several different cell types simultaneously to glean more information about the way that they interact and to uncover potential pathways to modify.

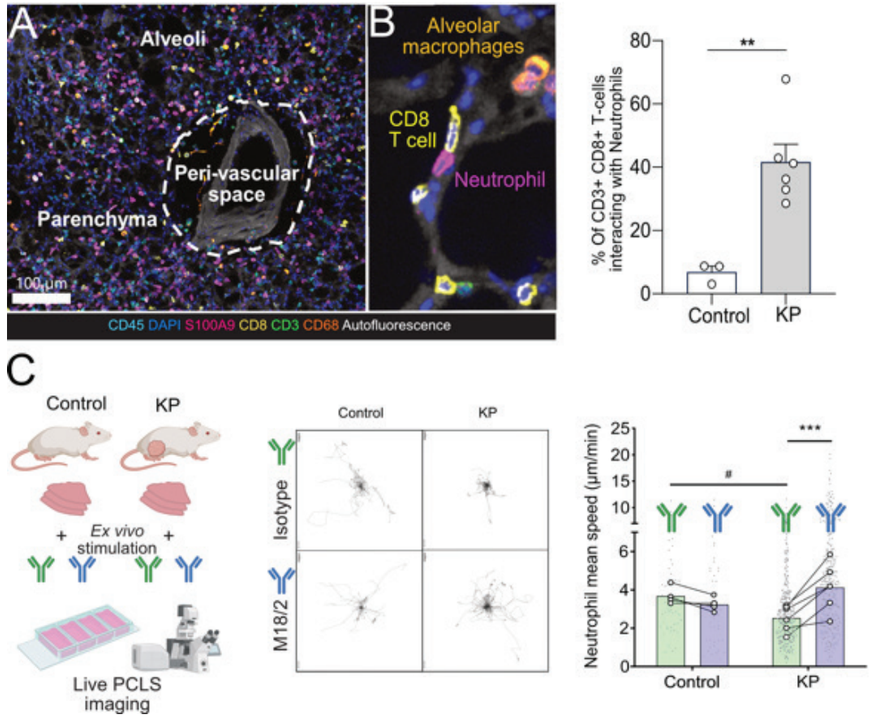

Figure 1. Neutrophil dynamics in the metastatic breast cancer pulmonary pre-metastatic niche. **A.** 3D multiplexed immunofluorescence of precision cut lung slice from a mammary (KP) tumour bearing mouse revealing abundant S100A9$^+$ neutrophils. **B.** CD8$^+$ T cells and neutrophils are commonly colocated. **C.** live imaging of ex vivo lungs from KP mice reveals slowed neutrophil motility that can be reversed by an antibody (M18/2) that activates β_2 integrin (an important immune cell adhesion molecule). Data reproduced from Fercoq, Cairns *et al.*, 2024, *bioRxiv*.

This year we preprinted work in which we probed the location and motility of neutrophils within the lungs of mice bearing spontaneously lung metastatic mammary tumours at a stage before overt lung metastasis could be observed. Using imaging, we found that the pulmonary capillaries were packed with neutrophils in mammary tumour bearing mice (Figure 1A). CD8+ T cells were in close contact with the neutrophils (Figure 1B). Live imaging revealed that the neutrophils moved more slowly than their counterparts in the lungs of healthy mice (Figure 1C). We are working on the hypothesis that this aberrant neutrophil intravascular motility, which we have evidence disrupts blood flow through the pulmonary capillaries, may protect seeding tumour cells and allow them to produce metastases (Fercoq *et al.*, 2024, *bioRxiv*). We are currently revising this work for publication and were pleased to receive interest and insightful comments from Simon Cleary on the "PreLights" Company of Biologists promising pre-prints website (***https://prelights.biologists.com/highlights/neutrophil-slowing-obstructs-the-capillaries-of-the-pre-metastatic-lung-in-breast-cancer/***).

In summary, by looking across multiple, relevant, cancer models, we aim to do three things: 1) uncover general mechanisms by which immune cells and their regulation contribute to the cancer microenvironment; 2) uncover cancers with the strongest or most manipulable interaction with particular immune cells; 3) monitor how treatment with immuno- and chemotherapeutic agents affects leukocyte localisation to develop better treatment schedules and combinations.

This year it was a pleasure to see Dr Marco De Donatis, our former PhD student who joined us for an additional year as post-doc to complete experiments, move on to an exciting post in immunotherapy at UCL, and two of our PhD students, Dr Desiree Zerbst and Dr Ryan Devlin be awarded their PhDs. We were also delighted that Dr Ximena Raffo-Iraolagoitia was promoted to Associate Scientist, well done and well deserved all! On a personal note, I was honoured that this team and the BAIR's excellent work was recognised by my own promotion to Senior Staff Scientist and the core-funding for the Leukocyte Dynamics Group research renewed. Thanks to all our colleagues, and the panel that contributed to this.

Publications listed on page 118

IMMUNE CELLS AND METASTASIS

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Pablo Casado Alonso[9]
Elizabeth Thompson[9]
Lewis Pennie[9]
Vardhini Rajkumar[9]
Nuria Serrano Ribes[10]

[1]CRUK Career Establishment Award
[2]CRUK Discovery Programme Award
[3]Worldwide Cancer Research
[4]MRC Programme Award
[5]CRUK Biology to Prevention Award
[6]NHS Lanarkshire
[7]Medical Research Scotland
[8]Indonesian Government Scholarship
[9]University of Glasgow
[10]University of Barcelona

Our lab focuses on a type of immune cell, called a gamma delta (γδ) T cell. γδ T cell refers to a variety of cell subsets with distinct properties and anatomical locations. There are γδ T cell subsets that kill cancer cells and other subsets that promote cancer progression. Our lab has ongoing projects aimed at understanding when and where these diverse γδ T cell subsets are important. We are exploring the involvement of γδ T cells in breast, colon, liver, and pancreatic cancers. In 2024, our lab published one research article, one commentary, one review article, and we contributed data to four collaborative studies led by other labs. We said good-bye to Rob who moved to Harvard for a postdoc position. We welcomed 10 new members to the lab.

Breast cancer

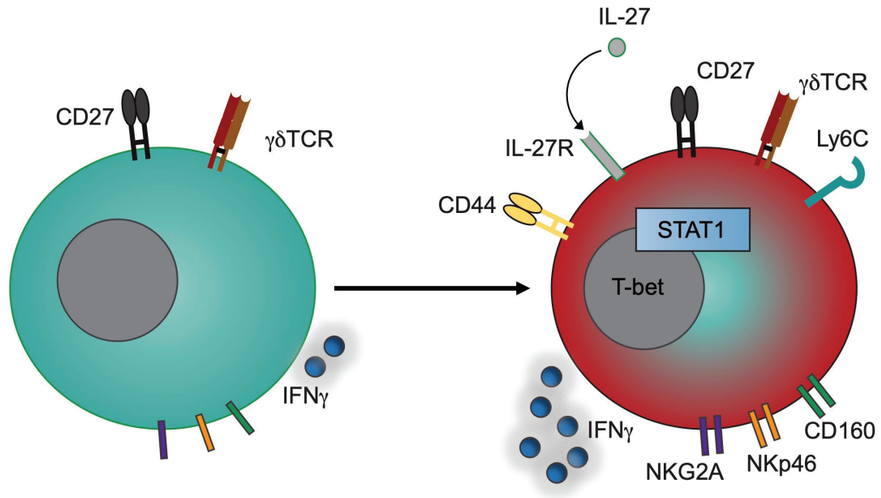
In mice, γδ T cells that express the co-stimulatory molecule, CD27, are committed to the IFNγ-producing lineage during thymic development, and in the periphery, these cells play a critical role in host defence and anti-tumor immunity. Unlike αβ T cells that rely on MHC-presented peptides to drive their terminal differentiation, it is unclear whether MHC-unrestricted γδ T cells undergo further functional maturation after exiting the thymus. This year, we provided evidence of phenotypic and functional diversity within peripheral IFNγ-producing γδ T cells. We found that CD27^{+}Ly6C^{-} cells convert into CD27^{+}Ly6C^{+} cells, and these CD27^{+}Ly6C^{+} cells control cancer progression while the CD27^{+}Ly6C^{-} cells cannot. The gene signatures of these two subsets were highly analogous to human immature and mature γδ T cells, indicative of conservation across species. We show that IL-27 supports the cytotoxic phenotype and function of mouse CD27^{+}Ly6C^{+} cells and human Vδ2^{+} cells, while IL-27 is dispensable for mouse CD27^{+}Ly6C^{-} cells and human Vδ1^{+} cells. These data reveal increased complexity within IFNγ-producing γδ T cells, comprising of immature and terminally differentiated subsets, that offer new insights into unconventional T cell biology (Figure 1).

Colorectal cancer

Using mouse models provided by Owen Sansom's lab, we have investigated the role γδ T cell subsets in colon cancer. One project focused on gut-resident cells that express the Vγ7 T cell receptor chain, because our previous work showed that these cells are inherently anti-tumorigenic cells. We have found that deletion of the tumour suppressor *Apc* induces changes in epithelial cells, causing reduced interaction between epithelial cells and Vγ7 cells. However, recent observations this year also broadened our scope to CD8αα αβ T cells of the gut, as it seems these cells may have redundant functions with Vγ7 cells. We are exploring the individual contributions of αβ and γδ T cells to anti-tumour immunity. Another project focused on a group of amphiregulin (AREG)-expressing γδ T cells that express either the Vγ4 or Vγ6 T cell receptor chains. By crossing Vil1-*Cre*^{ERT2};*Kras*^{G12D};*Trp53*$^{F/F}$;*Nicd1*$^{+/-}$ mice with γδ T cell knockouts, we found that γδ T cells promote tumour initiation in this model. Our experimentation has revealed specifically that Vγ4 or Vγ6 cells infiltrate tumours and these cells express AREG. We are working with the hypothesis that γδ T cell-derived AREG activates EGF receptor on cancer cells to induce their proliferation.

Pancreatic cancer

We have found that γδ T cells drive metastasis in the *Kras*$^{G12D/+}$;*Trp53*$^{R172H/+}$;*Pdx1-Cre* (KPC) mouse model of pancreatic cancer, and our work has been focused on overcovering the mechanism by which γδ T cells promote metastasis. We discovered that macrophages and fibroblasts are reduced in pancreatic tumours from γδ T cell-deficient mice,



indicating that γδ T cells regulate these cells in some way to support metastasis. Currently, we are investigating the mechanisms by which this occurs. At the same time, we are also exploring the role of IFNγ-producing γδ T cells in tumour-bearing KPC mice, as we have data to show that knockout of these cells accelerates cancer progression. We are performing killing assays and other *in vivo* experiments to determine which specific subset is responsible for these actions.

Publications listed on page 118

LOCAL AND SYSTEMIC FUNCTIONS OF THE ADULT INTESTINE IN HEALTH AND DISEASE

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Jade Phillips
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[2]Wellcome Trust
[3]China Scholarship Council
[4]EPSRC/MVLS
Royal Society

University of Glasgow

Research in our laboratory aims to elucidate the mechanisms by which intestinal stem cells (ISCs) adapt and respond to changes in their micro- and macro-environment, how the intestine senses and controls whole-body homeostasis, and how intestinal dysfunction can lead to broader organismal instability.

We use the fruit fly *Drosophila melanogaster* as a primary research model system due to its unparalleled genetic power and amenability for multi-organ *in vivo* studies combined with experiments in mammalian systems.

The adult intestine is a major barrier epithelium and coordinator of multi-organ functions. Stem cells constantly repair the intestinal epithelium by adjusting their proliferation and differentiation to tissue intrinsic, as well as micro- and macro-environmental signals. How these signals integrate to control intestinal and whole-body homeostasis is largely unknown. Addressing this gap in knowledge is central to an improved understanding of intestinal pathophysiology and its systemic consequences.

Combining Drosophila and mammalian model systems, the laboratory has discovered fundamental mechanisms driving intestinal regeneration and tumourigenesis and outlined complex inter-organ signalling regulating health and disease. We have three interrelated areas of research in the lab.

1 Identify and characterise stem cell intrinsic adaptations underpinning intestinal regeneration and tumourigenesis.

2 Elucidate interactions between the intestine and its microenvironment influencing intestinal regeneration and tumourigenesis.

3 Characterise how long-range signals from the intestine impact the whole-body in health and disease.

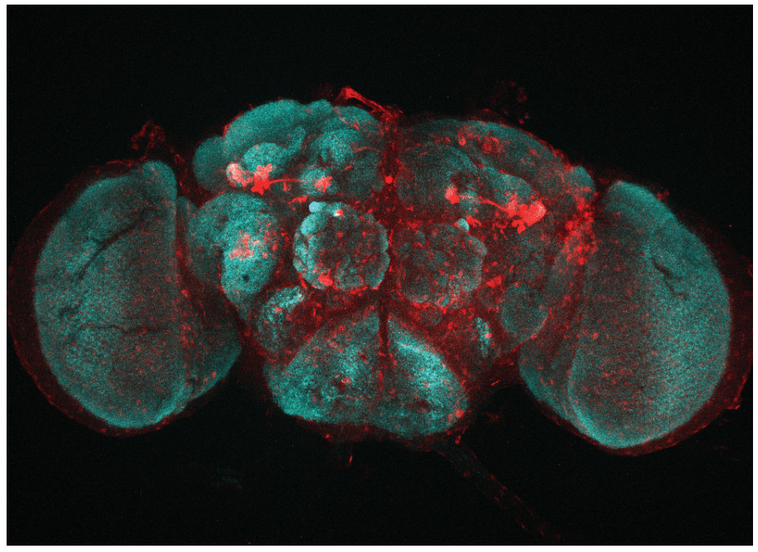

Figure 1: Gut/brain crosstalk in health and disease . Confocal image of the adult Drosophila melanogaster brain stained with the neuropil marker NC82 (Cyan), and a JAK/Stat signaling activity reporter (red).

Image credit: Dr Jack Holcombe

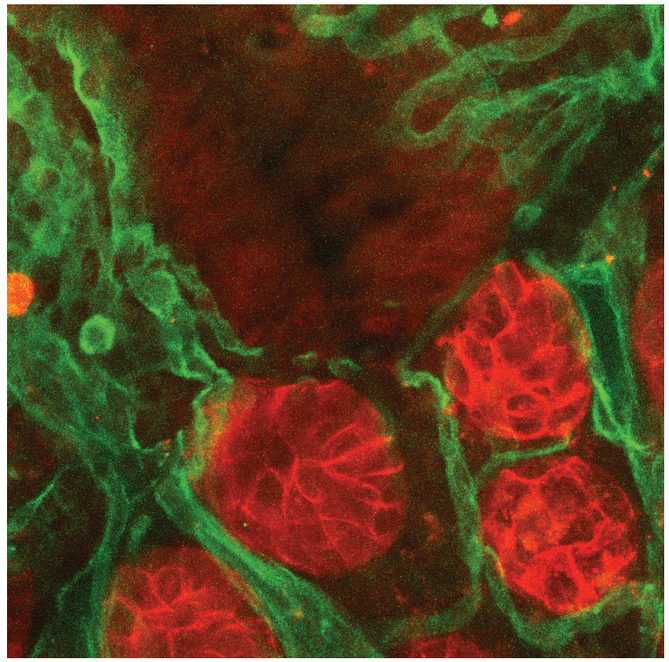

Figure 2: Gut/vasculature interactions in the adult intestine. Small intestinal epithelium (red) and associated blood vasculature (green).

Image credit: Jade Phillips

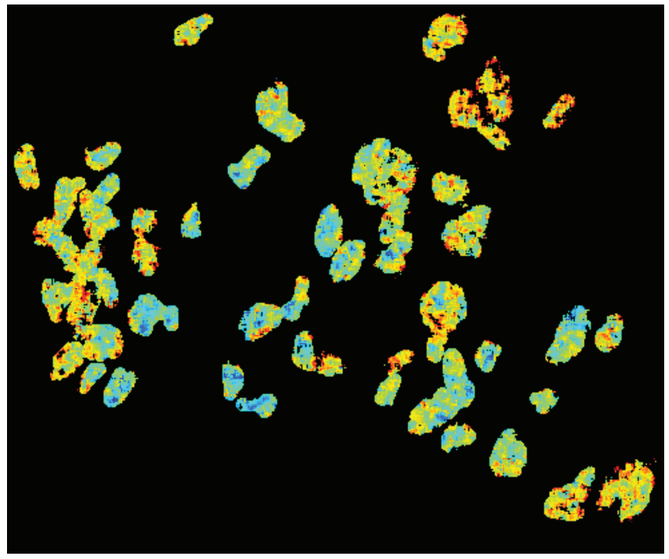

Figure 3: Metabolic adaptations of intestinal stem cells in health and disease. Oxidative phosphorylation FRET sensor in Drosophila adult intestinal stem cells (cyan and yellow).

Image credit: Yuanliagzi Tian

GENE REGULATION

Group Leader
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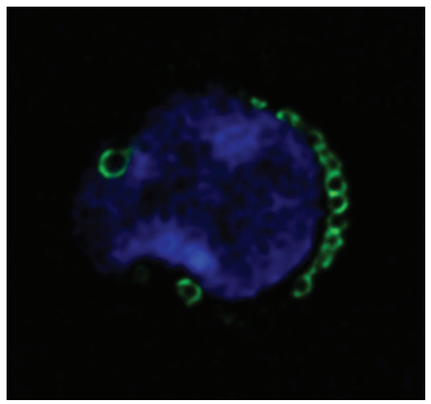
[1]Wellcome Trust Investigator Award
[2]ERC Consolidator Award
[3]Cancer Research Horizons
[4]MRC Studentship
[5]China Scholarship Programme

Precise and responsive gene regulation directs development, organ function and immune responses. Common oncogenic mutations result in deregulation of gene expression which can result in the acquisition of deleterious cellular behaviours and escape from growth control. We aim to understand how gene expression is regulated through the RNA cap, a potent structure formed on RNA polymerase II transcripts which impacts on transcription, RNA processing and translation. We investigate how the RNA capping enzymes are regulated by cellular signalling pathways and how they impact on gene expression and cell function. We explore the therapeutic value of targeting the RNA capping methyltransferases, identifying oncogenic pathways which render cells sensitive to inhibition of these enzymes.

How do the RNA capping enzymes function in health and disease?

Defining the mechanisms by which the RNA capping enzymes function and respond to cellular signalling pathways is key to understanding their role in tumour initiation and progression. We investigate the biochemical functions of the RNA capping enzymes and how they are regulated by post-translational modifications and co-factors. The development of therapeutic targeting approaches requires an understanding of RNA capping enzyme structure and interaction with ligands. We collaborate with Danny Huang to determine how RNA cap structures interact with proteins. We collaborate with Jo Birch (School of Cancer Sciences, University of Glasgow) to investigate the regulation of the RNA capping enzymes in Glioblastoma and Owen Sansom to investigate the role of the RNA capping enzymes in intestinal tumour initiation and progression. A key aim is to define the genetic alterations which increase sensitivity to RNA capping inhibition, thus indicating disease areas in which to target these enzymes.

Figure 1. Murine T Cells. IF analysis of TOMM20.

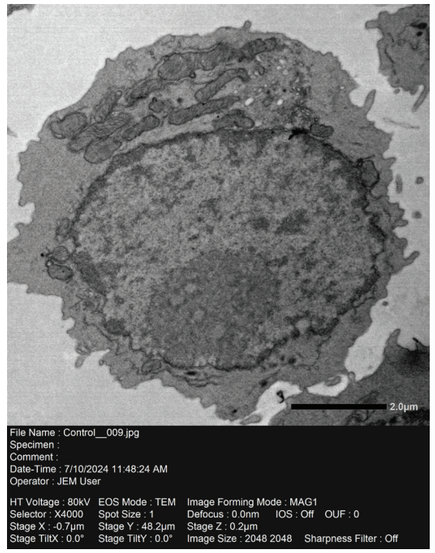


How do the RNA capping enzymes influence T cell function?

T cells are critical cells in the adaptive response to cancer and infection. When T cells interact with cognate antigens, gene expression and cellular metabolism increase massively, permitting rapid proliferation and the production of effector T cell populations required to target infection and cancer. We investigate how the RNA capping enzymes are upregulated during T cell activation, directing cell proliferation, differentiation and effector functions. The different RNA capping enzymes have distinct roles in gene expression during T cell activation and consequently have distinct roles in T cell function and fate decisions. In a tumour, the microenvironment influences RNA capping enzyme function as metabolites become limited. We collaborate with Ed Roberts to understand the role of the RNA capping enzymes in T cell responses in tumours.

Figure 2. Dopaminergic neurons derived from induced pluripotent stem cells. Cells engineered to express phospho-defective RNMT (mid panel) and phospho-mimic RNMT (right panel).

Image credit: Rajaei Almohammed, CRUK Scotland Institute.



How does RNA cap regulation co-ordinate gene regulation during differentiation?

Regulation of the RNA capping enzymes during differentiation co-ordinates the expression of genes associated with cell identity. During development and in the adult, regulation of the RNA cap methyltransferases has important roles in cell differentiation and cell function. These same mechanisms are re-used in tumour initiation and progression, influenced by metabolites in the tumour microenvironment. This year we have been investigating the role of RNA cap regulation in neuron development, function and in glioblastomas. The RNA cap methyltransferases have specific roles in neurons and glioblastomas with impact on proliferation, morphology and migration. In order to understand the gene-specificity of the RNA cap methyltransferases we analyse nucleotide-enzyme interactions using molecular biology and biophysical techniques. Our aim is to develop bespoke targeting strategies for the RNA cap methyltransferases for use in cancer and regenerative medicine.

Are the RNA capping enzymes viable therapeutic targets?

The RNA cap methyltransferases have influential roles in gene expression, cell proliferation, and pluripotency and differentiation. Targeting the RNA cap methyltransferases has selective roles in inhibiting the growth and proliferation of cancer cells. We are identifying oncogenic mutations in cancer models which sensitise cells to inhibition of RNA capping. We collaborate with Cancer Research Horizons to target RNA cap metabolism in colorectal cancer models. We collaborate with the Dundee Drug Discovery Unit and external partners to develop tool compounds to inhibit the RNA cap methyltransferases and use these to probe target disease areas.

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CANCER SYSTEMS BIOLOGY AND TUMOUR EVOLUTION

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Bioinformatician
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Postdoctoral Scientists
Shruti Khare
Gao Ran
Mengwei Li

University of Glasgow

Cancer remains one of the most complex, and devastating diseases of our time, characterized by its ability to evade treatment and adapt to an ever-changing biological landscape with diverse selective pressures. At the forefront of this challenge, our laboratory is pioneering the use of next-generation single-cell and spatial biology tools, paired with functional genomic technologies and relevant murine models, to unravel the developmental origins of cancer. Specifically, we investigate how damage-associated regenerative programs, activated in the context of chronic inflammatory diseases (fatty liver/MASLD, viral hepatis, colitis, IBD) contribute to cancer initiation and progression.

The Origins of Cancer: A Crossroads of Regeneration and Dysfunction

Recent work from our laboratory and others has elucidated remarkable foetal-like, developmental remodelling of the tumour microenvironment (TME) in human hepatocellular carcinoma (HCC) (Sharma *et al.*, 2020, *Cell*; Nguyen *et al.*, 2022, *Nat Commun*). Our current research is rooted in understanding how chronic inflammation, a hallmark of diseases such as colitis, chronic hepatitis, or pancreatitis, can activate similar developmental programs to establish a "pro-tumourigenic niche" or a fertile soil for tumourigenesis (Balakrishnan *et al.*, 2024, *J Hepatol;* Cappellesso *et al.*, 2022, *Nat Cancer*; Scolaro *et al.*, 2024, *Nat Cancer*). During these inflammatory processes, regenerative programs are activated to repair damaged tissues. However, when dysregulated, these programs can lead to aberrant cell growth, epithelial damage, endothelial-immune dysfunction, fibrosis and eventually tumour formation. Using advanced single-cell and spatial biology tools (single cell RNA-seq, scATAC-seq, spatial transcriptomics, proteomics and metabolomics, and multi-parametric flow cytometry), we study the cellular and molecular mechanisms underlying this transition from chronic non-healing wounds to cancer. These technologies allow us to map cellular interactions at unprecedented resolution, providing insight into how damaged epithelial cells communicate with their surrounding microenvironment, including immune cells, stromal cells, and endothelial cells. Finally, our understanding of the molecular mechanisms of cross-regulatory interactions between tumour cells and their ecosystem is paving the way for innovative therapeutic approaches that we, in close collaborations with clinicians, are implementing as part of clinical trials aimed at interrogating the efficacy of combining drugs targeting the tumour stroma (anti-angiogenics) along with immune checkpoint inhibitors. Initial data from these clinical studies on nasopharyngeal carcinomas (NPC) show striking improvement in anti-PD1 response when combined with anti-VEGFa therapy, compared to the use of anti-PD1 alone as monotherapy (Chong *et al.*, 2024, *Lancet Oncol*).

A Focus on Co-Evolutionary Mechanisms in the Tumour Microenvironment

Cancers do not evolve in isolation. It emerges and thrives in the context of its microenvironment, engaging in dynamic cross-regulatory interactions with surrounding cells. Our work centres on uncovering these co-evolutionary mechanisms that shape tumour initiation, tissue remodelling, and progression.

Key areas of focus include:

- **Endothelial Dysfunction:** Endothelial cells play a critical role in maintaining vascular integrity and regulating tissue homeostasis. In chronic inflammation, persistent endothelial dysfunction can promote abnormal angiogenesis, hypoxia, and endothelial anergy, fostering tumour growth and metastasis.
- **Fibrosis:** Fibroblasts, activated during tissue repair, can become cancer-associated fibroblasts (CAFs) that contribute to a fibrotic tumour microenvironment. This fibrosis not only supports tumour growth but also serves as a barrier to effective drug delivery, or immune-cell infiltration, making tumours more resistant to treatment.
- **Immune Dysregulation:** Chronic inflammation disrupts immune homeostasis, leading to immune suppression and the recruitment of tumour-promoting immune cells, such as pro-remodelling macrophages and regulatory T cells (Tregs). By studying how tumours exploit immune-stromal interactions, we aim to identify new therapeutic strategies targeting the tumour ecosystem to restore immune balance.

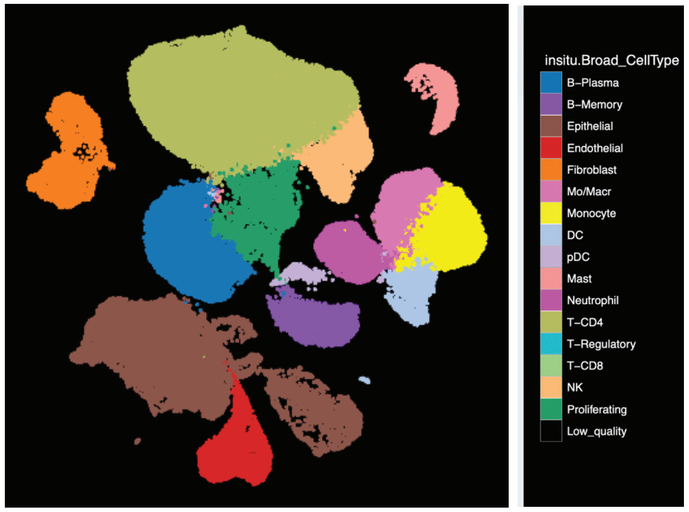

Figure 1. Cells to location: localizing cell types and cell states to their spatial coordinates in NPC

Bridging Technologies to Decode Tumour Evolution

Our laboratory employs an integrated approach, leveraging cutting-edge single cell and spatial multi-Omics technologies to study mechanisms of damage-associated chronic disease progression to cancer:

- **Single-Cell Analysis:** Tools like single-cell RNA sequencing (scRNA-seq, ATAC-seq) allow us to dissect cellular heterogeneity of disease-associated cell states (DACs) within tumours and their microenvironments, as well as gene regulatory networks and signalling pathways that specify the transcriptomic signatures of DACs
- **Spatial Biology:** Advanced imaging technologies enable us to visualize the geo-spatial organization and cellular interactions within tissue architecture, capturing the spatial dynamics of DACs or tumour cells with their ecosystem, including endothelial cells, fibroblasts and immune cells.
- **Functional Genomics:** CRISPR-based screens and drug libraries in relevant murine and patient-derived cell line/organoid models enable the identification of genes and pathways that drive tumour evolution, offering insights into potential therapeutic vulnerabilities.

From Discovery to Clinical Translation

Our ultimate goal is to translate these discoveries into tangible clinical benefits. By understanding the developmental origins of cancer and the role of co-evolutionary mechanisms, we aim to:

- Identify early biomarkers that predict tumour initiation and progression.
- Develop preventative therapies targeting the regenerative programs (especially those targeting the tissue/tumour ecosystem) that are hijacked during chronic inflammation.
- Create strategies to disrupt tumour-immune-stromal cross-regulatory interactions in order to prevent tumour progression into treatment-resistant, metastatic disease.

Publications listed on page 119

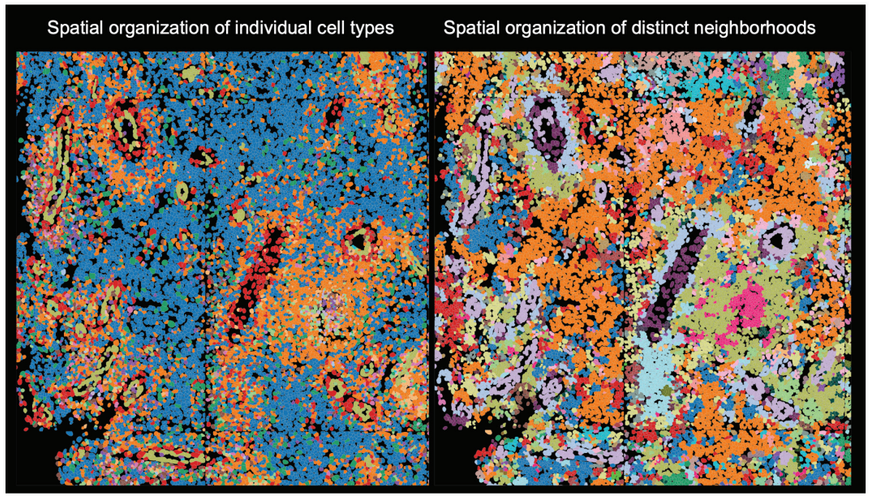

Figure 2. Spatial organization of individual cell type within a tumour (left); spatial distribution of distinct neighbourhoods of interacting cells (niches) identified within tumour biopsies

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METASTASIS AND CIRCADIAN RHYTHM

Group Leader
Zoi Diamantopoulou

Senior Scientific Officer
Gurman Pall

Post-doctoral Researcher
Sara Ortego

PhD student
Carolina Melo

Research in our laboratory, supported by a UKRI Future Leaders Fellowship, is dedicated to exploring the timing of metastasis. We aim to determine when metastasis occurs in various cancers, elucidate the molecular mechanisms through which circadian rhythm regulates the generation and metastatic spread of CTCs and identify gene vulnerabilities that could serve as targets for the development of novel anti-metastatic chronotherapeutics.

Metastasis is the leading cause of all cancer related deaths, accounting for nearly 12 fatalities every minute. Despite recent advances in early cancer detection, 50% of patients are either diagnosed with metastatic disease upon presentation or develop metastases after initial diagnosis with localised disease. The lack of effective anti-metastatic therapies poses a challenge in clinical practice, highlighting an urgent, unmet need that must be addressed promptly.

In our lab, we delve into metastasis through the study of Circulating Tumour Cells (CTCs). These are cancer cells that break away from the primary or metastatic tumour and through the blood circulation, they colonise distant organs thereby seeding new metastatic lesions. Thus, targeting CTCs holds immense potential for impeding metastasis, necessitating an in depth understanding of their biology to develop novel therapeutic strategies.

The rarity of CTCs in the bloodstream (average number of 5-10 CTCs per 7.5 ml of peripheral blood) poses a challenge in their detection and isolation. Leveraging our lab's expertise, we capture viable CTCs and scrutinise their expression profile and biological properties. We analyse samples from cancer patients and a range of cancer mouse models available at the CRUK Scotland Institute and we employ a combination of state-of-the-art microfluidics and robotic technologies, along with single-cell analysis methods, next generation sequencing, genetic engineering, CRISPR screens and imaging techniques to unravel the biology of CTCs and understand metastasis.

Recently, we demonstrated that CTCs disseminate during sleep, unveiling a key role of the circadian rhythm in metastasis. We analysed blood samples from hospitalised women with progressive breast cancer collected during the active (10:00am) and rest (4:00am) phases of the same day and we found a striking prevalence of CTCs during the nighttime. We also used different mouse models of breast cancer and examined spontaneous CTC generation over time. Similar to patients' data, we detected more CTCs during the mouse rest phase (corresponding to daylight time due to inverted circadian rhythm of rodents compared to humans) (Figure 1b-d). Additionally, we characterised a unique gene expression profile in CTCs induced by circadian rhythm regulated hormones during the rest phase, enhancing the metastatic potential of CTCs (Figure 2).

Building upon these findings, we delve deeper into the intriguing link between the circadian rhythm and metastasis, aiming to leverage the acquired knowledge to develop time-tailored personalised prognostic approaches along with effective anti-metastatic therapies adapted to patients' circadian clocks. Specifically, our research is structured around the three following interconnected questions:

1. Why is metastasis formed at a specific time of the day?
2. How can we block metastasis?
3. Will therapies be more effective if we administer them at specific times of the day?

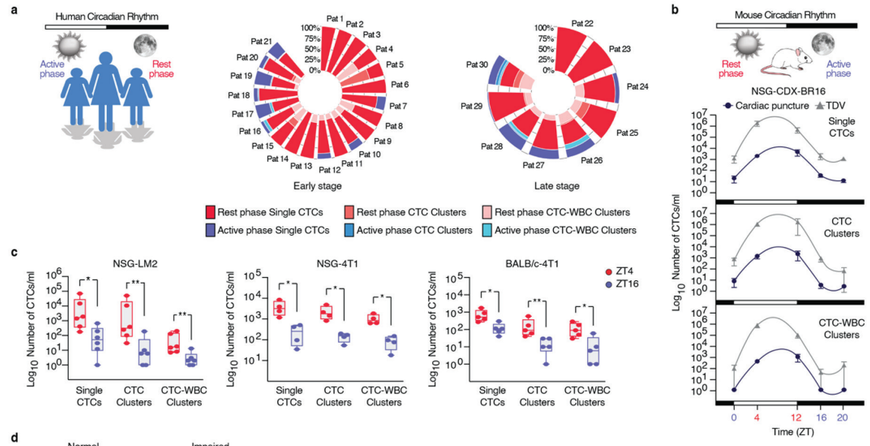

Figure 1. CTCs intravasate during the rest phase of the circadian rhythm. (A) Graphical representation of the human circadian rhythm. The white and black bars represent environmental light (active period) and dark conditions (rest period), respectively (left). The radial histograms show the percent of CTCs isolated during the rest or active phase in breast cancer patients. **(B)** Graphical representation of the mouse circadian rhythm. The white and black bars represent environmental light (rest period) and dark conditions (active period), respectively (top). Time kinetic analysis showing CTC counts in the NSG-CDX-BR16 breast cancer mouse model, from blood collected via cardiac puncture or tumour draining vessel (TDV) over a 24-hour time period. **(C)** Box plots showing the distribution of the number of CTCs collected at ZT4 or ZT16 in immunocompromised NSG-LM2 and NSG-4T1 or immunocompetent BALB/c-4T1 breast cancer mouse models. **(D)** Graphical representation of physiological (BL/6-EO771.lmb mice) versus impaired circadian rhythm (BL/6-Bmal1-/--EO771.lmb mice) (left). Graphs showing time kinetic analysis of CTC counts in the BL/6-EO771.lmb and BL/6-Bmal$^{1-/-}$-EO771.lmb mice. Data in panel "b" and "d" are presented as mean ± s.e.m.; for panels "c" center lines in the box represent the median; box limits represent first and third quartile; extremes of the whisker lines represent the minimum and maximum observed values. * $P < 0.05$, ** $P < 0.01$ by two-sided Mann-Whitney test. n represents the number of biologically independent mice.

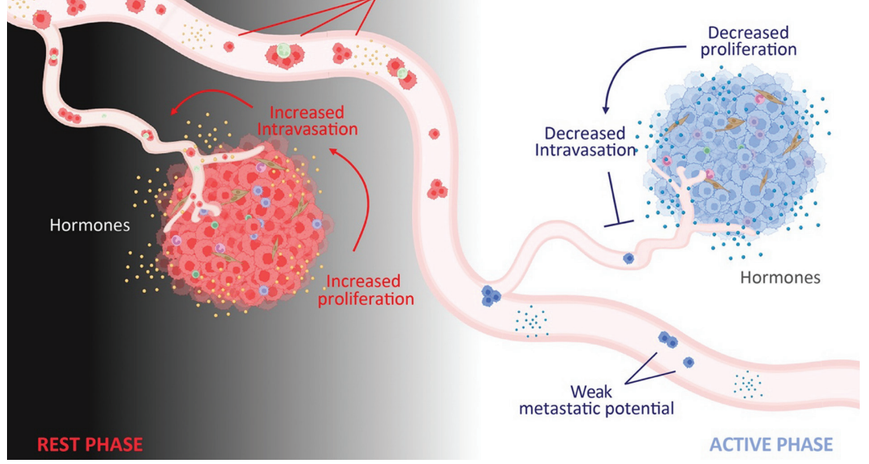

Figure 2. Proposed mechanism for the regulation of metastasis by the circadian rhythm.

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PHENOTYPIC PLASTICITY IN COLORECTAL CANCER

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QUEEN'S UNIVERSITY BELFAST

Our research team are focussed primarily on tumour-based discovery in colorectal cancer (CRC), utilising large collections of both human and preclinical mouse tumour tissue to interrogate molecular and morphological features to gain a better understanding of disease. Our work integrates molecular biology, computational analysis, and translational pathology, particularly in early stage localised disease, to uncover aggressive traits driving metastasis, and therapeutic resistance mechanisms, that in-turn can be used to guid more subtype-specific treatment strategies.

Phenotypic landscape of colorectal cancer.

Our research in 2024 led to the development of the pathway-derived subtyping (PDS) system, (Malla *et al.*, 2024, *Nat Genet*) which has offered previously unseen insights into tumour biology and patient outcomes. Unlike traditional gene-level classifiers, PDS used phenotypic signalling and pathway-level data to identify three subtypes: **PDS1**: Characterised by LGR5+ stem-rich, highly proliferative tumours with good prognosis, **PDS2**: Enriched for immune and stromal signalling, featuring ANXA1+ regenerative traits, and most interestingly **PDS3**: A previously overlooked slow-cycling subtype with reduced stem-like properties, increased differentiated lineages (e.g., enterocytes), and poor clinical outcomes. PDS3 tumours are characterised by transcriptional repression of many features previously defined as being essential hallmarks of colorectal cancer, while conversely exhibiting high levels of canonical epithelial differentiation reminiscent of a normal homeostatic balance and also more subtle traits that align with a neuro or squamous-like cell identities, all unique signalling profiles distinct from existing CRC subtyping systems (Figure 1).

The study also highlights the clinical relevance of transcriptional pathways, such as MYC targets (enriched in PDS1 and PDS2) and polycomb repressive complex (PRC) targets (elevated in PDS3). This axis reflects a stem-to-differentiation spectrum, with PDS3 tumours exhibiting diminished proliferation and stemness. Using disease-positioning to align these human tumour traits with a series of genetically engineered mouse models (GEMMs) from the Sansom lab, while PDS1 and PDS2 align well with these GEMMs, our study identifies PDS3 as being the least represented in these preclinical models and a range of *ex vivo* organoid models, which limits therapeutic exploration and underscores its clinical challenges (Figure 2). Prognostic analysis across large CRC cohorts confirmed the subtype's association with poor relapse-free survival, even when controlling for KRAS mutation status or microsatellite stability. This work emphasises the utility of integrating pathway-level subtyping with existing frameworks to capture overlooked tumour heterogeneity across stem and differentiated lineage states (Figure 3).

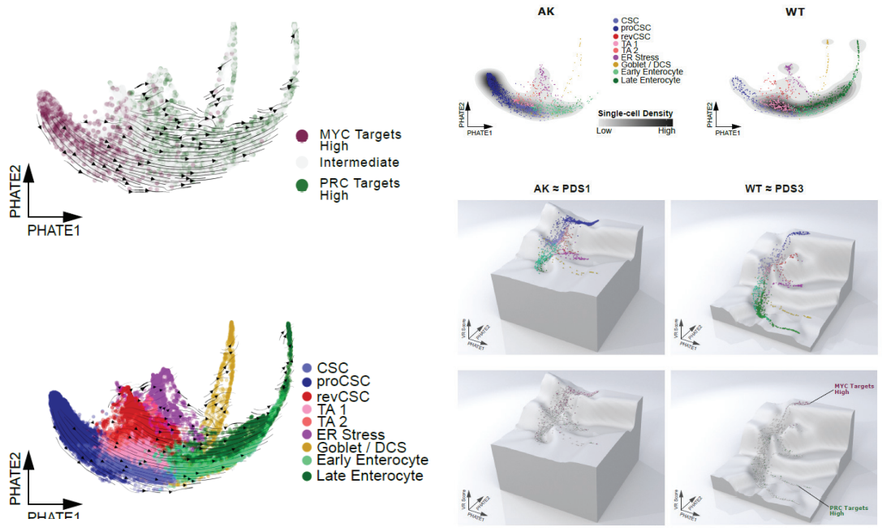
Disease positioning of human and mouse tumours.

Driven partly by the absence of robust disease-positioned PDS3 models described above, Owen Sansom's lab have generated a more comprehensive "gold standard" GEMM transcriptional cohort using n=1,200 samples from across a range of developmental stages (normal, precancer/short-term, cancer, metastasis) in both tissue and organoid and across a wide variety of genotypes (20+ genotypes, including combinations of conventional v serrated drivers). This unique resource has allowed our teams to begin to assess, within a heterogeneous collection of lesions, how mouse tumours recapitulate the phenotypic features observed during the development and progression of human CRC. Remarkably, in these preliminary analyses, using an unsupervised clustering method across all tissue samples has revealed a transcriptional pattern that appears to align with the well-understood disease trajectory of both the adeno-carcinoma sequence and, to a certain extent, the progression from non-invasive primary lesions to metastatic lesions.

In 2024, we performed a series of preliminary analyses, to directly address some of the outstanding criticisms of using GEMMs, regarding how well mouse models represent the range of important molecular and clinical subtypes, alongside how they recapitulate the phenotypic heterogeneity seen in human tumours. Using these data, in combination with a range of dual-species classifiers developed by our team, we have successfully disease-positioned mouse tumours according to human CMS, iCMS and the PDS approach. In addition to finding genotypes that consistently returned the same human tumour subtype, we also identified several fixed genotypes that were classified across a range of molecular subtypes (CMS, iCMS and PDS) and stem cell states. This is similar to the intrinsic and microenvironmental phenotypic heterogeneity observed in human tumours.

Figure 1. PDS signalling is associated with differentiation along an axis of colon epithelial lineage identities.

Figure 2: When projected onto a Waddington landscape, PDS signalling is associated with stem-like pluripotency and cell entropy, where AK genotypes (Apc and Kras mutant) drive stem cell features reminiscent of PDS1, whereas PDS3 maintains a more normal/WT balance of lineage identities.



Data analytic applications and analysis portals

Generation of transcriptional data has dramatically increased in the past decade, driving the development of analytical algorithms that enable interrogation of the biology underpinning the profiled samples. However, these resources require users to have expertise in data wrangling and analytics, reducing opportunities for biological discovery by 'wet-lab' users with a limited programming skillset. Although commercial solutions exist, costs for software access can be prohibitive for academic research groups. To address these challenges, we have developed a series of open source and user-friendly data analysis platforms for on-the-fly bioinformatic interrogation of transcriptional data by any user, regardless of bioinformatics skillsets, we have been developing a series of data applications to support our commitments to the FAIR principles.

Subtype Explorer (SubtypeExploreR, https://subtypeexplorer.qub.ac.uk). To complement the non-exhaustive PDS characterisations we presented in the *Nature Genetics* study, we developed the 'SubtypeExploreR' platform. This enables any user to interrogate transcriptional genes and/or signatures, including existing signatures from numerous databases or an unlimited combination of *de novo* unpublished classifiers, according to three different molecular CRC subtyping, including, PDS, CMS and iCMS across the bulk cohorts used in that study.

Molecular Subtyping Resource (MouSR, https://mousr.qub.ac.uk/). This internet-accessible analytical tool enables users to easily interrogate their data using an intuitive 'point-and-click' interface, which includes a suite of molecular characterisation options including quality control, differential gene expression, gene set enrichment and microenvironmental cell population analyses from RNA sequencing. The MouSR online tool provides a unique freely available option for users to perform rapid transcriptomic analyses and comprehensive interrogation of the signalling underpinning transcriptional datasets, which alleviates a major bottleneck for biological discovery.

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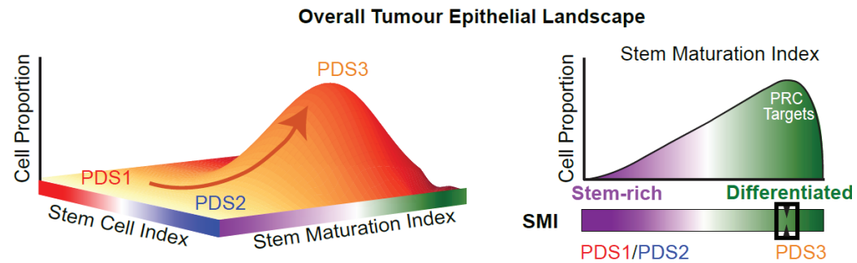
Figure 3: The colorectal phenotypic landscape can be defined using PDS from both a bulk and single cell viewpoint using a combination of signalling related to stem cell polarisation and epithelial cell differentiation status.



PANCREATIC CANCER EVOLUTION AND THERAPEUTIC DEVELOPMENT

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Pancreatic cancer is one of the most lethal cancers and will soon become the second cause of cancer death in the UK. Working at the interface between clinical care in the NHS and laboratory research, the overall aim of our research is to improve outcomes for pancreatic cancer patients by deepening our understanding of tumour-host interactions driving pancreatic cancer progression and response to therapy. To do this, we perform in-depth molecular and pathological studies of patient samples and use patient-derived preclinical models to create a solid platform of preclinical evidence to translate our discoveries into the clinic.

Pancreatic cancer is a cancer of unmet need that is fatal for most patients. In addition to late presentation, patients with pancreatic cancer develop rapid decline of their performance status, leading to ~60-70% of patients not receiving any anti-cancer treatment. Most patients succumb to the effects of metastatic disease imposed on the patient (host), including inappropriate inflammatory and immune response, and metabolic aberrations leading to muscle and fat wasting and functional decline. In addition, there are striking differences in outcomes based on other host factors such as age, gender, ethnicity and socioeconomic exposures. Interactions between the tumour and the patient (host) affected by pancreatic cancer are complex, from the formation of the very initial cancer cell to the ultimate demise of the host due to the overwhelming effect of the dissemination of metastatic disease. During this journey, the tumour and the host interacts and "co-evolve" in a symbiotic relationship. The trajectory and the outcomes of the co-evolution all depends on the tumour-host interactions temporally and spatially (Figure 1).

The overall aim of our research is to elucidate how tumour and host diversity drive co-evolution and outcome in order to develop better therapeutic strategies to improve the overall outcome of pancreatic cancer and to reduce cancer inequalities. To do so, we use routinely collected health care data, in-depth interrogation of patient samples using state of the art technologies (multiplex immunofluorescence, single cell and spatial sequencing, plasma proteomics) and preclinical patient-derived models for functional studies. Patient sample collection is ongoing as part of the national UK therapeutic development platform for pancreatic cancer (Precision-Panc), and the recently awarded Cancer Grand Challenge studying pancreatic cancer inequalities (SAMBAI, www.cancergrandchallenges.org/sambai).

We have identified systemic inflammation, host factors, and differential KRAS signalling as key drivers of rapid progression of the disease but with marked heterogeneity, which is being studied in more detail in our laboratory. In collaboration with the Le Quesne laboratory, we have developed a bespoke 50-plex immunofluorescence (mIF) protein expression panel using the Akoya Phenocyler system (Figure 2). The panel consists of antibodies targeting known molecular subtypes of pancreatic cancer (classical vs squamous/basal-like), cancer associated fibroblast (inflammatory, myofibroblastic-like, antigen presenting and metabolic), T cells (subtyping, checkpoints and functional state), B cells, and myeloid cells. Prognostically important spatial entropy, morphology and tumour microenvironment enrichment profiles have been identified and are being further evaluated. Moreover, in collaboration with the Yuan laboratory, we have identified the histomorphological landscape of pancreatic cancer using H&E images from multiple patient cohorts, using Histomorphological Phenotype Learning (a self-supervised learning network developed in the University of Glasgow).

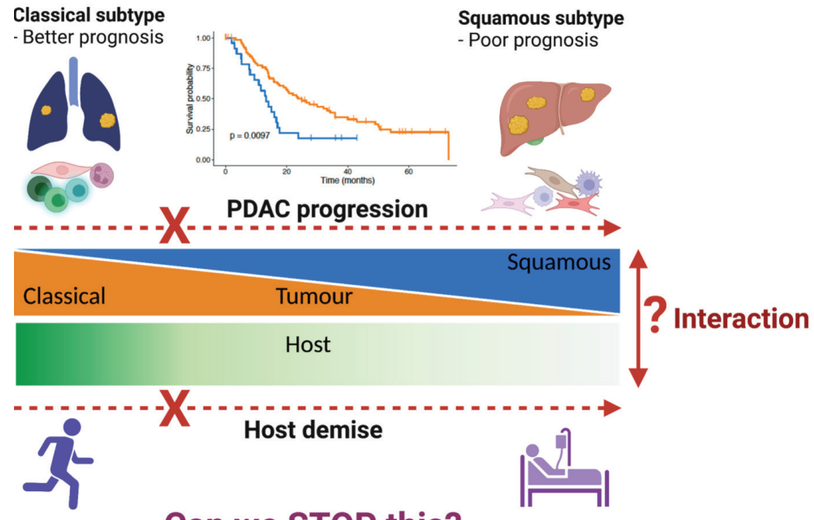

Figure 1. Co-evolution of tumour and host in pancreatic cancer. The overall goal of our research team is to understand tumour-host interactions driving distinct outcomes to develop better treatment strategies and reduce cancer inequalities.

As part of team SAMBAI, we will study how tumour-host interactions drive pancreatic cancer inequalities, with comprehensive measurements of social determinants of health, environmental, genetic and immunology factors that can help define the causes of disparate outcomes in patients of the African diaspora or those from deprived areas.

Clinically, I work as Consultant Medical Oncologist at the Beatson West of Scotland Cancer, am the pancreatic cancer lead of the Glasgow Experimental Cancer Medicine Centre, am principal investigator of multiple clinical trials and lead the molecular tumour board for Precision-Panc, the national therapeutic development platform for pancreatic cancer in the UK. Overall, this allows me to focus on developing personalised therapeutic strategies that emanate from discoveries in both basic science and reverse translation from clinical observation.

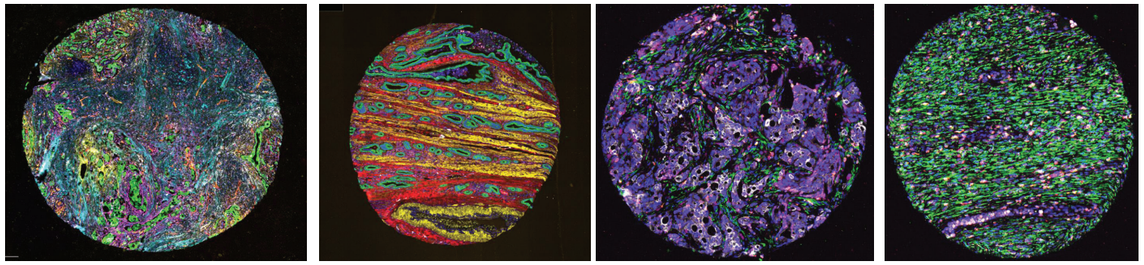

Figure 2. Representative multiplex immunofluorescence images of selected markers from bespoke 50-plex pancreatic cancer panel

INTEGRATIVE MODELLING

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Complex and dynamic interactions between cancer cells and elements of the tumour microenvironment shape tumour progression and contribute to therapy resistance. To unravel the biological complexity, and to uncover novel vulnerabilities to target, our lab focuses on developing diverse computational approaches, ranging from mechanistic modelling and computer simulations to spatial data analysis and machine learning. Our vision is that these approaches, in integration with clinical and pre-clinical experimental research, will increase our insights into the fundamental mechanisms underpinning tumour progression and therapy resistance and, ultimately, improve our strategies for stratification and treatment of patients.

The Integrative Modelling lab was established in August 2023. We are delighted to have welcome multiple team members to the lab in 2024. In our lab, we are interested in developing computational approaches to investigate the co-evolutionary dynamics and organisational principles of the tumour and its microenvironment. Our goal is to reveal a tumour's vulnerabilities through the lens of computational modelling and identify novel strategies to tackle therapy resistance. We collaborate broadly with cancer biologists, experimentalists, and clinicians, in an iterative manner, to ensure the biological relevance and translational value of our computational research.

Modelling co-evolutionary dynamics

The first strand of research evolves from our previous research in modelling evolutionary dynamics of tumours. We focus on developing mathematical and computational models to study co-evolutionary dynamics of the tumour and its microenvironment. Inference of dynamic co-evolutionary trajectories from molecular and spatial profiles of tumours in patient samples and pre-clinical experimental models will increase our insights into shared or divergent behaviours between subsets of tumours and can potentially reveal therapeutic targets and windows of opportunity for intervention.

Jayathilake Pahala Gedara, a postdoc who joined the lab in January 2024, has been investigating the crosstalk between tumour and its microenvironment in pancreatic cancer, with the goal to improve our understanding of resistance mechanisms and identify more effective treatment strategies. The computational model will be integrated with pre-clinical mouse experiments that tested the efficacy of drugs or drug combinations, in collaboration with Jen Morton's lab. The longer-term view is to establish an *in-silico* therapy testing platform for computationally screening treatment strategies for better outcomes and identifying promising candidates for experimental validation.

Philip Liu, a postdoc who joined the lab in September 2024, has been investigating early dynamics and determinants of colorectal cancer liver metastasis. The computational modelling will establish mechanistic insights into the dynamic integration of biological processes driving the distinct histopathological growth patterns, encapsulated or replacement growth, associated with better or worse patient outcomes, respectively. In integration with pre-clinical mouse experiments in Owen Sansom's lab, these computational models will have the potential to inform early preventive and interventive strategies to disrupt the growth of metastatic colorectal cancer within the liver.

Mapping organisational principles

In the second strand of our research, we focus on mapping organisational principles of the tumour microenvironment. Unravelling key cell behaviours and cell-cell interactions that sculpt the tumour microenvironment will potentially uncover novel therapeutic targets to combat tumour progression. We are interested in two levels of "mapping".

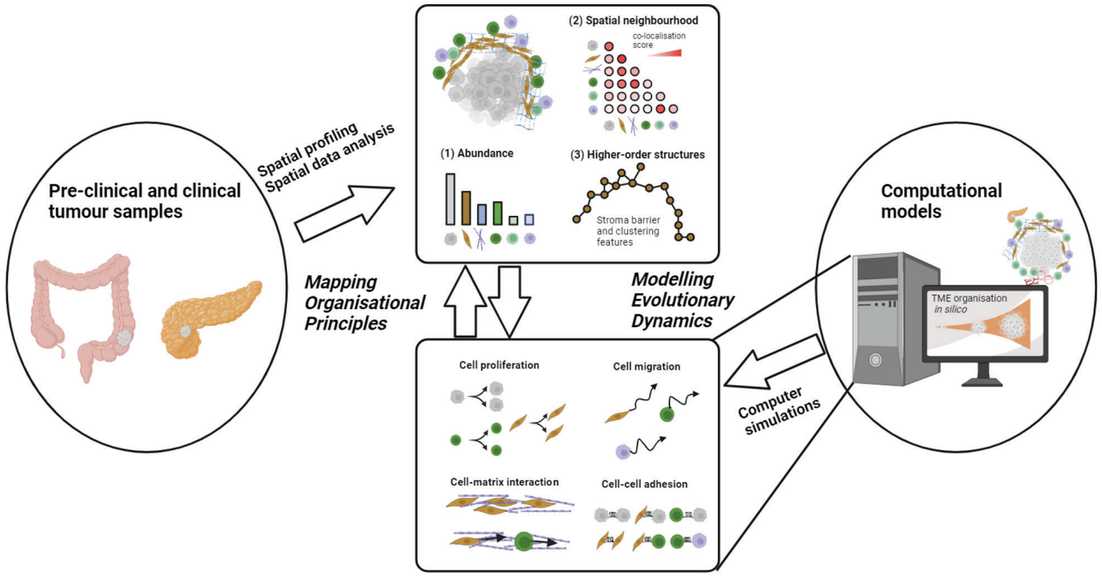

Figure 1. A framework for integrating computational approaches with pre-clinical and clinical work to investigate the evolutionary dynamics and organisational principles of the tumour microenvironment.

The first level of "mapping" involves spatial data analysis and machine learning methods. The rapid advances in spatial biology techniques, such as multiplex imaging and spatial transcriptomics, have deepened our insights into the spatial complexity of the tumour microenvironment. ***Anh Nguyen Phuong***, a PhD student who joined the lab in October 2024, has been investigating spatial biomarkers within the cellular ecosystems of colorectal cancer, in collaboration with Joanne Edwards's and Nigel Jamieson's labs in the School of Cancer Sciences, University of Glasgow. We are exploring further funding opportunities to grow this research area, in collaboration with John Le Quesne's lab.

The second level of "mapping" will be achieved through the integration of computational modelling and spatial phenotyping. Computer simulations of the mathematical and computational models will result in diverse co-evolutionary trajectories of the tumour and its microenvironment *in silico*. Linkage of these simulated tumour snapshots with molecular and spatial data of patient tumour samples will enable us to infer key cellular mechanisms and organisational principles. To facilitate this level of mapping through an integrative approach, modellers in the lab will also be developing quantitative data analysis and statistical inference frameworks, alongside their computational modelling work.

Concluding remarks

Cancer is a complex, and dynamically evolving, system. In the era of big cancer data, computational approaches are well positioned to tackle the complexity and distil key biological signals, alongside clinical and pre-clinical research, in an integrative and iterative manner.

We are very excited about contributing our integrative modelling perspectives and approaches to the multidisciplinary research at the CRUK Scotland Institute and wider cancer research community.

www.crukscotlandinstitute.ac.uk/payam_gammage

MITOCHONDRIAL ONCOGENETICS

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Mutations of mitochondrial DNA (mtDNA) are among the most common genetic events in all cancer, however their impact on disease initiation and progression is not understood. Mitochondria perform numerous metabolic functions, relying on faithful expression and maintenance of mtDNA, a small, multi-copy genome separate from the nuclear DNA that is contained exclusively within mitochondria.

Mutations of mtDNA and gross changes to mtDNA copy number can lead to profound metabolic alterations - one of the earliest identified hallmarks of cancer – and these changes are observed in >60% of tumours. In order to understand the possible links between mitochondrial genetics and metabolic dysfunction in cancer, our lab studies a range of cancer models using genetic and metabolic analyses alongside the development and enhancement of mitochondrial genome engineering tools and model systems. By better understanding the relationship between mtDNA mutation and cancer we are developing new therapeutic targets and approaches for clinical application, including the informed reallocation of existing treatments based on mtDNA genotype.

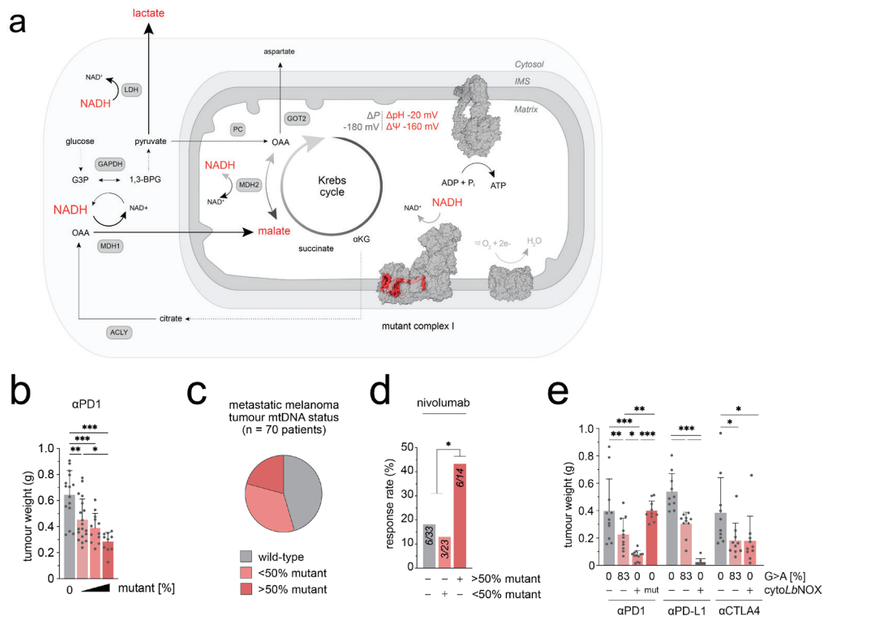

Figure 1. (a) Summary of the metabolic impacts in cancer cells resulting from truncating mutation in Mt-Nd5 of complex I. Redox imbalance due to diminished levels of complex I result in a saturated malate-aspartate shuttle and subsequent excess fermentation of pyruvate to lactate, coupled to loss of oxygen consumption at high levels of mutation. **(b)** Weight of control and increasing mtDNA mutant load tumours at a timed endpoint, treated with anti-PD1 checkpoint inhibitor, demonstrating increasing sensitivity of tumours as mtDNA mutant load increases. **(c)** Metastatic melanoma nivolumab (anti-PD1) clinical trial cohort mtDNA mutation status. **(d)** Treatment response rate of patients given nivolumab stratified by mtDNA mutant status demonstrates a major increase in response rate with high mtDNA mutation load. **(e)** Impact of several common immunotherapy drugs on timed endpoint tumour weights of mtDNA mutant tumours and tumours expressing the exogenous NADH oxidase cytoLbNOX. mtDNA mutant tumours and cytoLbNOX tumours are responsive to all forms of immunotherapy, while a catalytic mutant of cytoLbNOX is insensitive.

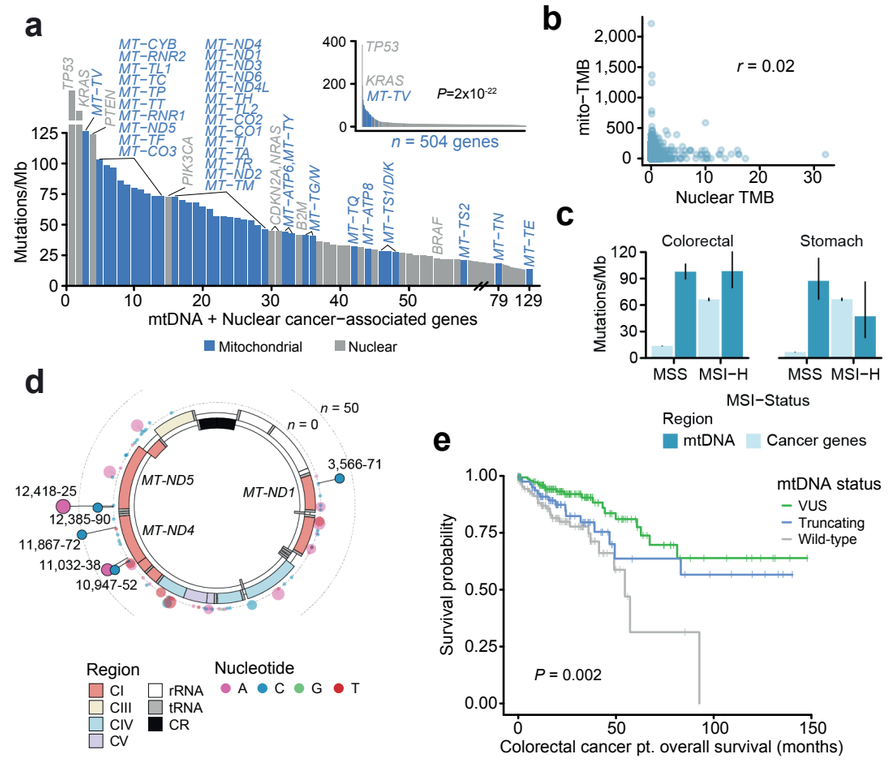

Figure 2. (a) Mutation rates (mutations/Mb) of individual mtDNA-encoded genes (blue) and nuclear-encoded cancer-associated genes (grey). Inset plot: mutation rates among 504 genes with mtDNA genes highlighted. Outer plot: closeup of the inset plot in the region containing all 37 mtDNA genes; commonly mutated nuclear cancer genes in this region are labelled for reference. **(b)** The correlation between TMB (mutations per Mb) among mtDNA (y axis) and nuclear-encoded, cancer-associated genes (referred to simply as cancer genes; x axis), (n = 3,624 well-covered pan-cancer tumours). **(c)** TMBs for somatic mtDNA mutations and mutations to cancer-associated genes are compared between microsatellite stable (MSS) and microsatellite unstable (MSI-High) tumours, for both (n colorectal cancer: MSI=65, MSS=318; n stomach adenocarcinomas: MSI=75, MSS=256). Although MSI-High tumours have elevated TMB for nuclear cancer genes, there is no effect on mtDNA TMB. Moreover, mtDNA TMB is similar to (or exceeds) that of nuclear cancer associated genes in both cancer types. Error bars are 95% exact Poisson confidence intervals. **d** Circular mtDNA genome annotated with locations of homopolymer repeat loci ≥5bp in length. Dot height from the circular mtDNA genome indicates the number of affected samples, dot colour indicates the identity of the repeated nucleotide (A, C, G, T), dot width indicates the length of the repeat region (5-8bp). The 6 solid-colour homopolymer loci highlighted are statistically enriched hotspots for frameshift indels, and when combined are the site of ~40% of all mtDNA truncating mutations in cancer. **e** Survival analysis of 344 Stage 1-3 colorectal cancer patients from The Cancer Genome Atlas (TCGA), stratified by mtDNA status (Wild-type n = 108; Truncating n = 84; VUS n = 152). Data from [Gorelick et al., 2021]. VUS, variant of unknown significance (any other potentially pathogenic mtDNA mutation that is not a truncating variant).

Defining the impacts of mtDNA mutations in cancer

Although current model systems for mtDNA mutations in cancer are limited, using model systems in hand we are addressing the effects of mtDNA mutations on cancer initiation, progression and behaviour across a range of established cellular, organoid and *in vivo* models of cancer.

Using cutting-edge mtDNA base editing technology, we have created the first *in vivo* models of mtDNA mutant cancer, bearing mutations in mitochondrial complex I, analogous to hotspot mutations found in our earlier work (Gorelick *et al.*, 2021, *Nat Metab*). We are assessing these in a number of cancer types, and have recently reported that mtDNA mutations are functional regulators of cancer metabolism in melanoma (Figure 1a), inducing a Warburg-like metabolic state, modifying the tumour immune microenvironment and controlling responses to immunotherapy in both animal models and in patients (Figure 1b,c) (Mahmood *et al.*, 2024, *Nature Cancer*).

In developing a mechanistic understanding of these impacts, we have subsequently devised strategies for sensitising non-mtDNA mutant tumours to immunotherapy using exogenous NADH oxidases, such as cyto*Lb*NOX, which demonstrate robust effects on the response of tumours to treatment (Figure 1d,e). These promising approaches to sensitising tumours to immunotherapy hold significant translational potential and have been protected with patent filings. This new approach to therapeutic sensitisation of cancers is in the process of commercialisation with Cancer Research Horizons.

Beyond experimental systems in the lab, using repurposed sequencing data from >40,000 tumours, we have shown that: i) mutations in mtDNA encoded genes are among the most common pan-cancer mutational events, comprising 25 of the 30 most mutated genes in all cancer (Figure 2a), that mtDNA mutational status is unaffected by nuclear DNA mutation burden or MSS/MSI state (Figure 2b,c), that recurrent hotspots define the patterning of severe mtDNA mutations (Figure 2d) and that mtDNA mutation state offers major prognostic value in colorectal cancer (Figure 2e) (Gorelick *et al.*, 2021, *Nat Metab*).

These findings illustrate some of the major impacts of mitochondrial genetics in cancer for the first time, shining a light on a whole additional genetic system of potential therapeutic targets that have been largely overlooked in cancer research to date.

Publications listed on page 121

UBIQUITIN SIGNALLING

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Post-translational modification with ubiquitin (Ub) initiated by sequential actions of Ub-activating enzyme (E1), Ub-conjugating enzyme (E2) and Ub ligase (E3) regulates diverse cellular processes, including signal transduction, cell cycle progression, apoptosis and gene transcription. Deregulation in the Ub pathway is often associated with human pathogenesis, including cancer. Our group uses structural biology and biochemical approaches to study the enzymes in the Ub pathway to understand their regulation, mechanistic function and mutation-induced deregulation. We anticipate that the knowledge gained from our structural studies will assist in the development of selective therapeutic targets within the Ub pathway.

Ubiquitin conjugation cascade

Covalent attachment of Ub involves three key enzymes, namely E1, E2 and E3 (Figure 1). E1 adenylates Ub's C-terminus in the presence of Mg2+ and ATP, followed by formation of a covalent thioester intermediate with Ub. E1 then recruits an E2 and transfers the thioesterified Ub to the E2's catalytic cysteine, forming an E2~Ub thioester intermediate (~ indicates the thioester bond). E3 generally consists of an E2-binding module (HECT, RING, RBR or U-box domain) and a protein-protein interaction domain that can recruit the substrate directly or indirectly. With this configuration, E3 recruits E2~Ub and the substrate to promote Ub transfer from the E2 to a lysine side chain on the substrate. In humans, there are ~600 RING E3s, and we are interested in uncovering their regulation and function and exploring the Ub system for cancer therapeutics.

Deregulation in CBL ubiquitin ligase

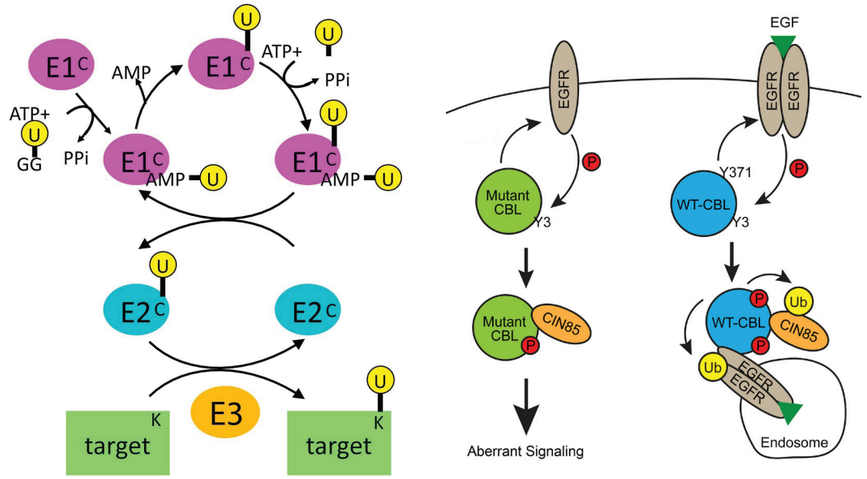
CBL proteins (CBLs) are RING E3s that negatively regulate receptor tyrosine kinases, tyrosine kinases and other proteins by promoting their ubiquitination and degradation by the proteasome or lysosome. Mutations in CBL have been observed in human patients with myeloproliferative diseases. Investigating the mechanism by which CBL mutants exert oncogenesis, we showed that CBL mutants inactivated E3 activity, thereby functioning as an adaptor to recruit other proteins such as CIN85 to elicit oncogenic signalling. Mechanistically, CBL mutants bound to receptor tyrosine kinases such as EGFR, which led to phosphorylation of CBL mutants' C-terminal tyrosines. Phosphorylated tyrosines induced conformational changes that enabled CBL mutant-CIN85 interaction. CBL mutants could not ubiquitinate CIN85, leading to deregulated CBL-CIN85 signalling which altered transcriptome landscape, that in turn upregulated PI3K-AKT signalling cascade to drive oncogenesis (Ahmed *et al.*, 2021, *Oncogene*) (Figure 2). Over the past year, we have characterized an inhibitory molecule that binds CBL mutants and block its oncogenic property in cells and in a mouse xenograft model. Ongoing works are to explore the potential of this molecule in both WT and mutant CBL-driven cancers.

MDM2 RING domain: regulation and targeting

MDM2 is a RING E3 that plays a critical role in the regulation of the p53 tumour suppressor protein by inhibiting p53's transcriptional activity and targeting it for proteasomal degradation. Approximately 50% of human cancers retain wild-type p53, but p53 expression is usually kept low due to amplification of MDM2 gene. Inhibition of MDM2-p53 interaction stabilises p53, resulting in elevated p53 activity that promotes cell cycle arrest and apoptosis in cancer cells. Small-molecule inhibitors targeting MDM2's N-terminal p53-binding domain are in clinical trials, but these compounds exhibit high on-target toxicities. We showed that inhibition of MDM2's E3 activity via mutagenesis led to p53 stabilisation but MDM2 mutants could still bind p53 and restrain its transcriptional activity. Upon stresses their interaction was abrogated leading to rapid p53 activation (Nomura *et al.*, 2017, *Nature Structural* and *Molecular Biology*). Expression of MDM2 E3-inactive mutant was tolerated in adult mice, despite high levels of p53. Upon γ-irradiation, p53 activity was rapidly

Figure 1. Enzymatic cascade for Ub modifications

Figure 2. Model showing mechanism of action of CBL mutant in driving oncogenesis



activated in various tissues, but most tissues were able to dampen p53 activity and regained homeostasis, suggesting inhibition of MDM2 E3 activity might reduce on-target toxicities (Humpton *et al.*, 2021, *Genes & Development*). In an effort to target MDM2 E3 activity, we showed that MDM2 adopted an autoinhibited conformation where its acidic-zinc finger regions formed intramolecular interaction with the RING domain to perturb its E2~Ub binding affinity and E3 activity. p14ARF is a negative regulator of MDM2 and binds to MDM2's acidic region. We showed that binding of p14ARF to MDM2's acidic region strengthened MDM2's intramolecular interaction and massively inhibited its E3 activity (Kowalcyzk *et al.*, 2022, Life *Science Alliance*). Our study provides the basis for p14ARF-mediated inhibition of MDM2 E3 activity (Figure 3) and reveals strategies for targeting MDM2 RING domain. Currently, we are developing MDM2 RING inhibitors via protein design.

DELTEX ubiquitin ligases

We have characterised the DELTEX family of ubiquitin ligases. They harbour a conserved C-terminal RING domain followed by a DELTEX C-terminal domain (DTC). Our work revealed that the DTC domain contains an ADP-ribose/NAD$^+$-binding pocket, enabling it to recruit ADP-ribose-modified substrates in cells and catalyze their ubiquitination (Ahmed *et al.*, 2020, *Science Advances*). Poly-ADP-ribosylation is an early event in the DNA damage repair pathway, and we showed that DELTEX E3s are involved in this process. We are currently investigating the underlying mechanism further. Beyond protein substrate ubiquitination, we also demonstrated that DELTEX E3s can catalyze direct ubiquitin modification of ADP-ribose and NAD$^+$ (Chatrin *et al.*, 2020, *Science Advances*), although the biological significance of this modification remains to be elucidated. Recently, we showed that DTX3L can bind single-stranded nucleic acids and catalyze ubiquitin modification at the 3'-OH group of ribose (Dearlove *et al.*, 2024, *eLife*). We are examining the functional relevance of this novel modification. Given the involvement of DELTEX E3s in DNA damage repair pathway, we hypothesized that these non-proteinaceous ubiquitination event may play a role in this process. Further characterization of these mechanisms could open avenues for therapeutic targeting in cancer.

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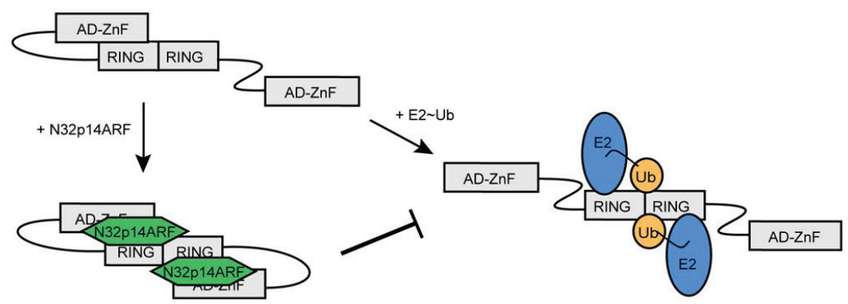
Figure 3. Regulation MDM2 E3 activity by p14ARF



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GROWTH FACTOR SIGNALLING AND SQUAMOUS CANCERS

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The transforming growth factor beta (TGFβ) superfamily can act as potent tumour promoters and tumour suppressors and their signalling pathways are frequently dysregulated in cancer. Work in our laboratory seeks to understand the molecular basis of how, when and where TGFβ superfamily signalling can act to both promote and inhibit tumour progression. Dysregulation of TGFβ signalling is particularly prevalent in squamous cell cancers (SCC) and we are investigating the molecular landscape and drivers of disease progression in cutaneous SCC (cSCC), Recessive dystrophic epidermolysis bullosa (RDEB) associated cSCC and Head and Neck SCC (HNSCC) using systems biology and biological functional approaches.

TGFβ signalling in cancer

TGFβ superfamily ligands are produced by a myriad of cell types and signal in autocrine, paracrine and systemic fashions to regulate a panoply of cell fate and biological processes during development, tissue homeostasis and in pathophysiological situations. TGFβ exerts its effects by activation of signal transduction pathways emanating from a heterotetrameric complex of TGFBR2 and TGFBR1 receptors whose formation is facilitated by ligand binding. TGFBR2 activates the kinase activity of TGFBR1 and this in turn phosphorylates SMAD2 and SMAD3, which then form hetero-oligomeric complexes with SMAD4, and regulate expression of hundreds of target genes that in turn mediate the biological effects of growth factor exposure. Inactivation of the potent tumour cell autonomous tumour suppressive effects of TGFβ signalling frequently occurs via genetic and epigenetic silencing of canonical signalling components whereas modulation of the pathway and its downstream target genes may enable autocrine, paracrine and systemic tumour promoting activity. Through transcriptome wide studies we identified *C1orf106* as a novel SMAD3 dependent TGFβ target gene that promotes clonogenicity in murine breast cancer cell lines and correlates with poor prognosis in human breast cancer (Figure 1, Strathearn *et al.*, 2024, *Cells*) and we are currently investigating the roles of other potential modulators and mediators of TGFβ tumour promoting activity.

TGFβ signalling in squamous cell carcinomas

In collaboration with Owen Sansom's and Irene Leigh's group (Queen Mary University of London) we have shown that TGFβ receptors are inactivated in 30% of sporadic cSCC and that TGFβ signalling can have potent tumour suppressive effects in the face of other mutational events *in vivo*. Despite TGFβ's powerful tumour suppressive effects in cSCC, 70% of tumours display no obvious inactivation of the canonical signalling pathway. Similarly, analysis of publically available HNSCC data sets indicate potential tumour suppressor roles of TGFβ signalling (loss/downregulation of canonical signalling components) in ~30% of tumour samples whilst the remaining ~70% of tumours show overexpression of TGFβ1 and many tumours upregulate TGFBR1 expression relative to normal tissue indicative of potential tumour promoting roles. Taken together, these observations indicate that TGFβ signalling may also act to promote tumour progression in both cSCC and HNSCC and we are focusing our initial efforts into understanding the potential tumour promoting effects of TGFβ signalling in cSCC and HNSCC in a panel of patient derived cell lines (PDCLs).

cSCC is a life-threatening complication for patients who suffer from recessive dystrophic epidermolysis bullosa (RDEB), a skin blistering disease caused by germline mutations in collagen VII, the anchoring fibril component in the skin. Unlike in sporadic cSCC, RDEB SCC tumours do not contain inactivating mutations in TGFβ receptors (Cho *et al.*, 2018, *Sci Transl Med*) pointing to a potential tumour promotion role in these cancers. Intriguingly, we have found that exogenous TGFβ stimulation inhibited proliferation of all RDEB cSCC PDCLs but that endogenous TGFβ signalling drove proliferation, clonogenicity, migration and invasion in the majority but not all of these cell lines (Dayal *et al.*, 2021, *BJD*). Targeting TGFBR1 kinase activity may have therapeutic benefit for patients with these tumours but in some it maintains tumour suppressive activity. Working in partnership with DEBRA we are building new models of RDEB cSCC, investigating the molecular processes by which TGFβ signalling acts to drive proliferation, migration and invasion in these tumours and striving to identify novel therapeutic susceptibilities of these aggressive cancers.

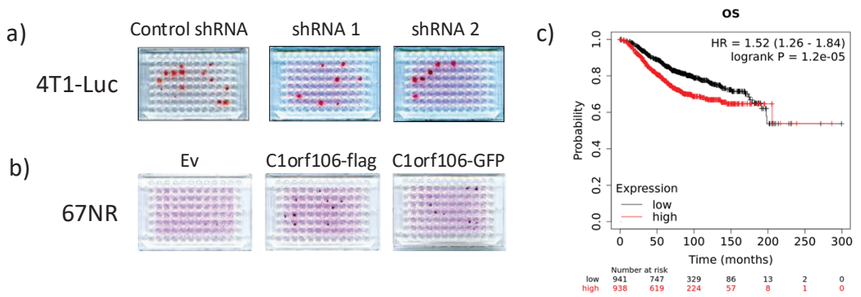

Figure 1. The TGFβ target gene C1orf106 promotes clonogenicity and correlates with poor prognosis in breast cancer. a. Knockdown of c1orf106 expression in metastatic 4T1-luc cells with two independent shRNAs inhibits colony formation. **b.** Overexpression expression of FLAg or GFP tagged c1orf106 in non-metatstic 67NR cells promotes colony formation. **c.** Kaplan-Meier survival analysis on human breast cancer patients split based on median *C1orf106* mRNA expression. OS= overall survival. (Adapted from Strathearn *et al.*, 2024, *Cells*).

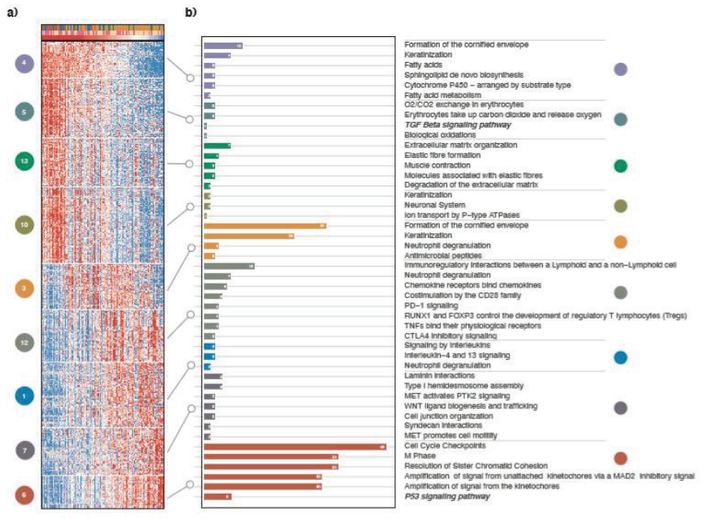

Figure 2. Pathways and processes associated with disease progression of the cSCC disease continuum. a. Heatmap of gene expression levels of genes in 9 core co-expressed gene clusters ordered by differentiated to progenitor like status (left to right). **b** GSEA analysis showing significantly enriched molecular pathways and/or processes in the 9 core co-expressed gene clusters. (Adapted from Bailey *et al.*, 2023, *Nat Commun*).

Deciphering drivers of SCC disease progression

The incidence of keratinocyte skin cancers represents a rising global health burden. Driven by UV mediated DNA damage, development of primary cSCC tumours can be preceded by pre-malignant Actinic Keratosis (AK). In contrast to most other epithelial malignancies, more than a third of patients develop multiple primary cSCC. Metastasis occurs in ~5% of cases, and there are few effective treatments for advanced cSCC, with five-year mortality rates of ~30% for metastatic disease. In collaboration with Irene Leigh, Catherine Harwood, Jun Wang (QMUL and Barts Cancer Institute), Charlotte Proby (University of Dundee), David Adams (Sanger Institute) and Peter Bailey (University of Glasgow), Crispin Miller and John Le Quesne we are carrying out a detailed characterisation of cSCC disease progression using a variety of next generation sequencing approaches coupled with spatial analysis of protein and RNA expression. Using whole exome sequencing (WES) we have previously demonstrated that both pre-malignant (Thomson *et al.*, 2021, *J Invest Dermatol*) and primary tumours possess remarkably similar complex genetic landscapes (Inman *et al.*, 2018, *Nat Commun*). Using bulk RNASeq transcriptomic profiling of 110 patient samples representing normal sun exposed skin, AK, primary and metastatic cSCC we have found that cSCC disease progression manifests as a disease continuum from a differentiated to a progenitor-like state (Bailey *et al.*, 2023, *Nat Commun*). K-Means clustering coupled with gene set enrichment analysis (GSEA) demonstrated that progression of cSCC is associated with the orchestrated modulation of key pathways and processes and reveals potential targets for therapeutic intervention (Figure 2). We are now investigating the transcriptional and genetic landscape of an independent cohort of primary cSCC tumours that did and did not metastasise and their matched metastases with a view to identifying mediators of metastasis.

In collaboration with the Glasgow Head and Neck Cancer group (GLAHNC,) and the Northern Head and Neck alliance we are seeking to understand the molecular basis of chemo-radiotherapy resistance, disease recurrence, lymph node metastasis and distant metastatic spread of HNSCC. Initial collaborative clinical studies are revealing the risk factors for developing HNSCC (Smith *et al.*, 2024, *Head and Neck*), changes in incidence rates (Smith *et al.*, 2024, *BJC Rep*) and the survival outcomes of laryngeal cancer patients (Rajgor *et al.*, 2024, *Clin Otolaryngol*). We are building on these studies and undertaking multiomic molecular profiling of clinically annotated patient samples from local site-specific cohorts and clinical trials and are developing pre-clinical experimental models.

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DEEP PHENOTYPING OF SOLID TUMOURS

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Solid tumours are complex assemblages of malignant cells, lymphocytes, fibroblasts, blood vessels and other tissue types, and are best thought of as complex neo-organs built around a never-ending cycle of injury and frustrated repair. To understand how malignant cells survive and spread in this potentially extremely hostile habitat, we must understand the microscopic environment at a cellular level and visualise the competing cellular strategies of malignant cells and their genomically normal stromal neighbours. We aim to answer a range of key questions in tumour biology by using the latest deep phenotyping technologies to directly observe and quantify cellular behaviours in intact tumour tissue.

We routinely develop highly multiplexed IF/ISH staining assays using Ventana autostainer platforms and collect multiplex images from human and mouse tumour tissues using Akoya Mantra and Polaris imaging platforms, as well as the FUSION ultra-deep imaging system. In essence, most of the technologies that we apply consist of three steps (Figure 1). First, we detect multiple RNA or protein targets with a range of immunofluorescent antibodies and probes. We then acquire high-resolution images, with separate layers for each marker of interest. These images are subsequently converted into quantitative data, typically single-cell quantitative measures and/or cellular phenotypes, obtained by the application of artificial intelligence image segmentation algorithms which we have created for the task. These spatial and quantitative cell data are used as the substrate for classical or more advanced modelling techniques intended to answer biological questions about tumour function.

Key projects:

1) Translational control in tumour cells

The dysregulation of mRNA translation is emerging as a key hallmark of malignant transformation, as tumour cells radically reprogramme their protein output by implementing translational control mechanisms associated with states such as cellular stress and altered nutrient availability. To what extent is mRNA translation regulation altered in human cells? Which hallmark behaviours are linked to which alterations in translational control? Which elements of the translational control machinery have promise as therapeutic targets?

We are examining numerous measures of translational control at the single-cell level in large collections of several common malignancies, and we are using the resulting images both to generate and to test hypotheses. For example, we have found that switching between expression of different mRNA helicases is associated with tumour cell proliferation and invasion as well as immune system evasion, and that stress signalling through eIF2 is intimately associated with tumour cell proliferation and invasion.

2) Tumour immunophenotyping

The most impactful development in cancer therapy in recent years is the introduction of immunotherapies. These treatments work by reversing the ability of tumour cells to mask themselves from the immune system which would otherwise rapidly destroy them. However, we are at present only partially successful in identifying which patients will benefit from these therapies. We believe that quantifying the degree of immune system engagement within tumour biopsy material is likely to improve our ability to do this; can we, by direct observation of complex cellular phenotypes in tissues, identify tumours which are actively evading immune system detection and/or destruction?

To achieve this, we are applying highly multiplexed panels of markers to identify tumour and immune cell phenotypes, for instance using our FUSION platform we can use upwards of 40 markers to distinguish specific cell phenotypes in the tumour microenvironment. We are then able to link the presence and relative spatial distribution of these cells to patient outcomes. We intend to apply these methods to cohorts of tissues from patients receiving immunotherapies with Glasgow's cancer treatment centre, and to see if we can improve our ability to predict patient response to immunotherapy, compared to current methods.

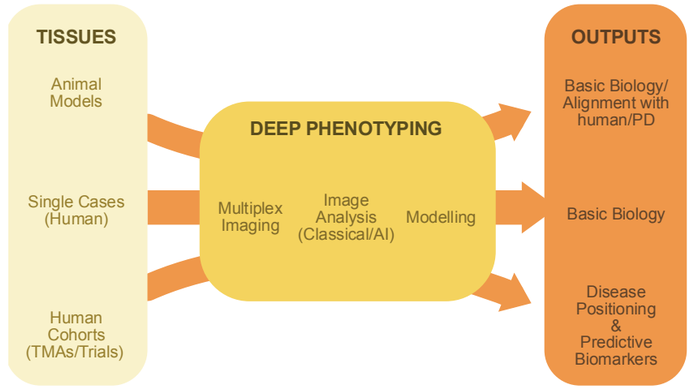

Figure 1. Workflow schematic of deep phenotyping methods. The basic pipeline (centre) is applied to a range of tissue types to achieve answers to diverse scientific questions.

3) Application of artificial intelligence to tumour microscopy

Self-learning artificial intelligence offers us the potential to reach deeply into the information present within microscopy images without necessarily knowing which features of the images are likely to be important a priori. These methods are potentially very powerful, and able to answer both clinical and basic scientific questions. Can we train machines to predict patient outcomes, and response to therapies?

We have accumulated very large collections of microscopy images from archival lung cancers and mesotheliomas, and, in collaboration with computer scientists, we are using these to train machine algorithms to attempt these tasks. In addition, we aim to use generative methods to identify image features which are particularly strongly associated with key tumour features (e.g. lethality, hallmark behaviours or genomic alterations). Furthermore, we are about to start applying these methods to highly multiplexed tissue images, which holds the potential for even deeper understanding.

4) Deep phenotyping of respiratory malignancies

We have particular interests in non-small cell lung cancer (NSCLC) and malignant mesothelioma. Both have high incidence in Glasgow and are in great need of improved therapies. We are using a combination of classical microscopy methods and multiplex methods to tackle key questions in these disease types. In particular, we are using linked RNASeq and multiplex image data to deconvolute gene expression in very large case cohorts, gaining unique insights across the breadth of human tumour variance.
Malignant mesothelioma is a difficult diagnosis to make in tissue biopsies, and we hope to improve this, as well as our ability to predict progression to invasive malignancy, by discovering novel biomarkers of malignancy, using a combination of classical methods and machine learning algorithms, and building upon Glasgow's flagship PREDICT-Meso physician-led study of early mesothelioma.

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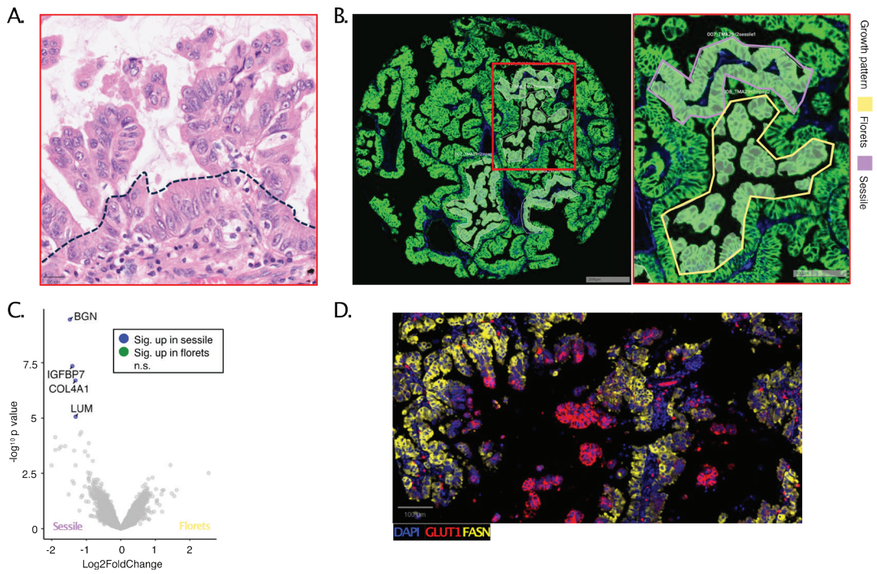

Figure 2. Use of spatial biology to reveal tumour cell plasticity in lung adenocarcinoma. Figure A: Haematoxylin and eosin stained representative image of micropapillary growth B: Example segment selection on Digital Spatial Profiler (Nanostring) using manual polygon selection of floret (yellow) and sessile (lilac) regions. C: Volcano plot of significantly differentially expressed genes (DEG). D: Representative multiplex immunofluorescent image (blue: DAPI, red: GLUT1, yellow: FASN) showing metabolic differences between sessile and floret regions.

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PROSTATE CANCER BIOLOGY

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Prostate cancer remains a major global health issue. It affects one in eight men in the developed world, and now accounts for more cancer related deaths in men than females dying of breast cancer. Research in our group employs a cross-disciplinary strategy to tackle clinically challenging aspects of aggressive prostate cancer, namely metastasis and treatment resistance. Our current research priorities aim to improve our understanding of the early stage of metastasis including colonisation of regional lymph nodes by prostate cancer. In addition, we are collaborating as part of the RadNet programme to study the molecular basis of radiation resistance in prostate cancer.

Defining the tumour microenvironment in promoting cancer metastasis to the regional lymph nodes in prostate cancer

Patients with high-risk localised disease have an increased risk of residual or recurrent prostate cancer despite radical treatment, and ultimately progressing to regional and distant metastasis, which resulted in shortened patient survivals. Molecular factors controlling the initiation of prostate cancer metastasis in the first instance to their subsequent colonisation in the regional lymph nodes within the pelvis and/or distant metastasis remains to be fully defined. It is well documented that the tumour microenvironment of prostate cancer tends to be immunologically cold, and the successes of immunotherapies in other tumour types have not been observed in prostate cancer. Hence, the relationships between the prostate cancer microenvironment and the respective draining lymph nodes represent a major focus of our multidisciplinary project.

The ORCHESTRATE (acronym for "investigating the tumOuR miCroenvironment of High-risk localisEd proStaTe canceR to identify AcTionablE pathways involved in cancer metastasis") project is a research programme funded through the Prostate Cancer UK Transformative Impact Award scheme. ORCHESTRATE was launched in 2024 and consists of five work packages (Figure 1), benefiting from our extensive translational infrastructure in clinical urology (Hing Leung, Mark Salji, Imran Ahmad), pathology (Jonathan Salmond) and imaging with laboratory expertise in preclinical models (Ed Roberts, Karen Blyth and Imran Ahmad), spatial biology (John Le Quesne and Nigel Jamieson, incorporating contemporary omics technologies) and facilitating informatics methodologies including machine learning (Ke Yuan), Figure 2. We will carry out parallel cross species analysis of clinically resected tumours and tumours derived from our preclinical models of aggressive prostate cancer. Figures 3 and 4 highlight expertise in multiplex phenotyping in both human and murine tumours, respectively. Hence, knowledge gained from our research will help identify better treatment strategies for patients with high-risk localised prostate cancer, with accompanying preclinical models for additional validation of putative mechanisms in cancer progression.

Schlafen family member 5 (SLFN5) and treatment resistant prostate cancer

We recently identified Schlafen family member 5 (SLFN5) as an AR-regulated protein in CRPC, with elevated SLFN5 protein expression (based on semi-quantitative immunohistochemistry analysis) in castration resistant prostate cancer, significantly correlated with poor patient survival outcome (Martinez *et al.*, 2021, *Cancer Res*). Our working model is that SLFN5 regulates the expression of LAT1, an essential AA transporter, and thereby intracellular levels of essential amino acids and mTORC1 signalling. Ongoing focus is to further define the molecular basis of SLFN5 mediated castration resistance, thus identifying novel therapeutic targets.

Besides tumour response to androgen deprivation therapy, SLFN5 has recently been implicated in repair of DNA double-strand-breaks (DSBs) by controlling 53BP1 topological arrangement at DSBs (Huang *et al.*, 2023, *Mol Cell*). Indeed, SLFN5 deficiency disrupts the DSB repair topology and impairs non-homologous end joining, telomere fusions, class switch recombination, and sensitivity to poly (ADP-ribose) polymerase inhibitor. We are therefore collaborating with colleagues within the RadNet Glasgow consortium, led by Anthony Chalmers, to characterise the role of SLFN5 in prostate cancer response to radiation therapy. With pump priming funding from RadNet Glasgow, we are creating a panel of radiation resistant human prostate cancer cell models to support future mechanistic studies.

Prostate Specific Membrane Antigen (PSMA) related Neuronal Metabolites in Treatment Resistant Prostate Cancer

PSMA converts NAAG (N-Acetylaspartylglutamic acid or N-Acetylaspartylglutamate), a highly prevalent neurotransmitter, to NAA (N-acetylaspartic acid) and Glutamate and may be important in radioresistance. We hypothesise that PSMA activity can be determined by NAA/NAAG measurement in urine. These neuronal metabolites may promote survival as a local acetate and glutamate source for sphingolipid dependent survival mechanisms driven by activation of glucocorticoid receptor.

A combined pre-clinical and clinical approach is being undertaken to investigate NAA/NAAG biology. Urinary NAA/NAAG pre and post radiotherapy may be clinically useful to explain differences in radiosensitivity related to PSMA activity. Surgical explant models of CRPC including bone metastasis will visualise NAA and NAAG within the tissue microenvironment using Mass Spec Imaging (MALDI / DESI).

Figure 1

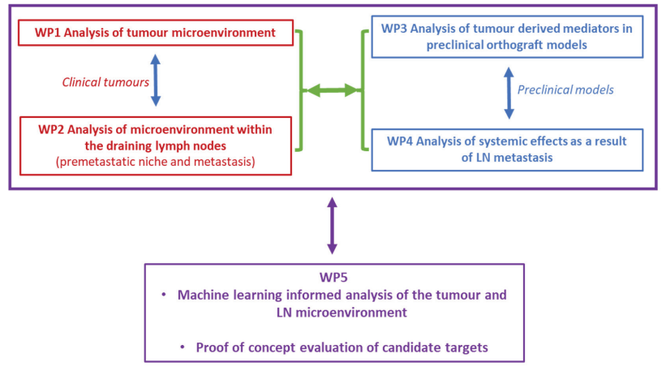

Figure 2

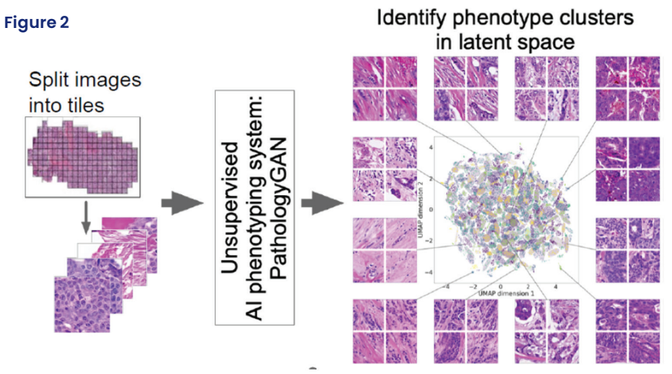

Figure 1. Overview of the five interconnecting work packages of the ORCHESTRATE project, leveraging the ability to carry out cross-species analysis on clinical resected tumours and tumours from pre-clinical models of aggressive prostate cancer.

Figure 2. Illustration of workflow to find phenotype clusters which could be used to refine histopathological classifications associated with lymph node metastasis.

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Figure 3

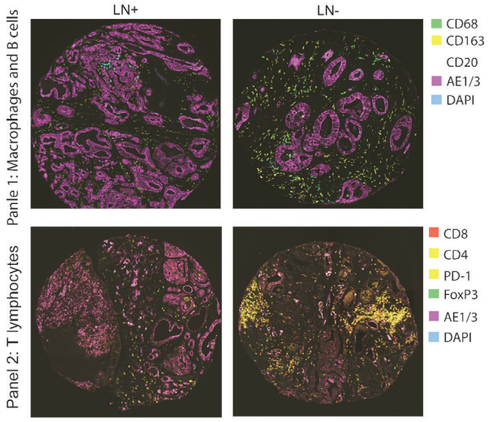

Figure 4

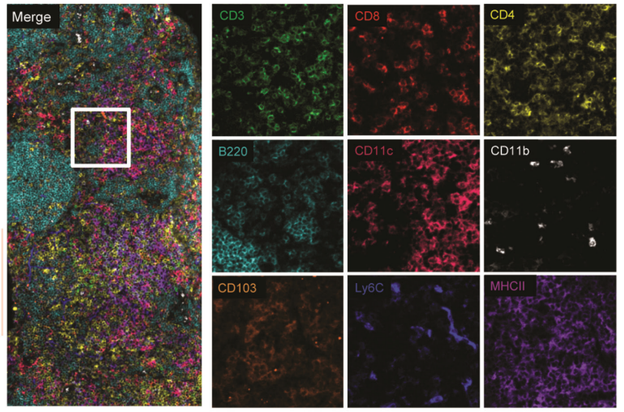

Figure 3. Representative multiplex immunofluorescence images showing reduced immune cell infiltration in patients with (left) compared to patients without (right) LN metastasis. Images are spectrally unmixed for Panel 1: macrophage & B cell (top) and panel 2: T lymphocyte (bottom). (Data generated in collaboration with Leah Officer-Jones and John Le Quesne)

Figure 4. Multiplex high resolution imaging of sections of murine lymph nodes, with subsequent segmentation and identification of key cell types carried out in HALO to support spatial statistical analysis. Images shows data from nine markers (identity provided in inserts) for illustration. (Data generated by Ed Roberts' research group.)

MOLECULAR IMAGING

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3 CRUK TRACC Programme
4 CRUK Glasgow Centre

Our lab develops new ways to visualise cancer – we create radiolabelled nutrients that image metabolic reprogramming, a hallmark of cancer, and use positron emission tomography (PET) to non-invasively characterise developing tumours. This year, we have focussed on developing technologies to non-invasively stratify colon cancer. Our goal is to classify tumours without the need for invasive biopsies, thereby guiding patient management towards the most effective treatment.

The primary focus of our work is to develop new methods to non-invasively image nutrient usage and then apply these techniques to investigate the causes and consequences of metabolic heterogeneity in mouse models of cancer. Our research has two main themes, first we develop and validate novel technologies such as new metabolic radiotracers and quantitative methods. Second, we exploit PET as a biological imaging modality and investigate the molecular mechanisms and vulnerabilities underlying regional tumour metabolism. The goal of our work is to validate imaging biomarkers for visualising *in vivo* metabolic phenotypes and, by investigating the liabilities of these phenotypes, determine if we can use metabolic imaging to identify susceptibilities that can be used to guide therapy in individual patients.

Stratification of colon cancer using metabolic PET imaging

The current approach for consensus molecular subtyping of colon cancer relies on gene expression profiling tissue, which is invasive and has limited ability to reveal tumour dynamics and spatial heterogeneity. PET imaging presents a non-invasive alternative, however, factors influencing PET imaging phenotype, the suitability of PET radiotracers for differentiating tumour subtypes, and the relationship between PET phenotypes and tumour genotype or gene-expression-based subtyping remain unknown. To address this we conducted a broad PET screening across a spectrum of colon cancer models with four metabolic tracers, [^{18}F]fluorodeoxyglucose ([^{18}F]FDG), [^{18}F]fluoro-ethyl-tyrosine ([^{18}F]FET), 3'-deoxy-3'-[^{18}F]fluorothymidine ([^{18}F]FLT) and [^{11}C]acetate, aiming to identify factors influencing imaging signatures and determine their relationship with genotype, tumour microenvironment and stage.

Our study revealed significant heterogeneity in PET imaging signatures, with distinct radiotracer profiles observed for different cancer models. Notably, oncogenic mutations, such as Kras and Apc loss, showed the most distinctive imaging features, with specific radiotracers like [^{18}F]FLT and [^{18}F]FET be particular effective at stratification of these respectively. Additionally, we found that the tissue microenvironment notably impacted [^{18}F]FDG uptake and higher uptake of [^{18}F]FET was observed in a metastatic model. Overall, this study establishes the feasibility of non-invasive molecular stratification using multiple radiotracer PET (Figure 1; Malviya *et al.*, 2024, *Clin Cancer Res*).

Multi-scale in vivo imaging of tumour development using a germline inducible triple-reporter system

Imaging reporter genes play a crucial role in visualising biological processes *in vivo*, including tumour development, cancer cell dissemination, and treatment response. Historically, incorporating reporter genes into the germline has relied on single imaging modality reporters operating over limited spatial scales. In response, we developed a novel platform technology addressing the challenge of multi-scale imaging of tumour development.

We created and validated a conditional triple-reporter mouse model (Rosa26$^{LSL\text{-}NRL}$), called the "Google-Earth" mouse, integrating imaging reporters for fluorescence, bioluminescence, and PET. This model also features inducible Cre-lox functionality, enabling precise spatiotemporal control of reporter expression. We demonstrated robust reporter inducibility across various tissues in the Rosa26$^{LSL\text{-}NRL}$ mouse, facilitating effective tracking and characterisation of tumours in liver and lung cancer mouse models. Utilising multimodal whole-body imaging, we accurately pinpointed tumour locations, guiding *in situ* lung microscopy to visualise cell-cell interactions within the tumour microenvironment.

This triple-reporter system establishes a robust platform technology for multi-scale investigation of biological processes within whole animals, enabling tissue-specific and sensitive cell tracking from whole-body to cellular scales. This technology is now accessible at the CRUK-Scotland Institute, supporting several new research programs (Dzien et al. 2024).

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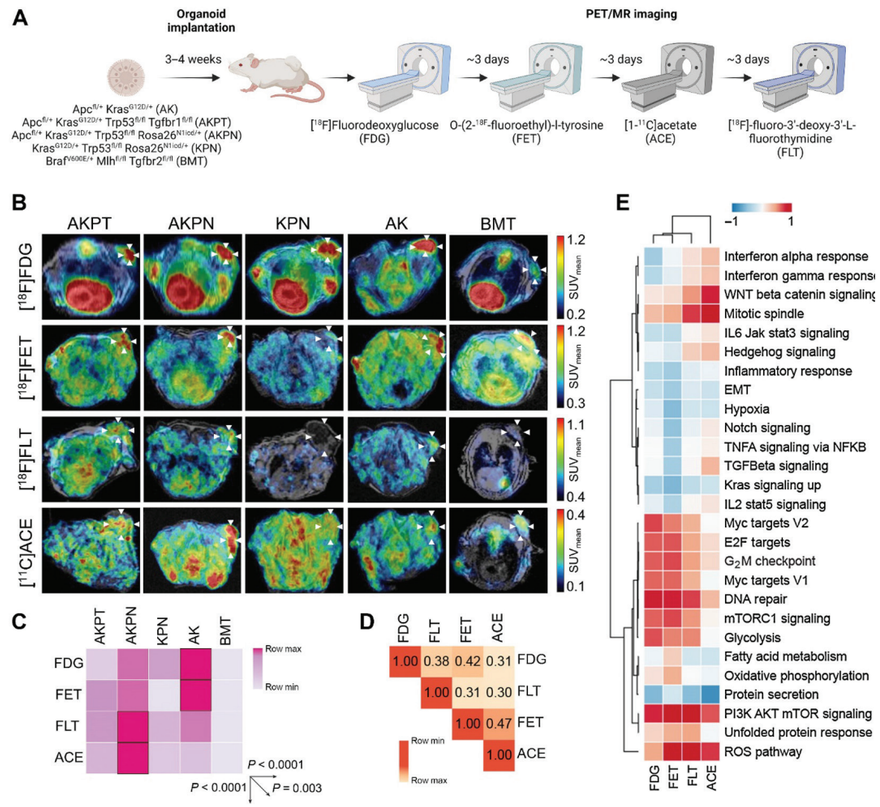

Figure 1. Distinct intermodel heterogeneity in PET imaging signatures. A, In the experimental imaging protocol, five colon cancer organoid models and four PET tracers were used to determine imaging signatures. Details of all mice used in these studies are presented in Supplementary Table S1. **B,** Representative transverse PET images from each model and tracer. The [18F]FDG PET/MR images of the KPN subcutaneous model are reproduced again in Figs. 4B and D and 5B for comparison against other tumors at different sites and stages. **C,** Imaging signature heatmap showing mean tracer uptake, models with highest tracer update highlighted with black outline (representation of the data matrix analyzed with two-way ANOVA). **D,** Correlation matrix of each tracer uptake based on Pearson correlation coefficient. **E,** Heatmap illustrating correlation of PET tracer uptake with gene expression in the Molecular Signatures Database (MSigDB) hallmark dataset, sorted by hierarchal clustering. (**A,** Created with BioRender.com.)

MITOCHONDRIAL REPROGRAMMING IN CANCER

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Tumours must overcome numerous metabolic challenges to thrive in nutrient-deprived microenvironments and to evade therapeutics. Mitochondria are dynamic organelles that provide the metabolic flexibility and plasticity demanded by cancer cells. Our overall objectives are to understand how mitochondria are reprogrammed at different stages of tumorigenesis and to reveal metabolic vulnerabilities in cancer by targeting mitochondrial metabolite transporters.

Intracellular biochemical reactions are compartmentalised to optimise energy efficiency and cellular adaptation to the environment. Essential metabolites, including amino acids and nucleotides, are transported both in and out of mitochondria under the control of dedicated solute carrier proteins. These metabolite transporters sit in the impermeable inner mitochondrial membrane and couple the metabolic reactions of the cytosol with the mitochondrial matrix. Mitochondrial solute carriers therefore represent crucial sites of cellular metabolic control that help govern tumour growth, survival and metastasis (Figure 1). This year, we studied how mitochondrial transport of amino acids and nucleotides is regulated in cancer. Our results have helped define the cellular contexts in which mitochondrial uptake of certain amino acids and nucleotides are essential for metabolic control with broad implications for epigenetic and innate immune signalling in cancer.

One of our specific goals is to understand how the mitochondria transport and metabolise nucleotides. Nucleotides are essential building blocks for the synthesis of mitochondrial DNA and RNA and we recently found that blocking mitochondrial nucleotide supply can suppress cancer cell growth (Grotehans *et al.*, 2023, *EMBOJ*). However, post doc Vanessa Xavier noticed that the inhibition of mitochondrial ribonucleotide supply also limits the build-up of mitochondrial double-stranded RNA (dsRNA) in cancer cells.

Mitochondrial double-stranded RNA in cancer

Mitochondrial dsRNA arises upon the hybridisation of long polycistronic mitochondrial RNA transcripts. Analogous to viral dsRNA, mitochondrial dsRNA is a potent immunogen when exposed to cytosolic dsRNA receptors and can drive inflammation. Therefore, mitochondrial dsRNA homeostasis is carefully regulated in healthy tissue to prevent aberrant innate immune signalling. Vanessa characterised the spatial distribution of dsRNA within mitochondria and found that mitochondrial dsRNA accumulated in proliferating cancer cells. This build-up of mitochondrial dsRNA was dependent on cell cycle progression and mitochondrial pyrimidine nucleotide salvage (Figure 2A). Next, Vanessa teamed up with Prof. John Le Quesne and the Deep Phenotyping team to search for evidence of mitochondrial dsRNA in the tumours of lung cancer patients. Using multiplex imaging of a patient tumour tissue microarray, they indeed detected an increase of dsRNA in malignant lung adenocarcinoma cells (Figure 2B,C). This work illuminated a link between mitochondrial dsRNA homeostasis and cellular proliferation in tumours (Xavier *et al.*, 2024, *Life Sci. Alliance*). The accumulation of mitochondrial dsRNA could be a novel marker of cell malignancy, and it will be important to determine how mitochondrial dsRNA influences tumour immunogenicity in future studies.

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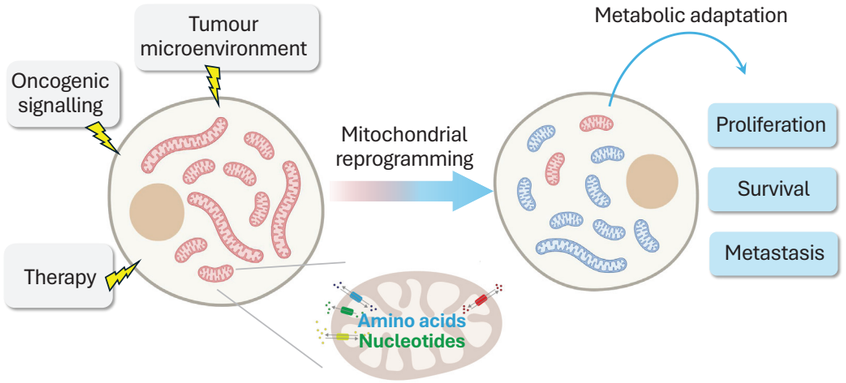

Figure 1. Mitochondrial reprogramming in cancer
Multiple mechanisms drive mitochondrial reprogramming during tumour development. Mitochondrial solute carriers support mitochondrial metabolic adaptation by transporting key metabolites including amino acids and nucleotides across the inner mitochondrial membrane.

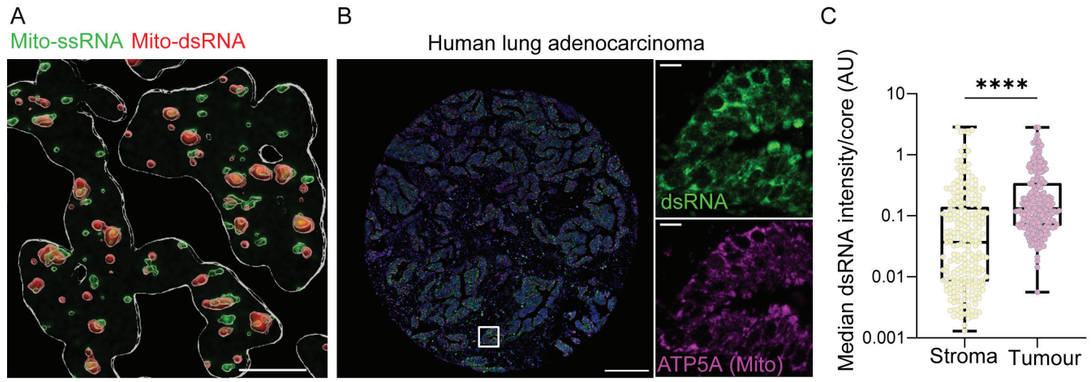

Figure 2. Cancer cells accumulate mitochondrial double-stranded RNA
A Super-resolution imaging of mitochondrial single-stranded (ss)RNA and double-stranded (ds)RNA foci in U2OS cells. **B** Mitochondrial dsRNA detected in a core from lung adenocarcinoma tissue microarray (TMA) by multiplex imaging using antibodies raised against dsRNA and ATP5A (mitochondrial protein). **C** Median dsRNA intensity in stroma and tumour regions per core of the TMA (N=233 cores with stroma, N=221 cores with tumour). This figure was adapted from Xavier *et al.*, 2024 *Life Sci. Alliance. DOI: 10.26508/lsa.202402764.*

MICROBIAL AND IMMUNE METABOLIC MODULATION

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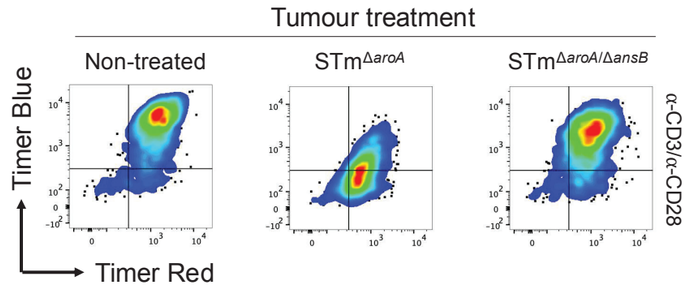
Our lab is interested in the interplay between microbes, epithelium and the immune system in the intestine. Bacteria have been used experimentally as cancer therapeutic agents since the work of William Coley in the early 1900's, yet only one bacterial cancer therapy (BCT) is in clinical use; BCG therapy for superficial bladder cancer. We are working toward understanding and improving BCTs by investigating mechanisms of bacterial adaptation to the tumour, direct effects of bacteria on tumour growth and effects on immune activation and responses. More broadly, we are interested in host-microbe interplay within the intestine (Figure 1).

The immune system protects us from infectious agents such as bacteria, viruses and fungi, as well as from malignant growth of our own tissues. In the intestine, we have both positive (commensals) and negative (pathogenic) interactions with bacteria. We are co-inhabited with trillions of microbes which, for the most part, do not elicit immune responses and exist in a symbiotic relationship with the host; and some bacteria even performing essential functions. The intestinal epithelium harbours innate sensors and is able to recognise and respond to pathogenic insults and help shape innate and adaptive immune responses. Intriguingly, many of these innate pathways can also act to suppress, or promote, tumourigenesis. This is where our intrigue lies; what microbial cues could we utilise to impair tumour growth and improve anti-tumour immunity? We use attenuated Salmonella typhimurium (STm) which selectively home tumours and efficiently reduce tumour growth. In particular, we study effects of STm on colorectal cancer (CRC) using both mouse models of CRC and tumour organoids (mouse and patient-derived). We aim to uncover mechanisms that both drive effective therapeutic responses as well as less-desirable side-effects, in an effort to best engineer STm therapy.

Figure 1. Word cloud generated from publication titles highlighting the laboratory's interests.

Metabolic suppression of tumours at the cost of T cell immunity

In previous work we demonstrated that attenuated Salmonella therapy altered the tumour metabolic landscape, with large reductions in a range of metabolites including sugars, TCA cycle intermediates and amino acids or their precursors (Mackie *et al.*, 2021, *JCI Insight*). From this we surmised that part of the mechanism of Salmonella therapy was metabolic competition – essentially the microbes could outcompete tumour cells for essential fuel sources and thereby limit tumour growth. Over the past few years one of the lab's research focuses has been to understand the role T cells play in BCT. Previous research had shown that T cells are not required for effective STm therapy, yet the mechanisms behind this have not been addressed. Further, there is effort to develop BCTs alongside checkpoint blockade therapies, thus it is important we resolve how T cells function during BCT. We indeed found that T cells from STm-treated tumours were dysfunctional; they could not sustain their activation and showed poor cytokine production and failed proliferation. This was not associated with defects in TCR signalling, but instead potent inhibition of glycolysis; upregulation of which is essential for T cell proliferation and gain of effector function. T cell metabolic dysfunction was due solely to asparagine depletion by bacteria, leading to depleted c-Myc protein; reversal of this depletion restored T cell function (Figure 2). Critically, STm-mediated c-Myc suppression was also detected in the tumour itself, which dampened tumour stemness and survival, highlighting an important 'double-edged sword' for STm BCT in which tumour control by bacteria comes at the detriment of adaptive immunity. These findings provide a strong rationale for addressing a previously unknown cardinal defect in Salmonella-based cancer therapies to yield more successful clinical outcomes. This work is now published in EMBO Molecular Medicine (https://doi.org/10.1038/s44321-024-00159-2). Future work will now aim to further dissect the efficacy of the asparaginase-deficient STm when in combination with immune checkpoint blockade therapies, or the asparaginase-sufficient strain with different timing regimens to improve tumour suppression.



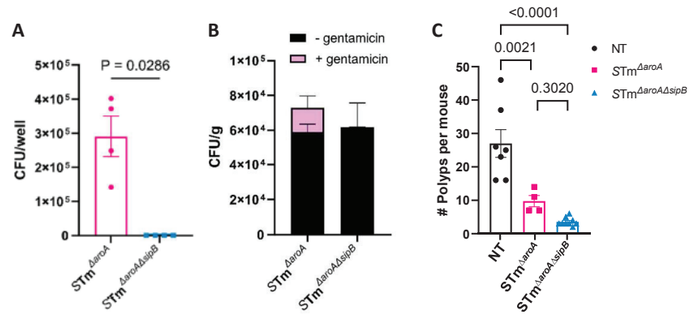


Figure 2. CD4+ T cells cultured in tumour conditioned medium from tumour organoids given the indicated Salmonella strains. Timer blue and red expression indicates persistent T cell receptor activation. Timer^{red+}Timer^{blue-} indicates terminated T cell receptor signalling. aroA=aromatase deficient STm; ansB=asparaginase.

Figure 3. A) Tumour organoids were infected with ΔaroA or ΔaroaΔsipB STm and CFU analysed. Loss of sipB ameliorates STm invasion. B) *Apc*$^{min/+}$ mice were infected with each STm strain and CFU in polyp tissue assessed. Gentamycin added to quantify STm residing intracellularly, the remaining are extracellular. C) *Apc*$^{min/+}$ mice were treated with indicated STm strains or control for 8 weeks, from 8 weeks of age. Polyp burden shows reduction in polyp number in STm treated mice.

Intra versus extracellular bacterial targeting

We had observed preferential invasion of Lgr5+ stem cells within the tumour and noted that in fact only a small percent of STm are intracellular; the vast majority reside in the extracellular spaces. Our questions were: why does STm preferentially invade Lgr5+ stem cells? And is intracellular invasion important or necessary for STm therapeutic effect? Using patient-derived colorectal cancer organoids we found that, like mouse-derived organoids, STm preferentially invade proliferating cells, and blocking cellular proliferation prevents STm invasion. We found this was due to part of the type III secretion system apparatus, and particularly SipB, which mediates tight binding to the host cell by interaction with membrane cholesterol. We have used invasion deficient STm to start to investigate the necessity of intracellular invasion for therapeutic effect of STm therapy (Figure 3). Invasion-deficient STm may represent a safer therapeutic avenue, decreasing the (very low) risk of bacterial dissemination in immune compromised cancer patients. However, some STm therapy strategies are focussed on using STm as a vehicle to deliver intracellular cargo – thus it is important to further understand how, how many and which cells are actually targeted intracellularly, and how much bearing that has on therapeutic success.

Epithelial innate sensors

Another avenue of interest for our lab are intracellular innate sensors that are expressed by epithelial cells, particularly how they contribute to, or control, tumorigenic growth. Previously, we have shown that a family of proteins called NLR apoptosis inhibitory proteins (NAIPs) suppress epithelial tumorigenesis in a cell-intrinsic manner. NAIPs belong to a family of inflammasome-forming proteins, and other groups have also identified tumour suppressive roles for other family members, suggesting a kind of innate sensing and checkpoint in epithelial transformation. Recently, we have been asking what effect loss of epithelial NAIPs, which we observed during tumorigenesis, might have on the intra-epithelial / tumoral immune response, particularly on intraepithelial lymphocyte populations. We have found some alterations in gamma delta T cells, which we aim to follow up in collaboration with the Coffelt group.

Publications listed on page 124

COMPUTATIONAL BIOLOGY

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Boyu Yu

Rapid advances in technology are leading to a wealth of high-dimensional data describing the behaviour of cells in normal and tumour tissue. We are using computational approaches to interrogate and integrate these high dimensional data in order to develop a more holistic view of the altered regulatory processes that lead to the development and progression of cancer.

A major focus of the Institute is to use multiple 'omics and imaging modalities to generate a more holistic view of the processes that occur in tumour tissue. The goal is to use these data to stratify patient populations to generate a more granular view of the underlying biology of a given tumour. We then use data to position our pre-clinical models of disease against these more tightly defined patient subsets, supporting forward translation from discovery science into the clinic, and back translation from the clinic into our experimental models. Holly Hall has been working, first with the Bird lab to position liver cancer models against patient cohorts, and then to do this at scale using pan-cancer public domain datasets. In parallel, Andrew Papanastasiou is developing novel algorithms and approaches to support the analysis of these more holistic datasets, applying a mixture of techniques from Artificial Intelligence, Large Language Models and Deep Learning. His work is showing that single cell and organoid data are also applicable to these disease positioning approaches as well as in *in vivo* models.

While considerable attention has been directed at the regulation of transcription, many of the downstream processes such as the control of RNA processing, splicing, and mRNA stability are also under tight regulatory control. The translational machinery that governs when, and how these mature mRNAs are translated into correctly folded proteins is similarly constrained. A critical question, therefore, is how is the information that defines these systems encoded within the genome?

Our work exploits the availability of a large and diverse cohort of well annotated genome sequences from different species. This allows comparative genomics to be used to pursue regulatory patterns from an evolutionary perspective. In parallel, the availability of large cohorts of DNA- and RNA-sequenced patient tumour samples makes it possible to explore the evolutionary constraints placed upon different regions of the genome by selection pressure from within the tumour environment. In both cases, the available data are now at sufficient scale to support classical- and neural-network based machine learning algorithms, and we are applying these in combination with mathematical models that draw upon ideas from information theory.

We are collaborating with the Bushell and Le Quesne groups to explore the role of regulatory sequences embedded within coding sequences, how mutations and changes in the regulatory machinery in and around these regions can impact on protein levels. Eva Freckmann is interested in how these regulatory patterns impact on gene expression across human tumours. Britt van Abeelen is exploring how patterns of tRNA usage interact with the translational control machinery and how these are altered in tumour cells. Boyu Yu is investigating the regulatory sequences embedded in the untranslated regions of protein coding genes, and how these sequences are used by cells to regulate mRNA stability and protein translation. In collaboration with Ke Yuan at the University of Glasgow Computer Science, and David Robertson at the University of Glasgow Centre for Viral Research (CVR), we have been supported by the DiRAC high performance computing facility who have enabled us to use their considerable computing power to build state of the art Large Language Models (LLMs) of biological sequences. Alex Pancheva has created an exciting LLM of RNA sequences that are starting to reveal candidate sequence elements with potential relevance in cancer.

We are also part of PREDICT-Meso, a £5m Accelerator project funded through a partnership between CRUK, Fondazione AIRC, and Fundación Científica de la Asociacion Española Contra el Cáncer (FC AECC). Mesothelioma is an incurable cancer that typically develops years after inhalation of asbestos dust and fibres. The factors that underpin the development of mesothelioma are currently poorly understood. We are applying computational approaches to study 'omics data arising from multiple tumour types including mesothelioma, colorectal and liver cancer samples.

Underpinning all these algorithms is a requirement to perform computationally intense calculations across thousands of genome sequences with matched transcriptome and proteomics data. We have worked with Naveed Khan to commission a High-Performance Computing system that underpins our data science efforts across the Institute.

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PRECLINICAL PANCREATIC CANCER

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[4]CRUK Scotland Centre

Pancreatic cancer is a major healthcare challenge, predicted to become the second most common cause of cancer death in the western world within the decade. The focus of our research is to better understand the disease and identify and test more effective therapies. We use genetically engineered mouse (GEM) models that recapitulate human tumours, in terms of both driving mutations and the immuno-suppressive tumour microenvironment, and adapt them to mirror heterogeneous subsets of the disease. These models provide a clinically relevant platform in which we trial novel tumour and microenvironment targeting therapies.

Pancreatic cancer kills over 430,000 people every year. It is one of the deadliest epithelial malignancies, and both incidence and mortality are rising. Indeed, it is predicted to be the second most common cause of cancer death within the next decade. In the UK alone, there are around 30 new cases every day. Less than 8% of those patients will survive their disease for five years, and only 1% are likely to survive beyond ten years. Despite improvements in surgical management and significant investment in clinical trials, cure rates have only minimally increased over the last 50 years, and current therapies are largely ineffective.

Research has helped improve our understanding of disease evolution, genetic alterations, and the tumour microenvironment. Activating mutations in *KRAS* are the most prevalent driver mutations, accompanied by loss of function of tumour suppressor genes. Some mutations found in subsets of patients may confer sensitivity to targeted therapies (Biankin *et al.*, 2012, *Nature*). For that reason, part of our work involves modelling gene mutations that are found in smaller subsets of human pancreatic cancer, with a view to understanding the biological consequences and therapeutic sensitivities associated with those mutations.

Another feature characteristic of pancreatic cancer is the dense fibrotic stroma that surrounds and supports the tumour cells and can account for up to 90% of the tumour volume. This microenvironment consists of fibroblasts and extracellular matrix (ECM) proteins as well as significant inflammation but a dearth of effector T cells. Each component plays an important role in pancreatic cancer progression, influencing tumour cell proliferation and survival, metabolism, migration, and immune surveillance (Candido *et al.*, 2018, *Cell Reports*; Steele *et al.*, 2016, *Cancer Cell*; Vennin *et al.*, 2018, *Gastroenterology*). Therefore, another aim of work in our lab is to investigate how stromal signalling impacts on the disease and how we might target it for therapeutic gain. Due to the complex nature of tumour-stromal interactions it is important to study this in vivo, in spontaneous tumours with a physiological microenvironment and immune response.

Radiotherapy in Pancreatic Cancer

The stroma can have profound effects on therapeutic response; however, therapeutic interventions may also have significant effects on the stroma. For example, radiotherapy can cause remodelling of the tumour microenvironment (TME) which may favour tumour growth and treatment resistance. Using our small animal radiotherapy research platform we have developed a protocol for tumour-targeted radiotherapy in GEM models of pancreatic cancer (Figure 1A). The use of radiotherapy in pancreatic cancer treatment has been limited thus far, however, this may be due to a lack of understanding of the effect of radiation on the pancreatic TME. Irradiation results in tumour cell death and release of tumour-associated antigens that can elicit a cytotoxic T cell response against the tumour. However, it can also drive the release of inflammatory cytokines and chemokines which can result in altered fibroblast secretory output, ECM remodelling, macrophage polarisation and a more immunosuppressive microenvironment. We have found that radiation alone can provide some survival benefit in pancreatic tumour-bearing mice (Figure 1B), however, this is accompanied by distinct cellular changes in the TME (Figure 1C).

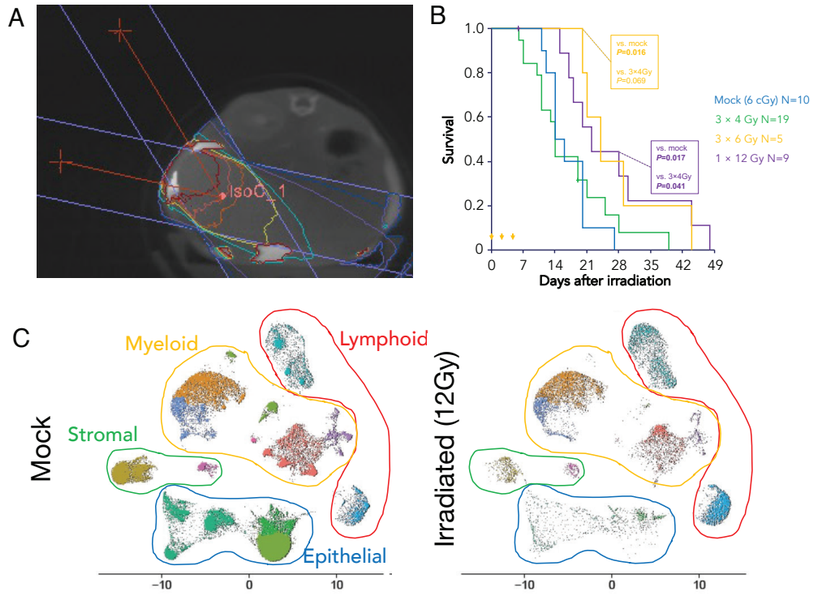

Figure 1. A Radiotherapy delivery plan using the small animal radiotherapy research platform. **B** Kaplan-Meier curve showing improved survival of pancreatic tumour-bearing mice in response to radiotherapy. **C** scRNAseq analyses show changes in cell populations post-radiotherapy in treated mice.

Thus, we are using our models to investigate responses in individual cells in the TME to determine the mechanisms controlling pro-tumourigenic immune and fibrotic responses with the aim of identifying rationale therapeutic combinations to promote anti-tumourigenic immune responses while inhibiting pro-tumourigenic immune and fibrotic responses. We are also using models lacking certain tumour suppressor genes to investigate whether certain mutations can render tumour cells more sensitive to therapy.

Targeting KRAS

By far the most common event driving pancreatic tumourigenesis is KRAS mutation. Previously believed to be "undruggable", the advent of mutant KRAS inhibitors could be transformative in this disease, particularly now that inhibitors have been developed for the most mutated form in pancreatic cancer (Hallin *et al.*, 2022, *Nature Medicine*). We have already observed that inhibition of multiple signalling pathways downstream of KRAS can have significant efficacy in tumour-bearing mice (Driscoll *et al.*, 2016, *Cancer Research*). However, resistance can develop quickly, concomitant with deregulation of signalling in both tumour and stromal cells. We are now investigating how these pathways can help tumour cells to adapt to therapeutic intervention and influence the response to treatment. For example, we have recently been investigating a protein called Gremlin1, an antagonist of bone morphogenic protein (BMP) signalling which is overexpressed in cancer-associated stromal cells and reported to be a driver of fibrosis in chronic pancreatitis (Figure 2A). Overexpression of the gene also correlates with decreased survival in pancreatic cancer patients. We found that treating pancreatic tumour-bearing mice with a therapeutic antibody against Grem1, in combination with an inhibitor of the KRAS target MEK, resulted in significantly slower tumour growth and tumour stasis in some mice, and a significant increase in survival (Figure 2B). We are currently investigating the mechanisms behind this synergistic efficacy using the Nanostring GeoMx spatial transcriptomic platform (Figure 2C). Our data have also led to a clinical trial to test this promising combination in patients with pancreatic cancer, which we hope will demonstrate the potential impact of this work for patient benefit.

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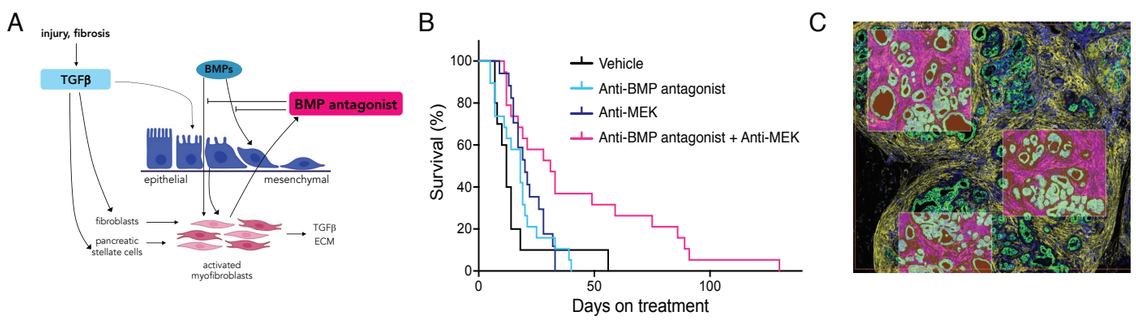

Figure 2. A Schematic of the role of BMPs in pancreatic cancer. **B** Kaplan-Meier curve showing improved survival of pancreatic tumour-bearing mice in response to combination therapy. **C** Region selection and cell type masking for GeoMx analysis. Mouse pancreatic tissue stained for CK19 (green, tumour cells), PDPN (yellow, fibroblasts) and DAPI (blue, CK19-PDPN- cells).

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MYC-INDUCED VULNERABILITIES/THORACIC CANCER RESEARCH

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University of Glasgow

Oncogenic signalling profoundly alters how cells respond to their environment, typically putting tumour cells under tremendous pressure to reconcile conflicting cues. For example, tumour cells must re-organise their metabolic pathways to balance competing needs for biosynthetic precursors with energetic homeostasis, commonly while surviving in a milieu of limiting oxygen and nutrients.

We use genetically engineered mouse models, primarily of lung cancer and mesothelioma, to understand how developing tumours cope with conflicting cues in their natural environment. Our overarching hypothesis is that oncogene-induced biological perturbations can be exploited for cancer therapy, even in the absence of direct suppression of driver oncogenes. We use deregulated MYC as our paradigm oncogene coupled with a mixture of candidate and RNAi-based approaches to identify induced vulnerabilities *in vivo* and *in vitro*, and are actively exploring several strategies for selective elimination of cells that overexpress MYC.

MYC in cancer

Overexpression of the transcription factor MYC occurs in a vast number of human cancers. The overexpression may arise from focal or broad chromosomal amplification, gene translocation, enhanced mRNA and protein stability, or indeed increased signalling through upstream regulatory factors such as Ras, Notch, or ß-catenin. In many *in vivo* settings, MYC overexpression is sufficient to initiate or exacerbate tumorigenesis and MYC is moreover typically required to sustain the cancerous phenotype. A successful therapeutic strategy that exploits MYC expression would likely have a tremendous impact on human health. To facilitate investigation of physiologically relevant levels of deregulated MYC expression in any tissue, we have generated and characterised Rosa26$^{DM\text{-}lsl\text{-}MYC}$ mice and deposited them with Jaxmice for unrestricted distribution to the broader scientific community.

MYC and KRAS drive immune evasion

How tumours evade detection by the immune system defines the underlying principle behind the therapeutic success of immunotherapy across a spectrum of cancer types. MYC is known to induce expression of PD-L1, which inactivates cytotoxic T cells upon binding to PD1, but new data from multiple labs, including ours, indicates that PD-L1 expression is not the sole immune evasion strategy deployed by MYC. In 2020, we showed that MYC and KRAS combine to suppress multiple cascades involved in cell communication with the immune system, with downregulation of the Type I Interferon pathway and of MHC I-dependent antigen processing & presentation forefront in these transcriptional responses. The transcriptional changes occur immediately upon acute activation of KRAS or modest overexpression of MYC in cell culture, and importantly, persist throughout tumour progression *in vivo*. Mechanistically, we identified repressive transcriptional complexes comprising MYC and MIZ1 binding directly to multiple key regulators of Type I Interferons in pancreatic ductal adenocarcinoma (PDAC). Genetic suppression of MYC or MIZ1 restore Interferon signalling, enabling PDAC tumours to elicit CXCL13 production in nearby macrophages and thereby recruit anti-tumour effector immune cells to limit tumour progression, resulting in extended survival. In the year since publication, this provocative finding of active suppression of the Type I Interferon cascade by the MYC/KRAS pathway has been reproduced in multiple cancer types, including lymphoma, breast, lung, ovarian and oesophageal cancers, indicating widespread use of this immune evasion strategy across many (all?) cancers. Pharmacological inhibition of MYC transcriptional repressive complexes may thus have benefit as a generic cancer therapy.

MYC-induced metabolic vulnerability

As part of a coordinated programme of cell growth required for cell division, MYC engages a number of biosynthetic programmes, such as ribosome assembly and protein translation, placing tremendous energetic demand upon the cell. In order to maintain energetic homeostasis, MYC upregulates glucose transporters and glycolytic enzymes, promoting the Warburg effect of limited glucose breakdown, and in parallel induces expression of glutamine transporters and exploits this pathway to maintain the citric acid cycle. The energetic strain that MYC deregulation thus places upon the cell is evident in progressive activation of the AMP-activated protein kinase AMPK, which plays a key role in maintaining energetic homeostasis. AMPK in turn inhibits TORC1 to attenuate the rate of macromolecular synthesis, effectively allowing cells to balance the rate of ATP consumption with ATP production. Importantly, the AMPK-related kinase ARK/NUAK1 is also required for maintenance of ATP homeostasis in cells wherein MYC is overexpressed. NUAK1 plays a specific role in MYC-dependent activation of AMPK and also maintains mitochondrial respiratory capacity. Suppression of NUAK1 thus impairs the ability of MYC-overexpressing cells to respond to declining ATP levels while simultaneously depriving cells of ATP-generating capacity, suggesting that suppression of NUAK1 may be an effective means to selectively kill cancer cells with high levels of MYC expression.

Oncogene cooperation during lung cancer progression

Lung cancer remains one of the deadliest forms of cancer worldwide, accounting for 18% of all cancer-related deaths, and the incidence of lung cancer is on the rise, especially in the increasingly industrialised and densely populated cities of emerging economies. Poor prognosis arises in large part from the combination of late disease detection and limited matching of patients with emerging targeted therapies. We have found that modestly elevating MYC levels in a KRAS-driven model of lung cancer is sufficient to drive progression to metastatic disease. This progression arises in part through increased transcription of promiscuous ERBB family ligands. We have identified an unexpected requirement for signal transduction through the ERBB receptor tyrosine kinase network for both establishment and maintenance of KRAS-mutant lung cancer. Our data suggest that KRAS-driven tumours actively seek ways to amplify signalling through the RAS pathway to sustain the tumour phenotype.

Inflammation and genetics of mesothelioma

Mesothelioma is a lethal cancer of the lining of the chest cavity that arises in people chronically exposed to asbestos. There are no effective therapies and patient survival is typically less than 18 months from diagnosis. Our lab has teamed up with respiratory physician Kevin Blyth to build an international network of clinicians and researchers with the common goal of improving patient outcomes for this dreadful disease. We have developed a new mouse model of mesothelioma that will enable us to investigate the interplay between asbestos-driven chronic inflammation and the major recurring mutations that are commonly found in human mesothelioma. Significantly, intrapleural injection of asbestos dramatically accelerates onset and severity of mesothelioma in our mice, even after homozygous deletion of 3 major tumour suppressor genes, indicating that chronic inflammation continues to contribute to mesothelioma beyond the acquisition of rate limiting mutations. This startling observation suggests that patients may benefit from interventions that aim to reduce inflammation, in addition to those directly targeting the tumour population.

Major developments in 2024

This year saw the development of multiple new genetically engineered mouse lines that will be incorporated into our work in the coming years. These include FLP-recombinase deletable alleles of the tumour suppressor genes Bap1 and Cdkn2a (with Doug Strathdee), along with a derivative of the latter that will enable controlled deletion of both Cdkn2a and the immediately adjacent Cdkn2b locus. Greatest excitement however is reserved for the generation of the Tandem Arrayed Regulator mouse in collaboration with the Mary Lyon Centre and the National Mouse Genetics Network. This allele will potentially transform how cancer is modelled in mice, from the current "all at once" approaches, to spatially and temporally controlled introduction of sequential genetic events allowed true modelling of tumour progression. We also tested a novel CRE- and Dox-dependent Cas9 allele that will enable controlled use of *in vivo* CRISPR techniques and potentially *in vivo* screening for new mutations that cooperate with known first hit mutations during progression of thoracic cancers. A major new initiative to commence next year will see the incorporation of all of these models into a Complex Models bioengineering hub, led by Susan Rosser of Edinburgh University.

Personnel wise, the departure of George Skalka was offset by the arrival of new postdocs, Mahnoor Mahmood and Shahnawaz Ali, funded by my CRUK programmes, REMIT and IAMMED-Meso, respectively. Funded by REMIT, Nadia Iqbal was also recruited to the lab of Seth Coffelt. Visiting M.Sc. student Sophie Heese made a valuable contribution to our ongoing work in lung cancer, investigating control of Hippo signalling via ERBB inhibition. Postdoc Sarah Laing presented at the 25th International Beatson Cancer Conference, narrowly missing out on the prize for best short talk, but PhD student Danielle McKinven won best Cancer Discovery talk at the School of Cancer Sciences away day. Several lab members also presented posters at the ISEH/ICEPH/ISEG international conference in Galway, Ireland while much of the lab also presented at the PREDICT-Meso, ECR's day.
The lab published 2 reviews and contributed to 2 publications, 1 in EMBO Journal and the other in Blood Cancer Discovery. I authored a policy article for Openaccessgovernment.com on the value of mouse models for cancer research, and I was appointed to the Action on Asbestos Board of Advisors.

Publications listed on page 125

INTEGRIN CELL BIOLOGY

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The microenvironment dictates how and where cancers originate and their spread throughout the body. The extracellular matrix (ECM) is an important component of the microenvironment and ECM components, such as fibronectin and collagen, are key to tumour initiation, growth and metastasis. Our laboratory is focussed on using mouse models to determine the molecular details of how the ECM influences initiation and metastasis of both liver and breast cancer and how the integrin receptors for the ECM control these processes *in vivo*. We report that integrin dependent deposition of fibronectin must occur early in tumorigenesis for cancers to propagate in the liver. Furthermore, we have found that, early in their development, breast tumours alter the metabolism of immune cells such as neutrophils to alter integrin behaviour. This leads to neutrophil recruitment to the lungs and subsequent metastasis to this organ. These studies highlight how drugs which target novel pathways controlling integrin function may be used to control both tumour initiation and metastasis.

Neutrophil pyrimidine metabolism leads to priming of the lung metastatic niche in breast cancer.

To identify circulating metabolites that may be the harbingers of metastasis, we profiled serum metabolome in the MMTV-PyMT mouse model of mammary cancer. This revealed that the circulating levels of the pyrimidine, uracil correlate closely with metastasis of mammary tumours to the lung. Further investigation indicated that the high level of circulating uracil in mice with metastatic mammary cancer emanates from neutrophils expressing the enzyme uridine phosphorylase-1 (UPP1). Indeed, the presence of a primary tumour in the mammary gland drives expression of UPP1 in neutrophils which leads to increased cleavage of the nucleoside uridine to yield ribose-1-phosphate and the pyrimidine base, uracil which is released from these neutrophils into the circulation. Moreover, we found that GM-CSF could drive increased UPP1 expression in neutrophils indicating the likelihood that this tumour-derived cytokine may be responsible for mediating increased UPP1 expression in tumour-bearing mice. We, therefore, studied the consequences of genetically deleting UPP1 on metastasis and found that MMTV-PyMT:UPP1$^{-/-}$ mice have significantly reduced lung metastases by comparison with their MMTV-PyMT:UPP1$^{+/+}$ controls. Mobilisation and recruitment of neutrophils to metastatic target organs is becoming established to prime these tissues for metastasis. We, therefore, visualised the expression of adhesion molecules and the movement of neutrophils in the lungs of mammary tumour-bearing mice and studied the influence of genetic deletion or pharmacological inhibition of UPP1 (using the specific UPP inhibitor, benzylacyclouridine (BAU)) on this. These studies indicated that the activation of UPP1 in neutrophils leads to upregulated surface expression of an integrin, CD11b which causes the neutrophils to adhere to, and become trapped in, the lungs of tumour bearing mice. These neutrophils then, in turn generate an immunosuppressed microenvironment in the lung which, we propose, favours metastasis to this organ. Conversely, inhibition or knockout of UPP1 leads to recruitment of neutrophils which are associated with increased T-cell numbers and an immunocompetent lung microenvironment consistent with decreased metastasis to this organ. These data indicate that pharmacological targeting of UPP may be an effective means to reducing lung metastasis in breast cancer (Figure 1A).

Restriction of mRNA translation is key to efficient initiation of liver cancer

The mRNA translation/protein synthesis machinery is known to drive cancer cell proliferation and tumour growth. This is thought to satisfy cancers' particular need to increase biomass. Mutated oncogenes also influence the selectivity of mRNA translation to favour synthesis of cohorts of proteins (such as cell cycle regulators) to further support cancer cell proliferation. These simple facts have fuelled the search for agents which oppose or moderate mRNA translation. Indeed, drugs which target the mRNA translation machinery – and the signalling linking mutated oncogenes to this – are now being evaluated in clinical trials as cancer therapeutics.

The genesis of tumours is not simply a process in which mutated oncogenes drive cell proliferation. Acquisition of mutated oncogenes may not, initially, drive tumorigenesis. Indeed, under many circumstances, oncogenes promote senescence (not proliferation) and the mutated cell's ability to overcome oncogene-induced senescence dictates whether tumorigenesis occurs. Importantly, senescent cells are highly secretory, including many extracellular matrix (ECM) components which are deposited locally. We have developed and utilised transgenic mouse models to show how the genesis of liver cancer requires an ECM-driven override of oncogene-induced senescence. The hyperproduction of secretory proteins associated with oncogene-induced senescence would be expected to require reprogramming of the mRNA translation machinery in cancer cells. Indeed, we identify that a negative regulator of mRNA translation initiation (eIF4A2) *restrains* synthesis of ECM proteins, thus maintaining membrane trafficking and facilitating ECM protein deposition. Importantly, in the absence of eIF4A2 senescent hepatocytes do not progress to proliferating liver cancer because of a lack of ECM deposition rather than a lack of production. As we have seen, lack of appropriate restraint of secretory protein synthesis following oncogene activation can delay tumorigenesis through matrix suppression (Figure 1B). Consistently, we demonstrate that administration of rapamycin shortly following oncogene activation strongly promotes senescence override and allows progression to proliferating liver cancer. Thus, although inhibition of protein synthesis may be an effective way to reduce tumour biomass and the growth of established tumours, it is important to consider that high levels of mRNA translation can extend a period of senescence occurring following oncogene activation. Thus, use of drugs which reduce mRNA translation, if administered shortly following oncogene activation, may awaken senescent cells, and promote tumour progression.

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1A

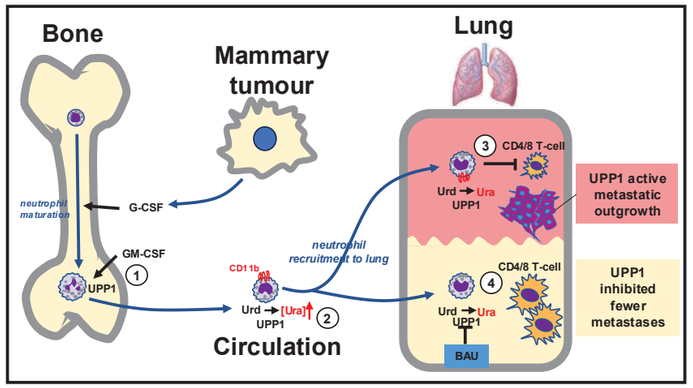

Figure 1A Neutrophil pyrimidine metabolism leads to priming of the lung metastatic niche in breast cancer. **[1]** Mammary tumours release factors such as G-CSF to drive neutrophil maturation in the bone marrow, and GM-CSF which leads to activation of uridine phosphorylase-1 (UPP1) in neutrophils. **[2]** Circulating neutrophils expressing UPP1 generate high levels of serum uracil (Ura). UPP1-expressing neutrophils express high levels of the surface adhesion protein CD11b (integrin αM). **[3]** CD11b leads to capture of UPP1-expressing neutrophils in the lung. These neutrophils suppress T-cells and metastasis in the lung. **[4]** When UPP1 is inhibited using benzylacyclouridine (BAU), T-cells number in the lung increase to suppress metastasis.

1B

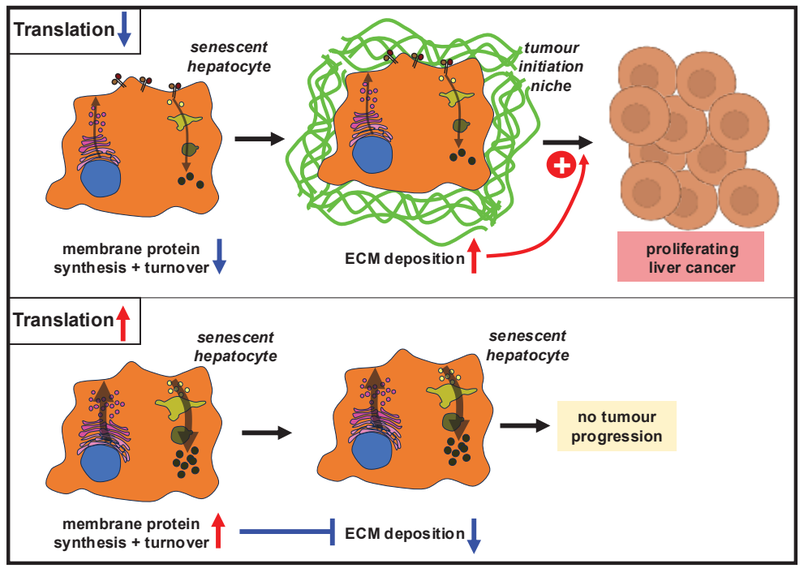

Figure 1B Restriction of mRNA translation is key to efficient initiation of liver cancer. When the translation of secretory mRNAs is restrained (upper panel), protein synthesis and membrane protein turnover is moderate. This maintains membrane trafficking and facilitates ECM protein deposition to promote progression of liver cancer from a senescent phase to form highly proliferating hepatocellular carcinoma. Conversely, when mRNA translation is rapid and unrestrained (lower panel), ECM deposition is inhibited and senescent hepatocytes harbouring oncogenic mutations remain senescent and do not progress to proliferating liver cancer.

CELL PLASTICITY & EPIGENETICS

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Phenotypic plasticity, the ability of a genotype to produce a variety of phenotypes, has been documented as a core biological process underlying numerous cellular events ranging from unicellular adaptation to multi-cellular organism development. In the context of cancer, phenotypic plasticity leads to the establishment of co-existing phenotypically diverse metastable states that could grant cancer cell populations with the capability to adapt to fast-paced environmental fluctuations in the absence of genetic divergence and thus fuel cancer development, metastatic spread and resistance to therapeutic paradigms. Given the crucial role that cell plasticity and non-genetically encoded phenotypes play in biology, our research aims to unravel the molecular mechanisms underlying such a phenomenon and to address its role as a key determinant during cancer onset and progression.

Over the past few years and by means of applying and developing multimodal single-cell technologies, our lab has demonstrated that clonal populations of a variety of cellular systems from diverse tissues of origin display multiple non-genetically encoded metastable states that can be ascribed to dramatically different cellular phenotypes. Motivated by our observations, and by applying our in-house developed lineage tracing technology (Figure 1A. BdLT-Seq (Shlyakthina, Blochl *et al.*, 2023, N*at Commun*; Shlyakthina, Blochl *et al.*, 2024, *STAR Protoc*)) we have recently shown that in *in vitro* transformation models driven by mutant HRAS, KRAS and NRAS, transcriptome states are inherited upon cell division but they can also be rewired into distinct states resulting in a progeny displaying diverging transcriptome profiles, thereby fuelling the generation of non-genetically heterogeneous populations. Moreover, we reported that the plastic capacity of transcriptome states to generate progeny with a transcriptome profile different from that of the parental cell is not stochastic, but it is rather encoded in non-genetic networks and restricted in an ancestry lineage-linked manner.

More recently, we have shown that restricted transcriptome plasticity and inheritance is not unique to cancer models and could be observed in non-tumourigenic cell systems suggesting its widespread and, perhaps, universal nature. Strikingly, and in line with BdLT-Seq data (Figure 1B), subcloning a parental – clonal – population of any of the cellular systems analysed gives rise to populations of cells that are enriched in subsets of transcriptome states (Figure 1C) that only partially recapitulate the heterogeneity observed in the parental clone, supporting the existence of a non-genetic molecular memory being transferred along cell divisions[3]. Strikingly, 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 may play a key role in shaping cancer onset, progression, and evolution.

Interestingly, by delving into the inner molecular workings underlying cell plasticity, we have uncovered that a large subset of long non-coding RNAs (lncRNAs) and a small fraction of intrinsically disordered proteins (Intrinsically Disordered Regions-containing proteins, IDRs) determine clonal molecular divergence, thus potentially acting as key players in cell plasticity and non-genetically supported cell and, thereby, populational adaptation (Shlyakthina, Blochl, Moran *et al.*, 2024, *bioRxiv*). Strikingly, we observed that divergent lncRNAs co-localize with cluster-specific IDR-proteins (Intrinsically Disordered Regions-containing proteins) within perinuclear structures and nucleate active components of major signalling pathways

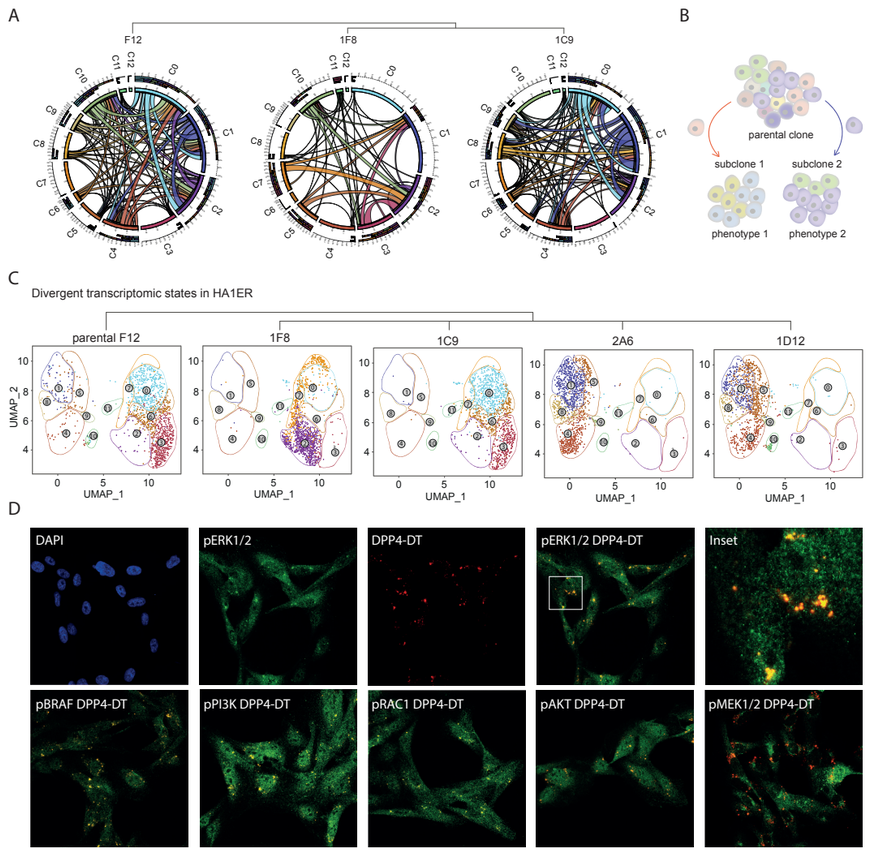

Figure 1. A. Lineage tracing (BdLT-Seq) was performed in the HA1ER clonal system. Data obtained from the founder clonal population (F12) and 2 subclones (1F8 and 1C9) is depicted as chord diagrams representing transcriptome state dynamics for cells belonging to a particular state/cluster at the beginning of tracing and their divergence after 4 days. Detected clusters are depicted (C0 to C12) and integrate the collapsed behaviour of all cells analysed that belong to each gene expression state. Origin clusters are shown in different colours (Day 0) and chords represent the endpoint cluster association (Day 4). **B.** Conceptual toy diagram depicting that BdLT-Seq data predicts that some clones will become enriched in a defined/ reduced number of states as verified experimentally in C. **C.** The founder clone from the HA1ER (F12) and divergent subclones (1F8, 1C9, 2A6 and 1D12) were subjected to scRNA-Seq. UMAP plots represent transcriptome states and their divergence among analysed subclones. Individual states are depicted in colour and numbered. **D.** HA1ER clone 1F8 was subjected to immunocytochemistry for activated signalling pathways coupled to RNA-FISH targeting DPP4-DT lncRNA. The upper panel depict individual channels (DAPI, pERK1/2 and DPP4-DT) and a merged image (pERK1/2/DPP4-DT) whilst the lower panels depict merged images for DPP4-DT/pBRAF, DPP4-DT/pPI3K, DPP4-DT/ pRAC1, DPP4-DT/pAKT and DPP4-DT/pMEK1/2. Inset is displayed for the upper panel.

Credits for figure
Bianca Blochl and Maxi Portal

(Figure 1D). These agregates co-segregate with phenotypic divergence, thereby pinpointing to a potential molecular device underlying phenotypic heterogeneity by modulating cell plastcity through signal integration (Signal Integration Portals - SIP). Indeed, altering the levels of diverging lncRNAs results in the redistribution of SIP-associated signalling cascade components within the cytoplasm, which is accompanied by a wide-scale transcriptome remodelling and shifts in phenotypic output. Therefore, we hypothesize that the repertoire of divergent lncRNAs present in each cell and their interaction with differentially expressed/localized IDR-proteins does facilitate the formation of membraneless condensates which depending on their molecular composition, will nucleate distinct signaling cascades in time and space, thus orchestrating phenotypic outcome in response to biological and/or therapeutic cues.

It is our hope that, due to the universality of our findings, our discoveries may prompt a new biotechnological revolution where biological control would move beyond genetic manipulation to finally harness the reprograming potential of the non-genetic compartment paving the way for the development of a new generation of anti-cancer agents.

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EPITHELIAL IMMUNE CROSSTALK IN DEVELOPMENT AND DISEASE

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In our lab we study the mammalian skin to elucidate the complex interactions between the epithelial cells and macrophages, both in the context of the developing skin and in inflammatory skin disorders. Embryonic macrophages derived from the yolk sac and foetal liver, enter the skin at E12.5 and contribute to both homeostasis and inflammation, rather than serving as bystander cells. We have been particularly focused on the metabolic crosstalk between epithelial cells and macrophages. The insights gained from these studies will pave the way for research focused on interrogating epithelial-immune metabolic crosstalk in cancer.

A major question in the field of inflammation is how you initiate an immune response and terminate it after a defined period. Compromising either the start or end of an inflammatory response can lead to debilitating states such as infection and sepsis or, at the other extreme, chronic inflammatory diseases. Both subtle and dramatic alterations in the sheet of epithelial cells that comprise the barrier of various organs in the body are known to elicit an inflammatory response. Though this phenomenon is well-documented, a mechanistic understanding of how epithelial homeostasis governs an inflammatory reaction is still far from reach. Over the past several years, we have attempted to address this using our integrin beta1 knockout mouse model. Of relevance is a strong inflammatory and wound response that is elicited in the integrin KO mice. A differentiating feature of this mouse model is the fact that immune/wound-healing response is elicited during embryonic development, at a time when there are no extrinsic wounds and no skin microbiome that may facilitate this process. The integrin KO mouse thus offers an excellent model system to understand the epithelial-immune crosstalk that contributes to excessive ECM remodeling.

Epithelial-immune metabolic crosstalk

In our recent study (Ayyangar *et al.*, 2024, *EMBO J*), using the epidermal integrin b1 conditional KO mice, we report that the epidermis and macrophages augment unique yet complimentary metabolic programs where the epidermis augments glucose uptake and glycolysis and the macrophages augment TCA cycle. This metabolic program is initiated by an early increase in reactive oxygen species (ROS) that aids in enhanced stabilization of glycolysis regulator, HIF1a (Hypoxia Inducible factor), in the epidermal compartment. Enhanced glycolysis in the epidermis leads to increased generation of glycolysis-end-product lactate that is subsequently exported and utilized by macrophages in the dermal compartment to augment a pro-remodeling fate that is characterized by increased MMP9 generation. Notably, inhibition of glycolysis and its regulators in the epidermis, TCA cycle in macrophages, and lactate-mediated crosstalk between the two compartments using metabolic drugs led to a remarkable reduction in the pro-remodeling fate acquisition in macrophages and in turn, skin inflammation. Mechanistically, we show that lactate augments the TCA cycle and MMP9 generation, in part, through NF-kB activation. **Impact:** this work provides us a pathway to understand the metabolic underpinnings of inflammatory skin diseases such as psoriasis, as well as the future development of metabolic drugs to treat skin inflammatory disease, such as atopic dermatitis and psoriasis.

Understanding the Metabolic Underpinnings of Inflammatory Skin Disorders

Psoriasis is a chronic hyper-proliferative inflammatory skin disorder that affects up to 2 percent of the world's population and the incidence seems to be increasing over time. Notably, some individuals suffering from psoriasis are at higher risk of developing other diseases, including arthritis and cardiovascular

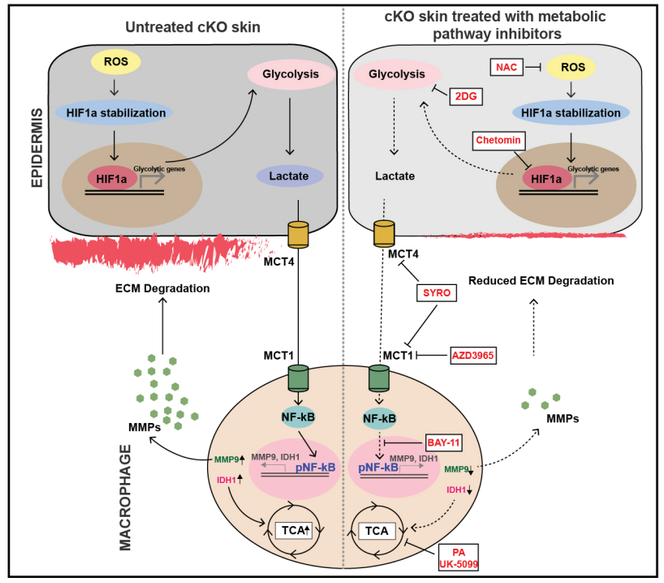
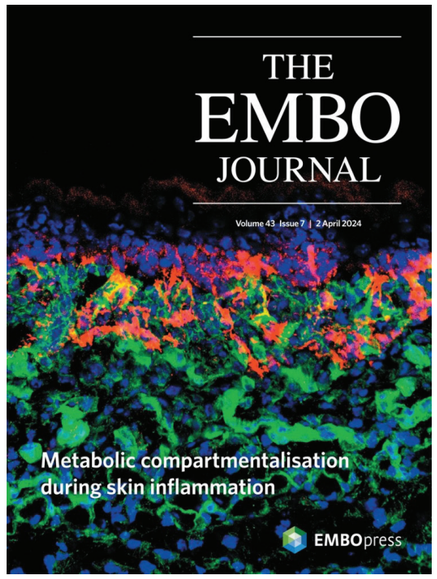


Figure 1. Under sterile inflammatory conditions in skin, early augmentation of ROS-HIF1a axis leads to enhanced glycolysis and lactate generation in the epidermal compartment. Consistently, inhibition of lactate transporters, epidermal-macrophage intrinsic metabolism and upstream glycolysis regulators in the epidermal compartment using small molecule inhibitors lead to inhibition of pro-remodelling fate and hence inflammation in the cKO skin.



dysfunction, a phenomenon termed as psoriatic march. The current line of treatment for psoriasis primarily involves the use of strategies that control inflammation or inhibit the adaptive immune response. Interestingly, there is increasing evidence of the association between psoriasis and metabolic syndrome which encompasses abdominal obesity, glucose intolerance, diabetes, dyslipidaemia, and high blood pressure, suggesting that psoriatic skin disease is driven by metabolic remodeling of the skin. However, the upstream regulators and downstream effects of the metabolic remodeling in the skin in inflammatory skin diseases like psoriasis remains poorly understood. To understand this better, we have generated an IMQ-induced mouse model of psoriasis-like dermatitis. Interestingly, preliminary analysis of IMQ-induced mouse models suggests that the early stages of psoriasis induction is associated with augmentation of glycolysis and increased expression of lactate transporters MCT1/4 (Monocarboxylic acid transporters) in the epidermal compartment of the skin. Notably, inhibition of lactate-mediated crosstalk using MCT1/4 inhibitor, Syrosingopine (Syro) and inhibition of ROS using ROS scavengers led to a significant reduction in psoriatic phenotypes. **Impact:** these results potentially open a new paradigm and lay the foundation for treating psoriasis in human patients with inhibitors against ROS-HIF1a-glycolysis-lactate axis that target innate immunity in the skin, which can be used in conjunction with the existing treatment methods or as a replacement for better therapeutic outcomes. We are currently testing the ability of metabolic drugs to attenuate the psoriatic phenotypes and have filed a patent encompassing this work.

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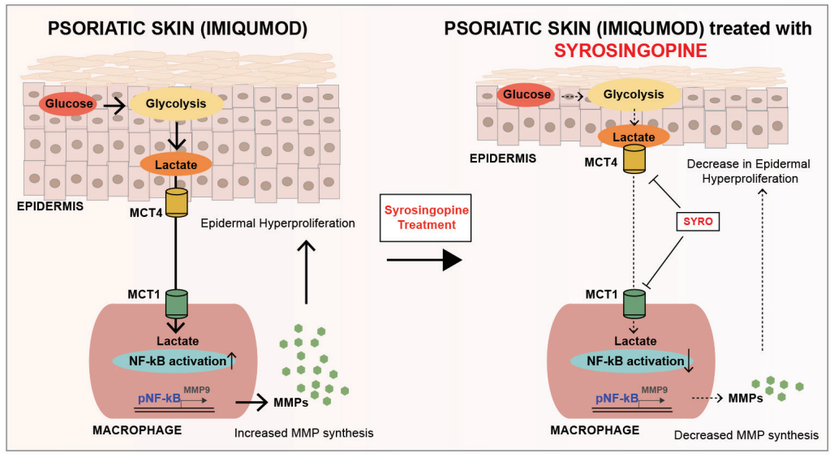

Figure 2. Schematic showing that epidermally derived lactate drives psoriasis through enhanced NF-kB activation in the macrophages that, in turn, leads to enhanced generation of MMP9. Inhibition of lactate mediated crosstalk between epidermis and macrophages using Syrosingopine attenuates psoriatic burden in imiquimod treated mice.

IMMUNE PRIMING AND TUMOUR MICROENVIRONMENT

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In recent years immune checkpoint blockade has led to dramatic patient benefit in a variety of cancers previously refractory to treatment. These therapies function by re-invigorating existing anti-tumour immune responses which have been rendered ineffective but only show efficacy in a subset of patients. By comparing robust immune responses against viral challenges with those raised against tumours we are unpicking how the tissue microenvironment is dictated and how this influences the lymph node to induce sub-optimal T-cell responses. Using these insights, we hope to define approaches to improve anti-tumour immune responses to expand the number of patients who can benefit from these therapies.

Our research primarily focuses on the role of dendritic cells (DC) and the initiation of anti-tumour immunity (Figure 1). DC progenitors develop in the bone marrow and traffic to the tumour where they sample tumour antigens before migrating to the tumour-draining lymph node and activating anti-tumour T-cells. We have previously shown that T-cells are sub-optimally activated in the tumour-draining lymph node and that improving DC functionality, and consequently T-cell activation, improves responses to immunotherapy. To understand how the tumour leads to sub-optimal immune activation, we are seeking to elucidate the mechanisms involved at each stage of the DC lifecycle.

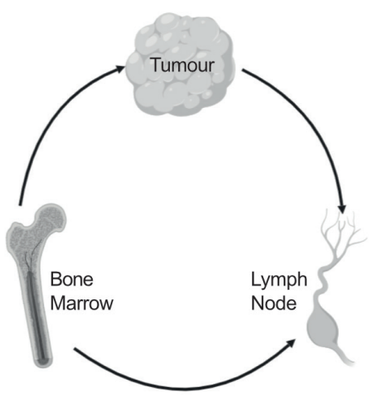

Figure 1. The DC lifecycle
DC precursors develop in the bone marrow and migrate to the tumour and the lymph node. Once within the tumour, they sample proteins from the microenvironment and then mature and migrate to the lymph node. There are the DC which migrated straight to the lymph node and those which migrated from the tumour coordinate to drive anti-tumour T cell priming.

Immune history and impacts on tumours

We have shown that there are long term changes in tissues after infections and have recently shown that these alter tumour development within the tissue. Using a model of influenza we have shown that several models of cancer within the lung are more aggressive if they occur after the resolution of a previous lung infection. Indeed, this was also the case after a more simple inflammatory response. This does not appear to be due to a history of inflammation as preventing this with paracetamol did not reverse this impact and so we are currently investigating which changes in the lung are responsible for this pro-tumorigenic environment.

DC recruitment to the tumour

Previous work has shown that patients with higher numbers of DCs infiltrating their tumours have better outcomes and responses to immunotherapy; however, it is unknown what controls their recruitment and number within the tumour microenvironment. We have identified trafficking receptors on precursor DCs and have generated an assay to screen receptors individually and in combination to identify those required for DC entry to both tumours and sites of infection. We are now unpicking which signals draw DC into different tissues and will next determine which cells are producing these signals both during viral infection, where immune responses are robust, and in the tumour, where the response is sub-optimal. We will finally seek to understand what induces expression of these signals and attempt to increase DC recruitment to the tumour in order to improve both initial priming in the lymph node and to augment repriming at the tumour site.

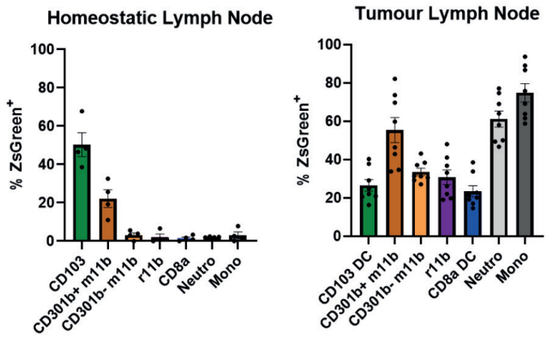

Figure 2. Tumour antigen is handled uniquely
ZsGreen expressed within the lung is carried to the lymph node by migratory DC, but the protein remains restricted to the migratory DC. When the same protein is expressed in a tumour, the protein is carried to the lymph node by migratory DC in a similar fashion but is transferred to other lymph node resident populations.

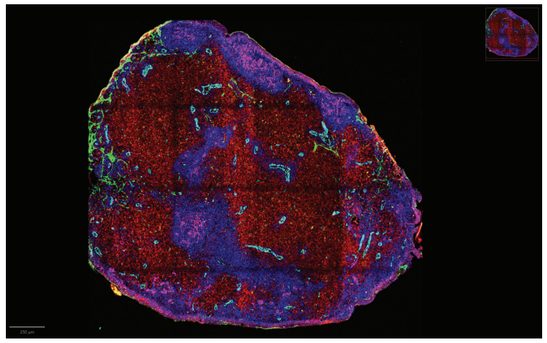

Figure 3. Lymph node organisation
A whole cleared lymph node stained for T cell, B cell and DC markers shows the organisation of a lung tumour-draining lymph node.

Antigen traffic to the lymph node

Beyond the number of DCs at the tumour site, how DCs carry tumour material to the lymph node, and how they distribute it, is also key to understanding how anti-tumour immune responses are generated. We have shown that the same protein, when expressed within a tumour cell, is handled differently than when expressed in normal tissue. Indeed, during normal development DCs restrict these proteins and do not transfer them to other DC subsets resident in the lymph node (Figure 2). During tumour development or viral infection, however, this protein is handed off to lymph node resident cells and we have shown that their activation mimics that of DC activated in the tissue (Figure 2). We have shown that this is due to co-transfer of this antigen alongside contextual cues communicating the nature of the challenge. This means that tumour derived dysfunction spreads to the lymph node leading to poor activation. We are now investigating how this transfer occurs and have seen that transfer relies on signals through specific costimulatory molecules in both cancer and in influenza infection. This implies that transfer relies on structures called tunnelling nanotubes which would allow transfer of co-packaged antigen and contextual information.

DC functionality within the lymph node

Finally, once the antigen has been trafficked to the lymph node, in order to drive effective anti-tumour immune responses, the lymph node must be highly organised, facilitating numerous specific cell–cell interactions. During tumour development the draining lymph node has been shown to be disorganised, and it has been proposed that several of these critical cell–cell interactions are disrupted. We have, however, demonstrated that the tumour-draining lymph node is capable of supporting robust immune responses, suggesting the problem is with the tumour-derived DC rather than with the node as a whole. In order to study how these cells interact differentially in the tumour setting, we have developed a protocol allowing us to stain the entire lymph node and to identify the location of critical cellular subsets within the 3D environment of the lymph node (Figure 3). We have also developed complementary approaches to allow identification of even more cell types within the lymph node microenvironment and are now building systems to allow robust analysis of tissue organisation. We have seen that in the cancer setting DC are only partially activated and this leads to them remaining excluded from regions where they normally fully activate T cells. Addition of inflammatory signals can drive relocalisation of these DC and improve the anti-tumour immune response. We now are investigating how this relates to human cancer by interrogating human lymph nodes.

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TUMOUR CELL DEATH AND AUTOPHAGY

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[4]Graduated in 2024

Our group is focused on understanding the factors regulating cell viability in cancer. Since inhibition of cell death mechanisms is a common event in tumour development, this poses problems for many forms of chemotherapy that utilise cell death pathways, leading to drug resistance.

We are investigating known cell viability and integrity regulators in several processes including apoptosis and autophagy, as well as searching for novel proteins and pathways that control cell homeostasis, tumour growth and chemosensitivity. We envisage knowledge gained from our studies will be translated and lead to improvement of existing clinical regimens or new targets for therapeutic intervention.

Autophagy in cancer

Autophagy (literally, 'self-eating') is a major catabolic process in the cell whereby cellular cargoes are delivered to and degraded in lysosomes allowing the cell to remove misfolded/damaged proteins and organelles that would otherwise be toxic for the cell. As such, autophagy is highly homeostatic and a significant factor in the preservation of cellular integrity.

The most characterized form of autophagy, and the focus of our work, is macroautophagy, which is often simply referred to as autophagy. The process is characterised by the formation of unique double-membraned vesicles, termed autophagosomes. The formation of autophagosomes is orchestrated via a series of evolutionarily-conserved **A**u**T**opha**G**y-related (ATG) proteins and as they grow they encapsulate cellular cargoes that are destined for degradation in the lysosome. Upon cargo digestion, the constituent parts of macromolecules are delivered back into the cytoplasm and can then either be recycled in biosynthetic pathways or further catabolized for the production of energy (Figure 1).

Due to its role in the preservation of cellular health and viability, autophagy protects against various forms of disease. In the context of cancer, the role of autophagy becomes complex. The consensus is that autophagy is tumour suppressive in normal cells and in the early stages of cancer. However, in established tumours, autophagy in tumour cells and associated stroma sustains the viability of tumour cells, hence in this context it promotes tumour maintenance. As a result, if we aim to destabilize tumour growth and viability by interfering with autophagy, it is imperative that we understand how and at what stages in different tumour types autophagy ceases to be tumour suppressive and switches role to support tumour growth and preservation, enabling appropriate intervention.

Identifying and understanding factors that regulate autophagy

Previous work by our lab, showed that p53 tumour suppressor (Crighton *et al.*, 2006, *Cell*), its related family member p73 (Crighton *et al.*, 2007, *CDD*), the hypoxia inducible transcription factor HIF-1α Wilkinson *et al.*, 2009, *Genes Dev*) and the chromatin modifier BRD4 (Sakamaki *et al.*, 2017, *Mol Cell*) are all regulators of autophagy, indicating that several key cancer-related pathways impact on autophagy. More recently, as well as identifying new autophagy regulators, our attention has also turned to understanding how known autophagy regulators affected the process. An example of this is our work on the leucine-rich repeat kinase 2 (LRRK2), which is frequently mutated in a high percentage of cases of Parkinson's disease and has also been implicated in cancer. In collaboration with the team headed by Prof. Jan Parys at KU Leuven, Belgium, we were interested in the mechanism of autophagy regulation by LRRK2. Our focus was on a cluster of phosphorylation sites where phosphorylation increases upon nutrient deprivation, as can occur in a developing tumour. We found that mutation of these phosphorylation sites impaired autophagy and lysosomal function, implicating the phosphorylation of these sites as a key event in starvation-induced autophagy. Interestingly, inhibition of LRRK2's own kinase activity also resulted in dephosphorylation of the phosphorylation cluster, but did not affect autophagy. As an explanation of these apparently contradictory results, we observed that mutation of the phosphorylation cluster resulted in increased LRRK2 kinase activity that was required to impair autophagy (Kania *et al.*, 2023, *CDDis*). These findings therefore provide insight into an additional control point for autophagy that is relevant to human disease. We continue to work on other known autophagy regulators to see how they function *in vitro* and *in vivo* and how they are affected by the novel autophagy regulators we identify.

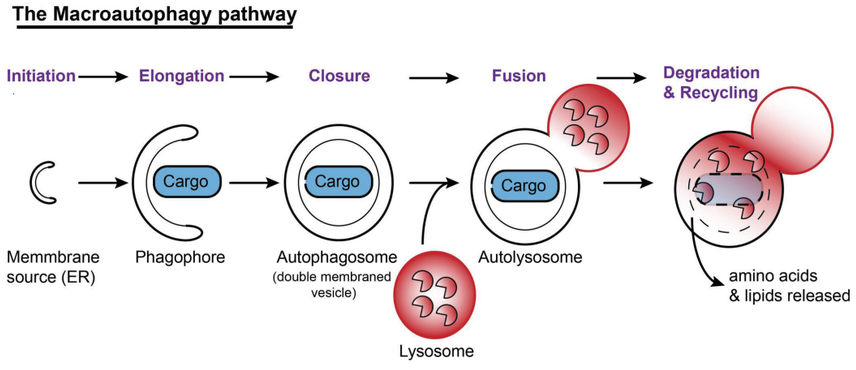

Figure 1: The (Macro) Autophagy pathway.
The process of macroautophagy occurs in the cytoplasm of the cells and proceeds through various stages to encapsulate cargoes destined for degradation. Ultimately, fusion occurs with a lysosome that provides hydrolases required for degradation. The breakdown products are then recycled or further catabolised.

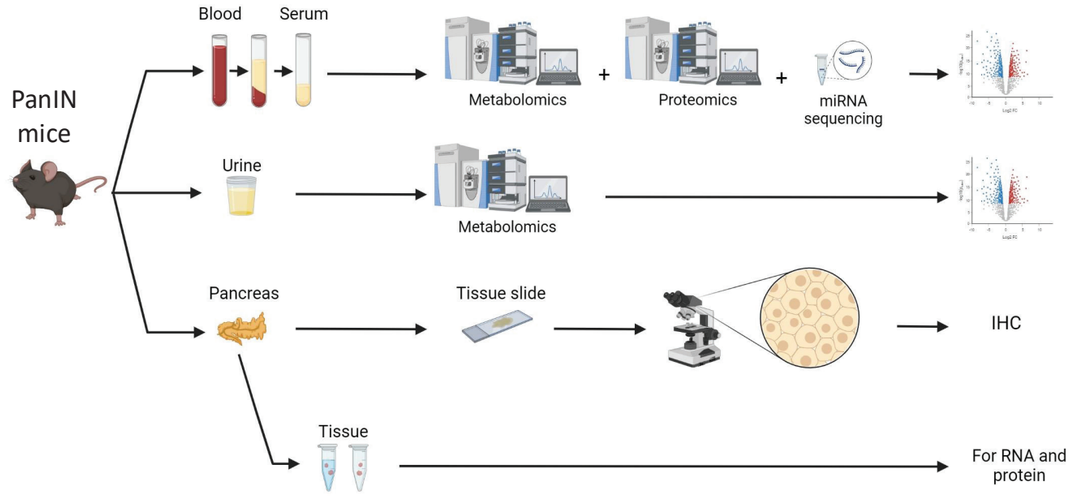

Figure 2: Utilizing autophagy to identify biomarkers of pre-cancerous pancreatic cancer.
Detection of pre-cancerous lesions enables the selection of individuals who are at risk and facilitates tumour formation at an early stage at which it can be treated. Mouse models were used to identify potential biomarkers in a mouse model with excessive Pancreatic interaepithelia neoplasia (PanIN) - the precursors of pancreatic ductal adenocarcinoma (PDAC). Identified factors were triaged and confirmed in an established mouse model of PDAC formation driven by mutant Ras and p53. The subsequent utility of these identified factors to identify human PanINs is currently being examined. IHC, immunohistochemistry.

Identification of potential biomarkers of pre-cancerous pancreatic cancer

It is widely accepted that the early detection of cancer results in more tractable therapeutic strategies, and as a result, better patient prognosis and reduced numbers of cancer-related deaths. Pancreatic ductal adenocarcinoma (PDAC) currently has very poor prognosis with only 7% surviving 5 years after diagnosis. Pancreatic intraepithelial neoplasia (PanIN) are considered to be the precursors of PDAC and the ability to detect PanINs would enable identification of patients at increased risk who can be monitored more frequently for the early stages of PDAC development.

Using a mouse model of autophagy that we previously described as having excess PanIN formation (Rosenfeldt *et al.*, 2013, *Nature*), we have utilized proteomics, metabolic mass spectrometry and microRNAseq to identify potential serum or urine biomarkers of PanIN formation (Figure 2). To triage the hits from these screens, we also analysed the serum and urine from genetically engineered mice that express the tumour-promoting genes mutant Ras and mutant p53 in their pancreata. Having successfully validated a number of hits, we are now examining if these potential biomarkers can be used to identify human PanINs and more importantly PanINs that are likely to progress to PDAC.

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COLORECTAL CANCER AND WNT SIGNALLING

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Colorectal cancer (CRC) is a heterogeneous disease comprising distinct molecular subgroups that differ in histopathological features, prognosis, metastatic propensity, and response to therapy. Leveraging omics and spatial biology technologies in state-of-the-art preclinical models harbouring key driver mutations, we are interrogating the molecular underpinnings of CRC initiation and progression to identify early-stage diagnostic biomarkers and develop stage- and subtype-specific targeted therapies.

To investigate the molecular pathogenesis and evolutionary trajectory of CRC, we have generated a suite of genetically engineered mouse models (GEMMs) that develop early-stage adenomas through to treatment-refractory, advanced CRCs, with GEMM- and patient-derived organoid cultures providing a tractable means to observe epithelial cell plasticity and adaptive therapeutic responses in real-time *ex vivo*. Through an extensive network of scientific and clinical collaborators, we have also expanded our disease modelling into other tissues, such as the pancreas, liver, and skin, to gain insights into the pathogenetic and molecular mechanisms underlying different disease domains and to evaluate potentially actionable therapeutic targets across tumour types. These disease-positioned mouse and organoid models are enabling the development and preclinical testing of prospective therapeutic approaches, the characterisation of patient-relevant biomarkers, and the identification of drug resistance mechanisms, and are integral to our overarching goal of translating promising preclinical findings into a tangible clinical benefit.

Preclinical evaluation of combinatorial therapeutic strategies in *Kras*-mutant tumour models

The RAS signalling pathway is commonly dysregulated in CRC and pancreatic ductal adenocarcinoma (PDAC), owing to the high prevalence of activating mutations in *KRAS*, as well as in melanoma, which typically harbours activating *BRAF* mutations. Such tumours thrive on the constitutive hyperactivation of RAS/RAF/MEK/ERK downstream signalling, which augments tumorigenic capacity by driving cell proliferation, survival, and invasiveness. Whilst KRAS has long been considered "undruggable," recently developed mutant-selective KRAS inhibitors have shown promising efficacy in certain disease contexts. Nevertheless, *KRAS*-mutant CRCs have proven refractory to conventional chemotherapies and single-agent kinase inhibitors, such as MEK- and EGFR-targeted monotherapies, lack efficacy in this setting. Thus, there is an urgent unmet need for the development of combinatorial therapeutic approaches for *KRAS*-mutant CRCs and other KRAS-driven tumours.

Recent studies have combined mutant-selective KRAS inhibitors with immunotherapies or other targeted treatments, and examined how combination strategies could overcome emergent resistance mechanisms. In collaboration with Karen Cichowski (Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA), we showed that concurrent inhibition of KRAS and the histone methyltransferase EZH2—an epigenetic silencer of key genes involved in differentiation—elicits robust and durable tumour regression in multiple human and mouse CRC models (Loi *et al.*, 2024, *Cancer Discov*). Notably, the EZH2-inhibitor tazemetostat potentiated the efficacy of various RAS-pathway inhibitors (the phospho-MEK inhibitor trametinib as well as mutant-selective KRAS inhibitors) only in tumours driven by mutations in RAS-pathway components, underscoring the selectivity of this drug synergy. Mechanistically, these drug combinations synergistically suppress Wnt-driven transcription and drive colorectal tumours into a Groucho/TLE4-mediated differentiated cell state, underpinned by the elevated expression of Wnt-pathway inhibitors, the induction of key markers of the secretory lineage, and the downregulation of stem cell–associated genes. This induction of differentiation, by the Wnt-pathway repressor TLE4, sensitizes cells to apoptosis via the expression of the proapoptotic protein BMF, eliciting clearance of tumour cells and ensuring the durability of the response. Importantly, these drug combinations are well tolerated with no toxicity towards the normal intestinal epithelium. Collectively, these studies suggest that combining epigenetic and oncogenic inhibitors targeting EZH2 and KRAS may hold therapeutic promise for the treatment of *KRAS*-mutant CRCs.

In metastatic melanoma, we explored how to overcome the emergent resistance to BRAF-targeted therapies, which commonly arises through the acquisition of activating hotspot mutations in *RAC1*. Such mutations sustain activation of the MAPK signalling pathway and, consequently, circumvent the effects of BRAF inhibition. Using GEMMs and patient-derived $BRAF^{V600E}$–mutant melanoma cell lines, we found that whilst PREX2 activity is dispensable for melanoma initiation and progression, targeting the PREX2/RAC1/PI3Kβ signalling axis sensitizes tumour cells to MAPK pathway–targeted therapies. These findings suggest that PREX2 or PI3Kβ inhibition could be combined with MAPK-targeted drugs to improve therapeutic outcomes in *BRAF*-mutant metastatic melanoma (Ford *et al.*, 2024, *Cancer Res*).

Overall, these studies are evaluating combinatorial therapeutic strategies and yielding multifaceted biological and molecular insights that have the potential to inform the development of clinical trials for *KRAS*-mutant tumours.

Kras allelic imbalance drives tumour initiation but suppresses metastasis and sensitizes to MEK inhibition in CRC and PDAC models

Whilst a plethora of studies have addressed how oncogenic *Kras* mutations drive tumorigenesis, our recent studies in GEMMs recapitulating CRC (Najumudeen *et al.*, 2024, *Nat Commun*) and PDAC (Fey *et al.*, 2024, *Cancer Res*) have revealed that the retention or loss of the wild-type *Kras* allele influences the function of its oncogenic counterpart, profoundly impacting disease trajectory and therapeutic outcome. Mechanistically, loss of the remaining wild-type *Kras* allele promotes the initiation of oncogenic KRAS-driven small-intestinal (Figure 1A) and pancreatic tumours, by increasing pro-proliferative downstream MAPK signalling (Figures 2A and 2B), suggesting that wild-type KRAS restrains the oncogenic effects of mutant KRAS.

Deletion of wild-type *Kras* in oncogenic $KRAS^{G12D}$–driven, aggressive tumour models accelerates tumorigenesis and shortens survival (Figure 1B),

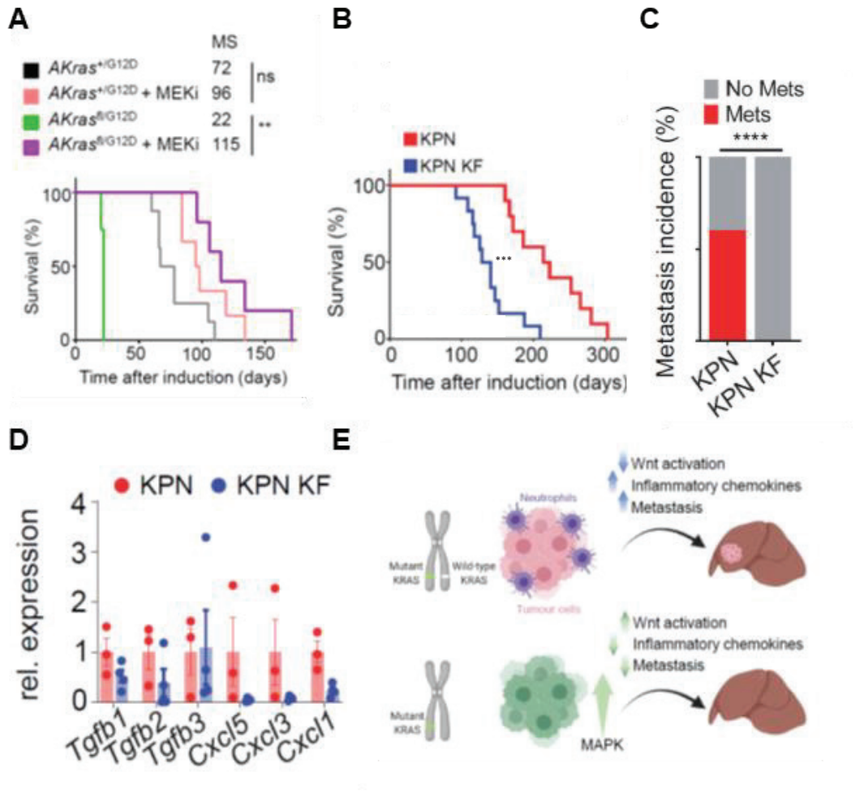

Figure 1: Loss of wild-type *Kras* sensitises to MEK inhibition and suppresses metastasis of $Kras^{G12D}$–mutant intestinal tumours. **A)** Kaplan–Meier survival curves for $VilCre^{ER}Apc^{fl/+}Kras^{+/G12D}$ ($AKras^{+/G12D}$) and $VilCre^{ER}Apc^{fl/+}Kras^{fl/G12D}$ ($AKras^{fl/G12D}$) mice, treated with MEK-inhibitor (MEKi) one day post tamoxifen-induction and aged until clinical endpoint. $AKras^{+/G12D}$, n=8 (2M, 6F); $AKras^{+/G12D}$ + MEKi, n=6 (2M, 4F); $AKras^{fl/G12D}$, n=4 (2M, 2F); $AKras^{fl/G12D}$ + MEKi, n=5 (4M, 1F). $^{**}P$=0.0050; ns, not significant; log-rank (Mantel–Cox) test. **B)** Kaplan–Meier survival curves for $VilCre^{ER}Kras^{+/G12D}Trp53^{fl/fl}Rosa26^{Nlicd/+}$ (KPN) and $VilCre^{ER}Kras^{fl/G12D}Trp53^{fl/fl}Rosa26^{Nlicd/+}$ (KPN KF) mice aged until clinical endpoint. KPN, n=10 (5M, 5F); KPN KF, n=12 (6M, 6F). $^{***}P=2\times10^{-4}$; log-rank (Mantel–Cox) test. C) Incidence of metastasis in KPN and KPN KF mice aged until clinical endpoint. KPN, n=11 (6M, 5F); KPN KF, n = 11 (5M, 6F). $^{****}P=1\times10^{-15}$; two-tailed chi-square test. D) Relative expression of transcripts encoding TGFβ ligands and chemokines in organoids derived from KPN and KPN KF tumours. KPN, n=3 (3M); KPN KF, n=4 (1M, 3F). Data, mean ± s.e.m. E) Schematic depicting the mechanisms whereby loss of wild-type KRAS activates Wnt signalling and reduces neutrophil recruitment, compromising the metastatic competence of KPN KF tumours. MS, median survival; M, males; F, females; s.e.m., standard error of the mean.

Figure 2: Loss of wild-type *Kras* in KPC *Kras*$^{G12D/fl}$ pancreatic tumours potentiates oncogenic KRAS-driven MAPK signalling and sensitizes to MEK1/2 inhibition.
A) Immunoblotting for indicated RAS-pathway components in KPC *Kras*$^{G12D/fl}$ and KPC *Kras*$^{G12D/+}$ tumours at endpoint. β-actin, loading control. Each lane represents PDAC tissue from an individual mouse of the indicated genotype. **B)** Transcript levels of indicated MAPK-pathway downstream target genes in KPC *Kras*$^{G12D/fl}$ and KPC *Kras*$^{G12D/+}$ tumours. Transcript levels were normalized to *Gapdh*. KPC *Kras*$^{G12D/+}$, n=5; KPC *Kras*$^{G12D/fl}$, n=6. Data, mean ± s.e.m. *P*=0.2 (*Etv4*), ***P*=0.0087 (*Etv5*), *P*=0.4 (*Dusp4*), ***P*=0.0087 (*Dusp6*), **P*=0.0152 (*Spry1*), **P*=0.0411 (*Spry2*); one-way Mann–Whitney U test. **C)** Kaplan–Meier survival curves for KPC *Kras*$^{G12D/+}$ and KPC *Kras*$^{G12D/fl}$ mice, treated with vehicle or MEK1/2 inhibitor (AZD6244), until clinical endpoint. KPC *Kras*$^{G12D/fl}$ + vehicle, n=5; KPC *Kras*$^{G12D/fl}$ + AZD6244, n=8; KPC *Kras*$^{G12D/+}$ + vehicle, n=6; KPC *Kras*$^{G12D/+}$ + AZD6244, n=5. MS, median survival; s.e.m., standard error of the mean.

yet remarkably the corresponding small-intestinal (Figure 1C) and pancreatic tumours are less likely to metastasize. In KPN KF (VilCre^{ER}*Kras*$^{fl/G12D}$*Trp53*$^{fl/fl}$*Rosa26*$^{Nlicd/+}$) small-intestinal tumours, loss of wild-type *Kras* activates Wnt signalling and reduces neutrophil recruitment to the premetastatic niche (Figure 1D) blunting metastasis formation, compared with KPN lesions retaining the wild-type *Kras* allele (VilCre^{ER}*Kras*$^{+/G12D}$*Trp53*$^{fl/fl}$*Rosa26*$^{Nlicd/+}$; Figures 1C and 1E). In the *Kras*$^{G12D/fl}$*Trp53*$^{R172H/+}$Pdx1-Cre (KPC *Kras*$^{G12D/fl}$) mouse model of PDAC, loss of wild-type *Kras* accelerates tumour initiation, giving rise to slow-growing, poorly metastatic tumours that exhibit a desmoplastic stroma with increased immune-cell infiltration, in contrast to the highly immune-excluded KPC *Kras*$^{G12D/+}$ tumours.

Notably, loss of the wild-type *Kras* allele in our CRC and PDAC models increases MAPK signalling (Figures 2A and 2B), which sensitizes them to MEK1/2 inhibition (Figures 1A and 2C). However, these findings unveil only a short window of therapeutic vulnerability, as pancreatic tumours eventually become resistant and progress rapidly. Conversely, retention of wild-type *Kras* dampens tumour dependence on MAPK signalling and confers resistance to MEK inhibition *ab initio* (Figures 1A and 2C), limiting treatment options for this group.

Together, these two studies advocate for patient stratification by *KRAS* allelic status in addition to screening for *KRAS* mutation status. Such patient selection strategies will discern those patients (whose tumours have lost the wild-type *KRAS* allele) that are most likely to benefit from targeted inhibition of downstream effector signalling.

Disease positioning of preclinical models and patient tumours

In collaboration with Philip Dunne's group (Queen's University Belfast and CRUK Scotland Institute), we integrated human and mouse multi-omics data to align our GEMMs to the consensus molecular subtypes (CMS) of human CRC, affirming that they recapitulate key features of disease progression and response to therapy (Amirkhah *et al.*, 2023, *Br J Cancer*; Malla *et al.*, 2024, *Nat Genet*). We employed a biological pathway–level approach and gene ontology—rather than relying on individual gene-centric biomarkers—to identify three biologically distinct pathway-derived subtypes (PDS1–3) of CRC (Malla *et al.*, 2024, *Nat Genet*). We found that PDS1 tumours are enriched for canonical *LGR5*$^{+}$ stem-like signatures and MYC downstream targets, whereas PDS2 CRCs are characterised by the expression of regenerative markers and elevated stromal and inflammatory signatures. This analysis also recognised the hitherto unknown PDS3 class: a slow-cycling, highly differentiated subset of canonical/CMS2 CRCs, which lacks discernible stem-like populations but is instead enriched for enterocyte and enteroendocrine lineages. Molecularly, PDS3 tumours exhibit reduced levels of components of the polycomb repressive complex (PRC), which results in the upregulation of PRC target genes that promote cellular differentiation. Given that none of our existing mouse models align with human PDS3 biology, we are developing tractable GEMMs to gain insights into the biological complexity of this newly recognised group of CRCs with the worst prognosis.

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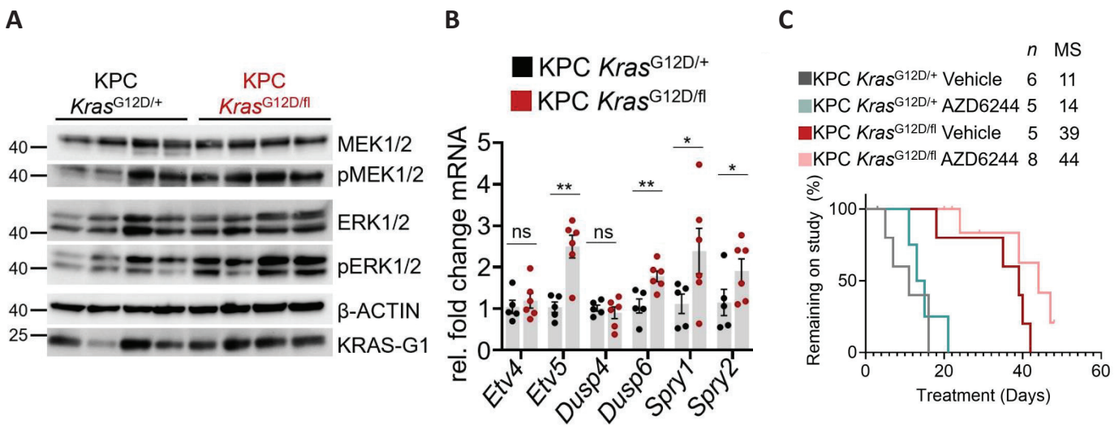

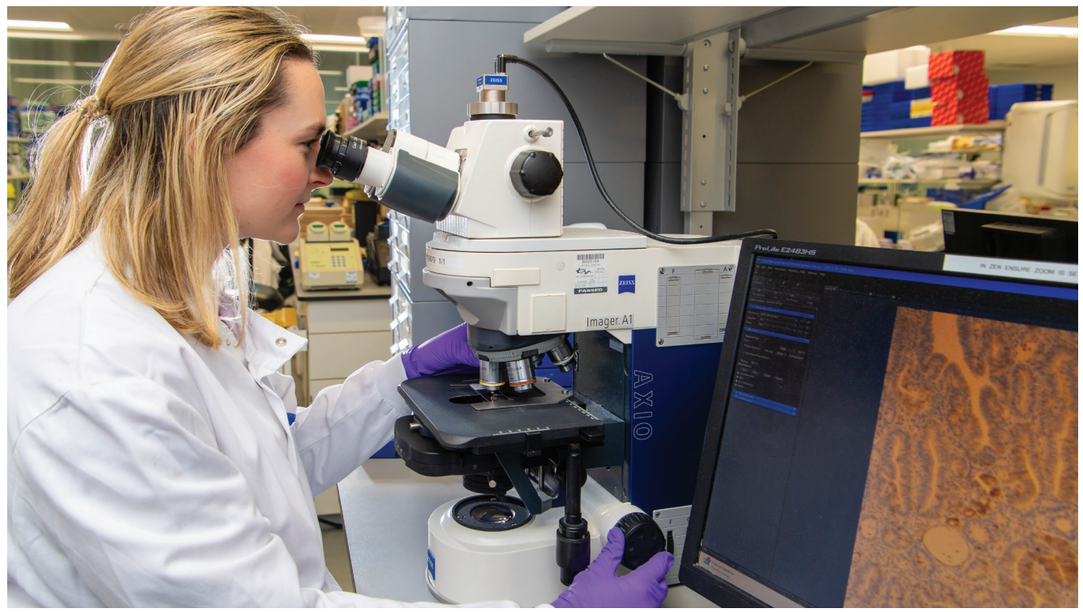

ADVANCED COLORECTAL CANCER

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Patients die from colorectal cancer due to spread/metastasis to other organs, in particular the liver. Our team studies patient tissues accessed at the time of surgery and generates models to better understand the mechanisms underlying colorectal cancer progression in patients with locally advanced rectal cancer and liver metastases with a view to developing and assessing novel targets for therapy.

Colorectal cancer (CRC) is the second most common cause of cancer-related death in the Western world. Disease that is localised to the colon can be treated with surgery. Despite this, 40% of patients will suffer from disease recurrence. Recurrence usually occurs at sites distant from the colon, most commonly liver and lungs and is called metastatic disease. Most patients who die from colorectal cancer do so due to metastatic disease. Unfortunately, treatment options remain limited for these patients, with surgery remaining the best strategy if disease is diagnosed early. Our team is focused on understanding why disease recurs following surgery, the patterns of recurrence and whether the disease can be subtyped to permit development of better therapies for patients.

Assessing the heterogeneity of colorectal liver metastases

Assessment of human colorectal liver metastases (CRLM) suggests that different subtypes exist. These can be detected histologically and separated into 'immune', 'stromal' and 'canonical' using transcriptomic analysis (Pitroda *et al.*, 2018, *Nature Comms*). Patients from the immune subgroup do very well following surgical resection and can be cured of their disease. It is likely these patients may also respond to commonly used immunotherapies; however, this is as yet still to be clearly elucidated. We are making efforts to accurately subtype the disease in our patients (Figure 1), and we have partnered with Nanostring to assess the heterogeneity of these subtyped tumours.

We have identified that CRLM in certain patients were profoundly immunosuppressed with very few activated T cells evident within the microenvironment of these tumours (Figure 1, Patient C), while others had significant upregulation of adaptive immune responses particularly at the edges of metastases (Patient B). We observed higher numbers of myeloid cell populations within the microenvironment of immunosuppressed and stromal tumours including neutrophils and macrophages, using immune cell deconvolution techniques and confirmed using IHC. These patients had contrasting survival based on their immune response, with patients able to obtain long term survival following surgery for liver metastases if they displayed a strong adaptive immune response (Wood *et al.*, 2023, *Cancer Res*), while patients with neutrophils surrounding metastatic disease had very poor survival following surgery. This represents an area for further study with a view to moving these observations into real-time to help guide decision-making for patients in the future. Having established the utility of these technologies, we are now studying neutrophil biology within individual patients. We have focused on identifying single cell RNA sequencing data of neutrophil populations and are identifying transcriptional pathways that drive neutrophil development in the context of CRLM (Figure 2A). We have shown that there are many different transcriptional states of neutrophils (Fetit *et al.*, 2024, *Cancer Res Commun*) and certain transcriptomic profiles that are only seen in liver metastases. We have performed Nanostring COSMX analysis to provide single cell level data with spatial resolution and are currently mapping neutrophil populations identified using bioinformatic approaches to tissue in attempts to identify pathogenic neutrophils in this context (Figure 2B). We have supported the development of a liver service for Glasgow as clinicians and now are able to access tissues from patients suffering from liver metastases. We want to understand the disease in these patients and to do this we have generated models targeting neutrophils in the disease. Additionally, we are trying to interrogate cancer cell types and states that associate with neutrophil types that influence prognosis. In collaboration with Kevin Myant in Edinburgh, we have observed that common mutations may lead to increased cancer cell plasticity and are now exploring whether under certain conditions this leads to neutrophil rich metastases with poor outcome.

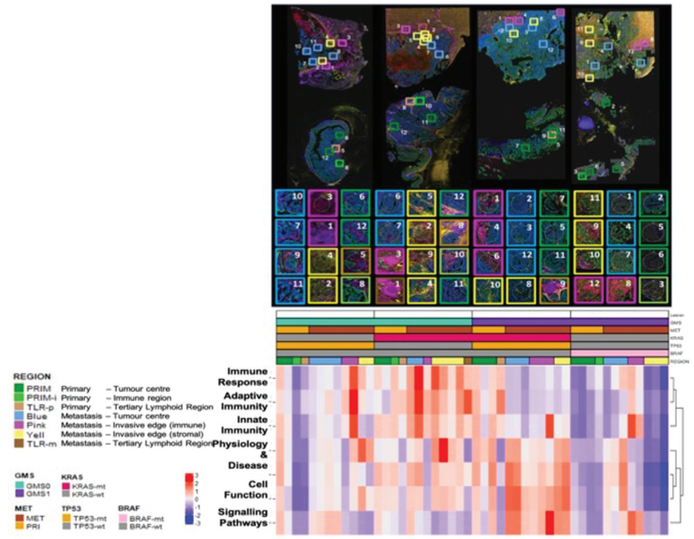

Figure 1. Transcriptomic assessment of heterogeneity, primary and metastatic sites in 4 patients

Modelling immunosuppressed metastatic CRC and understanding microenvironmental influences for therapeutic gain

We have worked closely with the Sansom laboratory and have been involved in the development of state-of-the-art models of CRLM. Using orthotopic transplantation techniques we can mimic human disease to provide a model of stromal rich metastasis for assessment of anti-metastatic therapies *in vivo*. Our previous work together has revealed that neutrophils were key cellular regulators of the metastatic microenvironment in CRC (Jackstadt *et al.*, 2019, *Cancer Cell*), regulating an immunosuppressed microenvironment as we observed in patients with very poor outcomes. However, the mechanism by which those neutrophils functioned to progress metastatic disease and how to manipulate them *in vivo* remains unknown. We have performed RNA sequencing of neutrophils from sites within our 'KPN' model and found differentially expressed genes within neutrophils associated with metastases. We are currently investigating whether inhibition of specific genes expressed by neutrophils *in vivo* influences their behaviour and progression of metastases. Others have shown: cooperation of gamma delta T cell populations in promoting neutrophil function at metastatic sites (Coffelt *et al.*, 2015, *Nature*); that production of transferrin by neutrophils supports metastatic cells (Liang, Li, & Ferrara, 2018, PNAS); the role of neutrophil extracellular traps in awakening dormant tumour cells (Albrengues *et al.*, 2018, *Science*); and that neutrophils can accompany tumour cells to metastatic sites and help them establish (Szczerba *et al.*, 2019, *Nature*). Modelling these immunosuppressed stromal metastases will allow us to understand immunosuppressive mechanisms using intravital imaging and whether they can be overcome through directly targeting neutrophils in this model. *Ex vivo* study of neutrophil function is being developed to further characterise these cells in this context. T cell-directed therapies are currently being trialled in combination with neutrophil-directed therapies to assess impact on metastatic progression with a view to taking forward for patient benefit in future.

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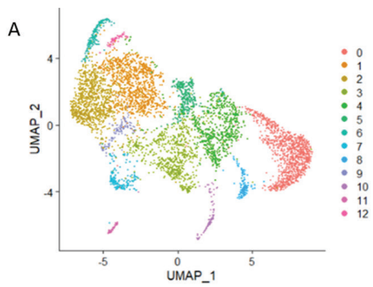

Figure 2. Single cell assessments of neutrophils in CRLM
A – UMAP showing neutrophil populations in CRLM
B- Nanostring GeoMx representation of gene expression at single cell level in tissue

MITOCHONDRIA AND CANCER CELL DEATH

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The best way to treat cancer is to kill it. Indeed, most cancer therapies work by killing tumour cells, be it directly or indirectly. Nevertheless, combined issues of toxicity and resistance limit the effectiveness of anti-cancer therapies. To address these, our research centres on understanding how mitochondria regulate cancer cell death and inflammation, with the goal of improving cancer treatment.

Mitochondria, cell death and cancer

Apoptosis requires caspase protease activity, leading to widespread substrate cleavage and rapid cell death. During apoptosis, mitochondrial outer membrane permeabilisation (MOMP) occurs, a crucial event that is required for caspase activation. Following MOMP, mitochondrial intermembrane space proteins, such as cytochrome c, are released into the cytoplasm where they cause caspase activation and apoptosis. Given its key role in controlling cell survival, mitochondrial outer membrane integrity is highly regulated, largely through interactions between pro- and anti-apoptotic Bcl-2 proteins. Cancer cells often inhibit apoptosis by preventing MOMP, often through upregulation of anti-apoptotic Bcl-2 proteins. Importantly, this can be exploited therapeutically – newly developed anti-cancer therapeutics called BH3-mimetics target these apoptotic blocks.

How do cells engage oncogenic sub-lethal apoptotic stress?

While apoptosis has potent anti-tumour activity, we have previously shown that sub-lethal apoptotic stress can trigger caspase-dependent DNA-damage having oncogenic effects. This occurs through limited MOMP in a few mitochondria – what we termed minority MOMP. Nonetheless why some mitochondria selectively permeabilised remained enigmatic. Mitochondrial fusion protects cells from sub-lethal apoptotic stress, whereas fission has the opposing effect. Moreover, we found that loss of mitochondrial function serves as an intrinsic priming signal, sensitising mitochondria to permeabilization.

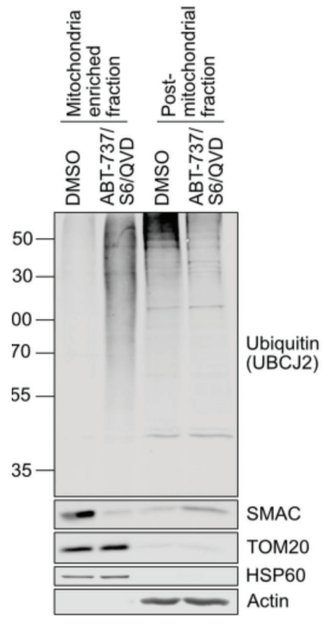
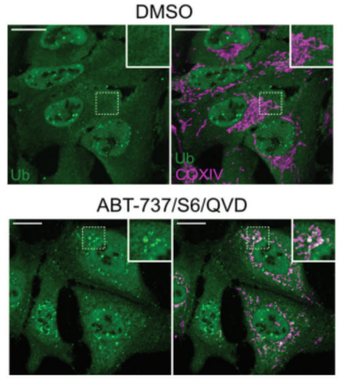


Figure 1. Mitochondria are ubiquitinated following outer membrane permeabilization
SVEC cells were treated to undergo mitochondrial apoptosis (ABT737/S6) and mitochondria were probed for ubiquitin by western blot (left) or imaging (right, ubiquitin: green, mitochondria: cyan). Following MOMP, extensive mitochondrial ubiquitination is observed.



By targeting mitochondrial dynamics and/or function these findings offer new strategies to both prevent oncogenic sub-lethal stress as well as enhance the tumour killing capacity of anti-cancer therapies.

Mitochondrial permeabilization promotes inflammation that engages anti-tumour immunity

Mitochondrial apoptosis is a silent form of cell death. Importantly, even in the absence of caspase activity, MOMP leads to cell death through progressive mitochondrial dysfunction. Our previous research has shown that such caspase-independent cell death (CICD) is immunogenic and can be harnessed to trigger anti-tumour immunity. Underpinning this immunogenicity is that permeabilised mitochondria activate myriad inflammatory pathways. We have investigated how MOMP engages inflammation. Our recent data shows that upon permeabilization mitochondria are extensively decorated with ubiquitin (Figure 1). Ubiquitination occurs in a promiscuous manner targeting many mitochondrial inner and outer membrane proteins. While traditionally considered a degradative post-translational modification, ubiquitination can also serve non-degradative signalling functions. Indeed, we find that mitochondrial ubiquitination serves to recruit the NF-κB adaptor molecule NEMO leading to activation of pro-inflammatory NF-κB signalling (Figure 2). Perhaps stemming from the bacterial ancestry of mitochondria, this process displays striking analogy to how our cells cope with intracellular bacteria, where invading bacteria are ubiquitinated leading to a protective NF-κB inflammatory response. We are currently investigating how mitochondrial-ubiquitination driven-inflammation contributes to the immunogenicity of tumour cell death.

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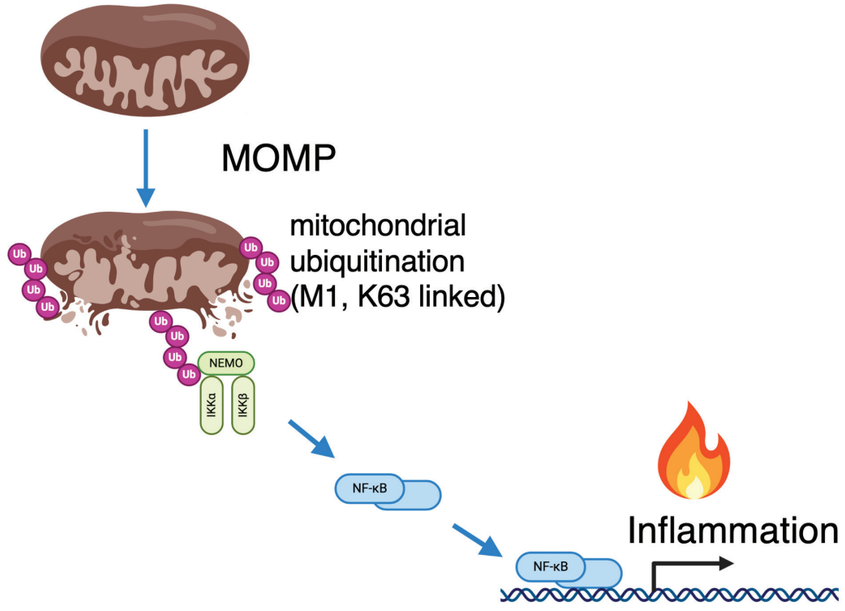
Figure 2. Mitochondrial ubiquitination drives inflammation following MOMP
Upon MOMP, mitochondria are extensively ubiquitinated. Ubiquitination serves to recruit the NF-κB adaptor molecule NEMO to mitochondria, promoting NF-κB driven-inflammation.



METABOLIC CROSSTALK IN CANCER

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Cancer is a multifactorial disease with widespread effects on patients' health. Cancer cells undergo metabolic rewiring to sustain continued proliferation and to survive in hostile environments. This includes alterations in the uptake and utilization of nutrients and metabolites. As such, the tumour microenvironment is important for metabolite supply to cancer cells and the presence of a tumour affects the normal function of its host organ. In addition, cancer is associated with systemic metabolic changes that can dramatically impact quality of life for patients and their fitness to undergo treatments. Research in our laboratory focuses on metabolic crosstalk between the host and tumours, ultimately aiming to develop new, more efficient therapies.

Implications of the gut microbiome in cancer

Gut microbiome dysbiosis is associated with various malignancies and this has implications for cancer onset, progression and therapy sensitivity. We previously showed that enzymes of tumour-associated bacteria metabolize commonly used anticancer drugs. Depending on drug-specific pharmacology, this results in decreased (e.g. gemcitabine) or increased (e.g. fludarabine) anticancer activity. This argues for careful consideration of the microbiome as a therapy-modulator. In addition, microbial metabolites are emerging as key players in cancer. Using untargeted metabolomics, we recently demonstrated that gut microbiota affect not only the local intestinal metabolic environment but also profoundly affect the blood metabolome (Figure 1).

We study metabolic interactions between microbiota and host cells using preclinical cancer models and patient samples, focusing on how microbial metabolites impact disease onset, progression and sensitivity to therapies. Because of its unique association with the gut microbiome, we have a particular interest in colorectal cancer (CRC). Dietary patterns with reduced fibre intake and high intake of processed food, sugar, fat and red meat affect gut microbial metabolism, and increase an individual's CRC risk. CRC is the second most common cause of cancer-related death worldwide, and there is an urgent need for better prevention strategies and therapies.

Certain gut microbiota produce genotoxic metabolites which affect the host intestine and may therefore contribute to CRC. Colibactin is a well-studied toxin produced by pks⁺ *Escherichia coli* which induces a characteristic mutational signature. We recently contributed to a collaborative study, reporting how specific bacterial adhesion of pks⁺ *E. coli* to the host epithelium is critical to exert its genotoxic

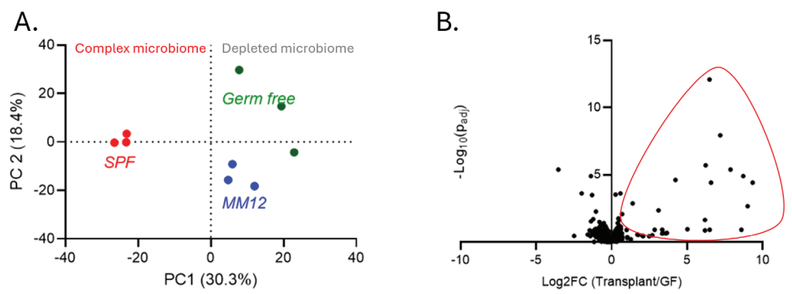

Figure 1. Untargeted metabolomics shows microbiome dependent alterations in blood metabolites
(A) PCA of serum metabolomics of germ-free mice and mice with a minimal (MM12; defined community of 12 bacterial strains) or complex microbiome (SPF).
(B) Volcano plot showing alterations in circulating metabolites in germ-free mice after introduction of a complex microbiome by faecal microbiota transplantation (FMT) with stool of SPF mice. Red shape indicates metabolites that are increased in animals that received an FMT.

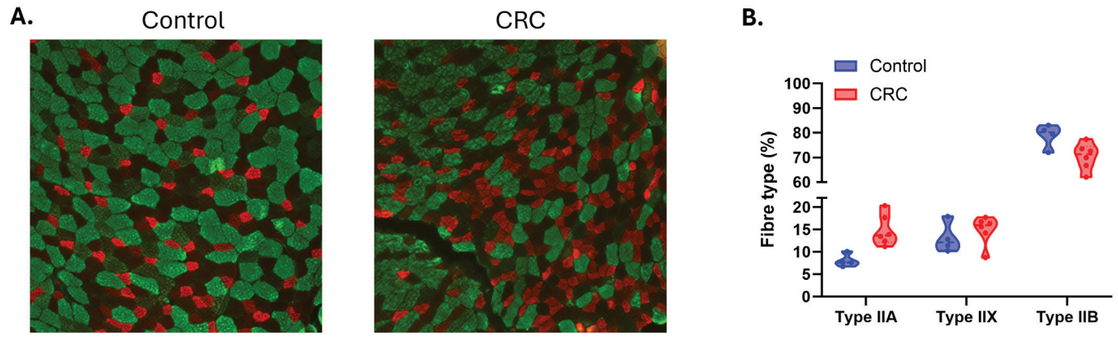

Figure 2. CRC affects muscle myofiber distribution
(A) Immunofluorescence images and (B) myofiber distribution of tibialis anterior muscle sections of control and tumour-bearing mice. Type IIA, 2X and 2B fibres are shown in red, no staining and green, respectively.

effects. This highlights the potential value of anti-adhesive therapies to reduce the risk of CRC or to impair disease progression (Jans *et al.*, 2024, *Nature*).

Metabolic determinants of cancer-associated cachexia

Cancer cachexia is a wasting syndrome defined by ongoing loss of skeletal muscle mass, with or without loss of fat mass, which cannot be restored by conventional nutritional support. This involves hypercatabolism, systemic inflammation, and ultimately leads to functional impairment. As such, cancer cachexia reduces quality of life, fitness for treatment and associates with increased mortality. Cachexia is part of the common functional decline affecting 80% of advanced cancer patients and is responsible for 30% of cancer deaths. At present, there is no cure and the underlying mechanisms of this debilitating condition are poorly understood.

Studies report that up to fifty percent of CRC patients experience cachexia during their disease. There is a lack of representative preclinical models of CRC-associated cachexia, and this impedes the development of novel, efficacious treatments. We use genetically engineered and orthotopic transplantation mouse models of CRC and study how these recapitulate important features of cachexia. Tumour-bearing animals of selected models show inability to thrive, loss of lean muscle and fat mass and pronounced alterations in tissue composition (Figures 2&3). Our ambition is to harness these models to identify targetable metabolic determinants of cachexia.

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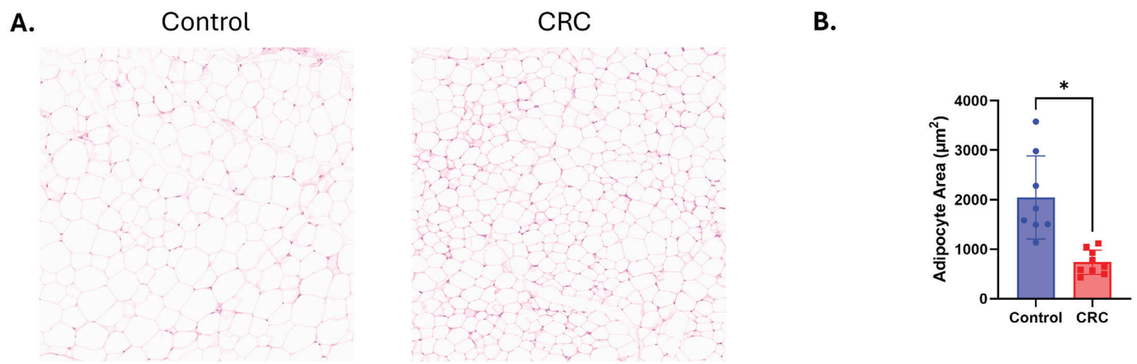

Figure 3. CRC affects adipose tissue architecture
(A) H&E images and (B) adipocyte area of inguinal white adipose tissue of control and tumour-bearing mice.

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ARTIFICIAL INTELLIGENCE IN CANCER RESEARCH

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Modern-day cancer research is generating an unprecedented amount of data, from high-resolution medical images to large-scale genomic and transcriptomic datasets. Harnessing this data through advanced AI, machine learning, and statistical models opens new avenues for discovery and translation into clinical practice.

Our work focuses on developing state-of-the-art methods tailored to cancer research challenges, particularly in the analysis of imaging and sequencing data. By addressing critical questions, such as identifying predictive biomarkers and uncovering novel therapeutic targets, we aim to drive progress in precision oncology and improve patient outcomes.

Mapping histomorphological phenotype across cancer types

Pathology slides are pivotal for cancer diagnosis, capturing vast information about cell shapes, interactions, and tissue structures. However, current diagnostic practices underutilize this data due to reliance on broad annotations from pathologists. Manual labelling of cells or tissue patterns across slides is infeasible.

To address this, we developed Histomorphological Phenotype Learning (HPL), an AI system trained on unannotated pathology slides using self-supervised learning (Claudio Quiros *et al.*, 2024, *Nat Commun*). HPL extracts generalisable features from millions of image tiles, clustering them into Histomorphological Phenotype Clusters (HPCs), which represent distinct tissue and cellular patterns. HPCs can predict cancer types, patient outcomes, and correlate strongly with molecular signatures.

HPL has proven effective in mapping cancer phenotypes. In collaboration with the Le Quesne lab, we demonstrated its utility in lung adenocarcinoma, capturing known growth patterns and uncovering subclasses, such as immune-activity-dependent solid growth patterns. Impressively, HPCs outperform IASLC (International Association for the Study of Lung Cancer) recommended grading when predicting recurrence-free survival on an external validation cohort.

Beyond lung cancer, in collaboration with Le Quesne and Tsirigos (NYU) labs, we have demonstrated HPL's utility in mesothelioma (Seyedshahi *et al.*, 2024, *bioRxiv*), colorectal cancer (B Liu *et al.*, 2024, *bioRxiv*) and osteosarcoma (Coudray *et al.*, 2024, *Clin Cancer Res*). We are also working on pancreatic cancer with David Chang (School of Cancer Sciences, UoG), prostate cancer lymph node metastasis with Hing Leung, and radiotherapy response in rectal cancer with Campbell Roxburgh and Joanne Edwards (Cancer Sciences, UoG).

Leverage multiplexed images to improve AI models for routine pathology slides

Multiplexed imaging techniques like CODEX provide detailed molecular insights but are cost-prohibitive for large-scale use. We developed TriDeNT, an AI model that transfers features learned from multiplexed images to H&E images, improving the performance of H&E-based AI models (Farndale *et al.*, 2023, *arXiv*). TriDeNT has shown up to a 101% improvement in downstream tasks.

To address the scarcity of matched multiplexed and H&E data, we used generative AI to digitally restrain H&E images as IHC images. This approach enabled training on synthetic matched datasets, achieving up to a 5.6x error reduction compared to real multi-modal data (Farndale *et al.*, 2024, *arXiv*).

Protein language model based in silico deep mutational scan

Protein and RNA sequences, like human language, can be represented as sequences of words—amino acids or nucleotides. Transformer-based protein language models (PLMs) have demonstrated remarkable potential, with public protein databases now comparable in size to datasets used for training language models like GPT-3. PLMs, such as ESMFold, have achieved breakthroughs in predicting protein folding structures.

We explored the pretrained ESM model's capabilities in predicting variant effects using ~20 million SARS-CoV-2 sequences curated during the pandemic (Lamb *et al.*, 2024, *PLoS Comput Biol*). Remarkably, ESM-2, a version that had not encountered SARS-CoV-2 during training, accurately predicted variant effects from a single sequence. Using the Wuhan-1 spike protein as a backbone, we conducted an *in silico* deep mutational scan, quantifying the impact of amino acid changes at every position. The predictions revealed conserved and highly mutable regions, aligning well with traditional statistical metrics like entropy, which rely on observing mutations across multiple sequences. ESM-2's ability to derive such insights without prior exposure to SARS-CoV-2 highlights its generalizability and utility for variant effect prediction.

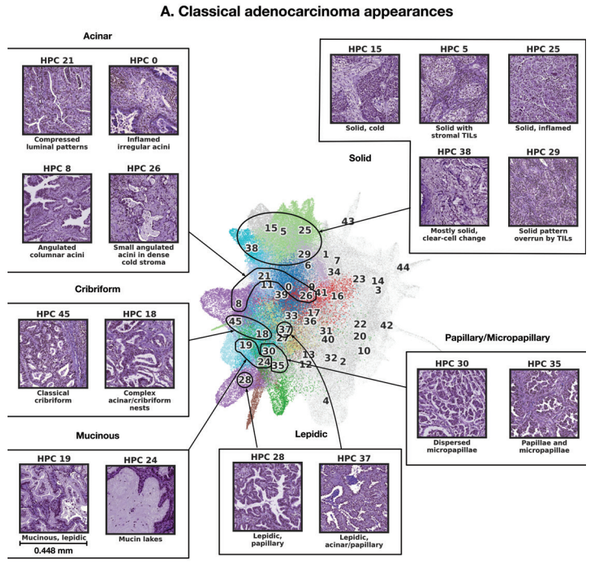

Figure 1. Uniform Manifold Approximation and Projection (UMAP) dimensionality reduction of lung adenocarcinoma tile vector representations labelled by HPC membership). HPCs of interest are coloured, while other HPCs remain grey. The consensus was obtained after independent annotations of HPCs by 3 pathologists.

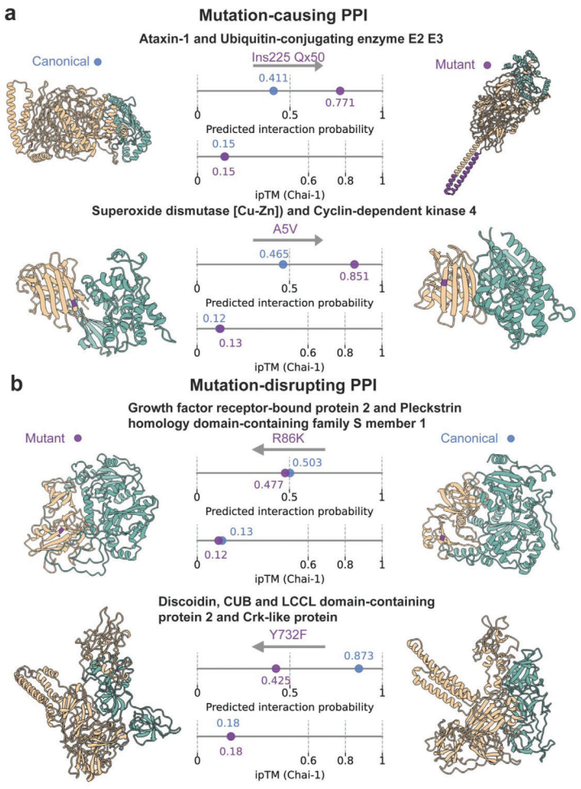

Figure 2. Demonstration of PLM-interact detecting changes in human PPIs associated withmutations. These PPI structures are predicted using Chai-1. Here, the mutated amino acids are highlighted in purple. Prediction interaction probabilities exceeding 0.5 indicate the proteins interact, while below 0.5 indicate non-interact. Chai-1's ipTM scores give the structure prediction confidence where <0.6 indicates failed predictions.

Predicting protein-protein interactions

Current approaches to PPI prediction use protein language models trained on single sequences, which lack the ability to model interactions between proteins. This limitation is akin to models capturing relationships among words but not between paragraphs. To address this, we developed PLM-Interact in collaboration with the Robertson lab (Centre for Virus Research, UoG) and Craig Macdonald (Computing Science, UoG) (D Liu *et al.*, 2024, *bioRxiv*). Trained on protein pairs with labelled interaction status, PLM-Interact achieved a 16%-28% improvement in accuracy (AUPRC) across multiple species, including humans, mice, and yeast. Importantly, it detected interaction-disrupting/causing mutations that eluded advanced tools like AlphaFold3, showcasing its potential for both prediction and mechanistic insight.

Future work

In the future, we will focus on the following directions

1. *AI for histology and spatial deep phenotyping*
   We aim to map all histologic patterns across all mouse models and cancer types generated locally and beyond. On the human side, we will work with NHS GGC to train pathology foundation models on their vast pathology slide archive. The mouse and human models will be used for disease positioning. We are also working on an HPL-like AI model trained with spatial proteomics data to combine molecular and morphological insights for a deeper understanding of tissue organization.

2. *Large language models for biological sequences and their interactions.* We aim to develop a suite of protein and RNA language models that could better predict the effect of mutations that were previously overlooked due to lack of recurrence across patients.

3. *Comprehensive in silico tumour model.* Building a digital twin of biological systems is an emerging paradigm that promises to transform experimental research. Our goal is to develop *in silico* tumour models that replicate the biological behaviour of mouse models. These digital twins would enable researchers to simulate experiments virtually, generating high-quality data that mimics real-world observations.

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TUMOUR MICROENVIRONMENT AND PROTEOMICS

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[3]CRUK Early Detection and Diagnosis Project Grant

High grade serous ovarian cancer (HGSOC) and triple negative breast cancer (TNBC) have limited treatment options, as only few targeted therapies effectively kill cancerous cells and patients frequently develop resistance to standard therapies. The tumour microenvironment actively supports cancer pathology and is populated by a variety of cell types that also offer alternative routes for therapy.

Our research focuses on cancer-associated fibroblasts (CAFs), as we and others have shown that they play a major role in modulating cancer pathology. CAFs strongly influence the function of cancer and other stromal cells by secreting extracellular matrix (ECM) components and modifiers, soluble factors and extracellular vesicles (EVs). We aim to understand the molecular mechanisms through which CAFs support cancer, and envisage targeting CAFs in combination with other anti-cancer therapies as a promising strategy to stop cancer growth and metastasis.

Our research primarily focuses on the role of CAFs in HGSOC and TNBC. These tumours contain vast regions of stroma, which are densely populated by CAFs, while CAFs were shown to play active roles in the progression of both diseases. Importantly, HGSOC cells and TNBC cells have few recurrent mutations, therefore limiting the availability of targeted therapies against cancer cells. As such, CAFs offer a valid alternative therapeutic opportunity in these tumour types (Santi *et al.*, 2018, *Proteomics*; Domen *et al.*, 2021, *Cancers*). We aim to decipher how CAFs create a tumour-promoting microenvironment and how we can block this process to make the tumour microenvironment unfavourable to cancer growth and tumours more vulnerable to therapeutic treatments; our overarching goal is to determine strategies that target CAFs to stop cancer.

CAFs can originate from normal fibroblasts resident at the site where the primary tumour develops. When a tumour starts developing, normal fibroblasts become activated into CAFs, and become able to secrete a plethora of soluble factors and ECM components that influence the function of surrounding cells and actively support cancer progression (Figure 1) (Kugeratski *et al.*, 2022, *Science Signaling*; Santi *et al.*, 2018, *Proteomics*). While CAFs are the results of the reprogramming of normal cells, we aim to find ways to revert CAFs to a normal cell-like phenotype that does not support cancer and that improves response to therapies.

For our research, we mostly use CAFs that we isolate from tumour tissues that are kindly donated by patients for research purposes, and we develop clinically relevant models to study their functions (Neilson, Cartwright *et al*, 2023, *Matr Biol Plus*). Our group has a strong expertise in mass spectrometry (MS)-based proteomics (van den Biggelaar *et al.*, 2014, *Blood*; Patella et al., 2015, Mol Cell Proteomics; Diaz *et al.*, 2017, *J Cell Sci*; van der Reest, Lilla *et al.*, 2018, *Nat Commun*), and we integrate this innovative technology in our research to tackle the above questions and provide new levels of understanding of CAF biology.

CAF – tumour blood vessel interaction

The vasculature of solid tumours is often responsible for the progression and aggressiveness of disease. Initially, tumours recruit blood vessels to obtain nutrients and oxygen to sustain proliferation. Later on, the tumour vasculature becomes leaky and provides a route for cancer cells to escape and form distant metastases.

Endothelial cells (ECs) line the inner layer of the vessel wall and regulate the functionality and growth of the vessel. Tumour blood vessels are typically embedded within a CAF-rich stroma, such that ECs are exposed to factors that CAFs secrete. We have found that CAFs influenced EC function by transferring functional proteins through a specific subset of EVs that are bound to the ECM that they produce. In particular, CAFs could transfer membrane-bound proteins that

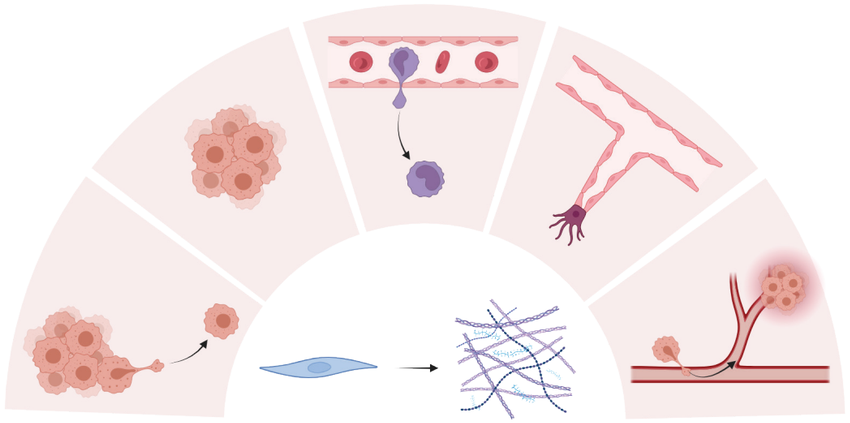


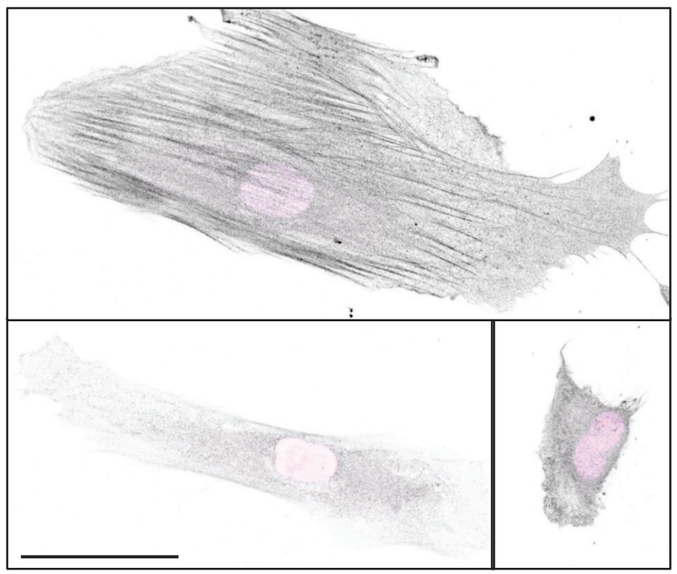
Figure 1. CAFs influence the behaviour of cancer and endothelial cells. Cartoon showing that cancer associated fibroblasts (CAFs), particularly those that secrete abundant ECM, influence various aspect of tumour development. Our works have shown that CAFs regulate mechanisms such as (from left to right): growth and invasive behaviour of the cancer cells, recruitment of immune cells into the tumour from the circulation, sprouting angiogenesis, cancer cell intravasation from the primary tumour to form distant metastasis.

confers the ability to the endothelium to interact with monocytes, which influence aspects of tumour progression, including antitumor immunity and metastasis. We therefore discovered a novel mechanism that could be targeted in CAFs to oppose the formation of tumour-promoting microenvironment (Santi *et al.*, 2023, *BioRxiv*).

CAFs & metabolism

Altered metabolism is a hallmark of cancer. In the last few years, it has emerged that, in addition to the metabolism of cancer cells, the metabolism of stromal cells is also an important regulator of cancer pathology (Kay *et al.*, 2023, *Curr Opin Biotechnol*; Kay *et al.*, 2021, *Front Oncol*; Kay & Zanivan, 2021, *Curr Opin Syst Biol*). Epigenetic regulators, such as histone acetylation and methylation, play major roles in determining cell phenotypes and functions, including in CAFs. An interesting aspect of cell metabolism is its link to epigenetics, as it provides acetyl and methyl groups as substrates for histone modifications. We found that CAFs produced high levels of acetyl-CoA, a source of acetyl groups for protein acetylation, and that this triggered the activation of a transcriptional programme resulting in the production of tumour-promoting ECM (Kay *et al.*, 2022, *Nature Metabolism*). We are now further investigating the potential of targeting mechanisms activated downstream of acetyl-CoA production in CAFs to block tumour development.

Figure 2. CAFs have different phenotypes. αSMA staining (marker of myCAF) of patient-derived HGSOC CAFs in culture suggesting that the bigger cell is a CAF with a myCAF phenotype while the elongated one is a CAF with an iCAF phenotype. Scale bar = 50 µm.



OmGel: a clinically relevant tool to study CAF biology

In patient samples, CAFs have different phenotypes and functions (Figure 2), some of which are interchangeable. A key example are myCAFs, which produce abundant ECM (Figure 1), and iCAFs, which have an inflammatory gene expression signature. Not much is known about iCAF functions in cancer because of the difficulty to maintain their phenotype in normal culture conditions. In collaboration with the Salo team at the University of Helsinki, we have developed omentum gel (OmGel), a clinically and physiologically relevant ECM made from the omentum (a major organ where HGSOC cells metastasise and that is removed during debulking surgery) of patients with HGSOC (Neilson, Cartwright *et al.*, 2023, *Matr Biol Plus*). We showed that OmGel has unprecedented similarity to the ECM of HGSOC tumours and that it supports HGSOC cells' invasive behaviour. Importantly, CAFs cultured with OmGel maintained an iCAF phenotype. Therefore, OmGel will uniquely enable us to study iCAF functions to advance our knowledge on their role in cancer.

News

This year, Paula was selected to present her work on the role of EP300 in CAFs as oral presentation at the EACR Cancer Metabolism Conference in Bilbao and Emily has been invited to presented her work on the role of proline metabolism in the tumour microenvironment at the Scottish Metabolomics Network Annual Symposium.

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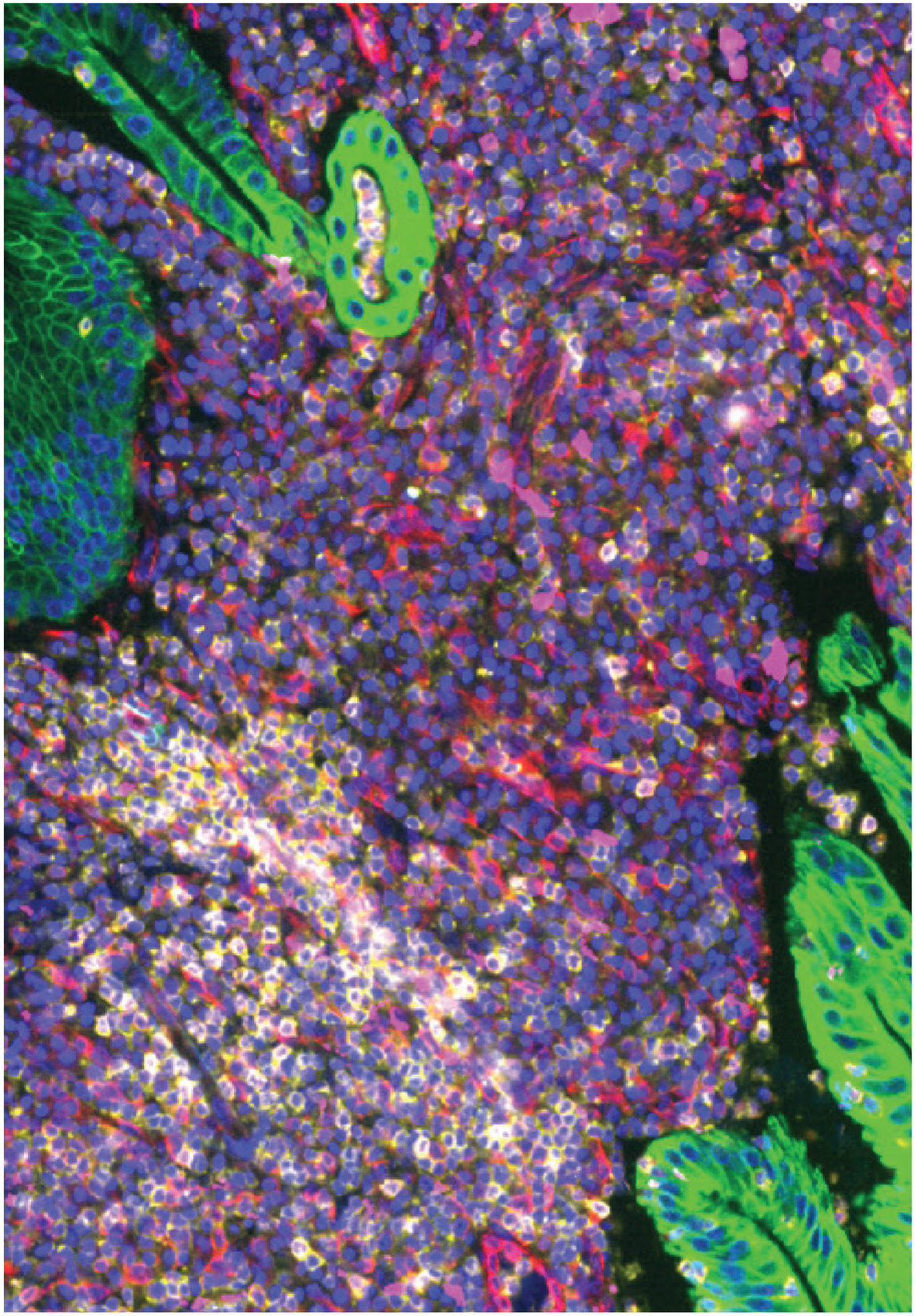

ADVANCED TECHNOLOGIES

TRANSGENIC MODELS OF CANCER

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Our lab strives to recapitulate human cancer in preclinical mouse models and interrogate all aspects of disease progression within a biological context. With the ultimate aim of identifying novel therapeutic approaches for patient benefit, we use physiologically relevant models to validate *in vitro* discoveries. This involves state-of-the art genetic, and refined transplantation models, often in combination with *in vivo* imaging modalities, which allow us to study how oncogenic pathways, altered metabolism and the tumour microenvironment contribute to cancer, and how these can be exploited for earlier detection and therapeutic gain.

Modelling cancer *in vivo*

The Institute is internationally renowned for scientific excellence using mouse models of cancer in a physiologically relevant way to gain insights on complex human diseases. This is important considering that tumour cells exist in a highly dynamic microenvironment which involves an intricate crosstalk between tumour cells and their neighbouring tissue compartments. Cancers spontaneously grow at their site of origin, invade surrounding tissue, and colonise distant organs which occurs through a complex array of processes that are distinct between different tumour types. Studying this multifaceted behaviour in a dish has limitations and requires advanced models in which tumours arise and mature in their natural environment. In this way, tumour cells directly and spatially co-evolve with stromal fibroblasts, immune cells, and the endothelium, recapitulating a more accurate tumour microenvironment, while being exposed to metabolic limiting conditions, and must negotiate biological barriers in order to metastasise. Many anti-cancer drugs, although effective in simplified tissue culture models, fail in the clinic because the nuances of taking these drugs into the whole animal setting cannot be ignored. Our lab utilises genetically engineered mouse models (GEMMs) with the same genetic alterations present in human cancers and share the same pathology and metastatic spread seen in patients. We have expertise in orthotopic xenograft and syngeneic models permitting the interrogation of tumour cell/immune interactions. Monopolising these preclinical models, in combination with *in vivo* imaging, our lab collaborates with colleagues to translate *in vitro* discoveries.

Research Collaborations

The lab is involved in diverse projects across all strategic themes of the Institute, from probing metabolism as a cancer vulnerability to studying the interplay within the tumour microenvironment, as well as modelling early disease. Targeting cancer cell metabolism offers an exciting therapeutic potential. A highlight this year was a collaboration with Saverio Tardito's lab (Ackermann *et al*., 2024, *EMBO Mol Med*) showing that selenocysteine production promotes lung metastasis in a model of triple-negative breast cancer (TNBC). TNBC cells evade ferroptosis, a lipid peroxidation-driven cell death, by secreting monounsaturated fatty acids. Inhibiting selenoprotein synthesis, reinstated ferroptosis and reduced lung metastasis *in vivo* (Figure 1). With Payam Gammage and his team, mutations in mitochondrial DNA were similarly shown to change the metabolic landscape, impacting the tumour immune microenvironment and revealing enhanced sensitivity to checkpoint blockade therapy in mouse models of melanoma (Mahmood *et al*., 2024, *Nat Cancer*). Also, in long-standing collaborations with Jim Norman's lab, neutrophil-specific uridine phosphorylase 1 (UPP1) and metabolic rewiring in breast cancer micrometastases reshaped the tumour microenvironment by influencing immune suppression, as well as extracellular vesicle production, and invasive microenvironments (Whyte *et al*, 2024, *bioRxiv*; Gounis *et al*., 2024, *bioRxiv*).

We have also been exploring the role of cancer-associated fibroblasts (CAFs) to drive metastasis in collaboration with Sara Zanivan's group. One such study showed that CAF-derived extracellular vesicles (EVs) deliver proteins to endothelial cells, modulating cancer, stromal, and immune cell interactions to influence tumour pathology (Santi *et al*., 2024, *Sci Signal*). In conjunction with Gareth Inman's lab, a novel TGF-β target gene, *C1orf106* (*INAVA*) was shown to drive tumour-promoting activities in breast cancer, enhancing migration, invasion, and tumour initiation (Strathearn *et al*., 2024, *Cells*). Collaborations with institute alumni also continue to yield fruitful results. With Mike Olson it was found that caspase-resistant ROCK1 prolongs survival in a B-cell lymphoma mouse model by creating a proliferation-suppressive bone marrow environment (Mardilovich *et al*., 2024, *Dis Model Mech*); while Karen Vousden's lab have shown how TIGAR modulates ROS dynamics to affect tumour behaviour and stromal interactions in pancreatic cancer (Cheung *et al*., 2024, *PNAS*).

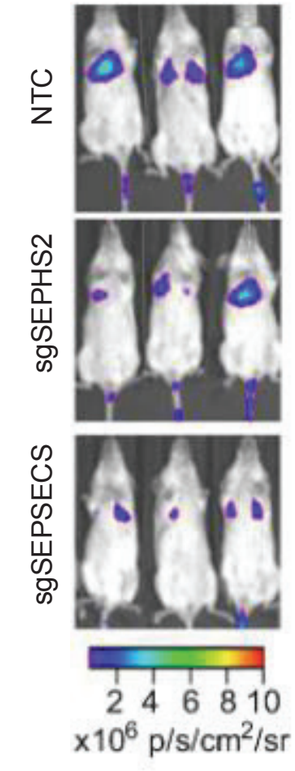

Figure 1. Representative IVIS images showing lung metastasis burden of MDA-MB-468 breast cancer cells post-intravenous transplant. Experimental groups include non-targeting control (NTC), sgSEPHS2 (targeting Selenophosphate Synthetase 2), and sgSEPSECS (targeting O-phosphoseryl-tRNA^Sec selenium transferase). Note the reduced tumour burden in the lungs of the targeted groups compared to the control group. Taken from Ackermann *et al*., *EMBO Mol Med* (2024): 16:2749-2774.

In a national collaboration with colleagues at Glasgow, Oxford, London and Belfast we co-lead the MRC's National Mouse Genetic Network (NMGN) Cancer Cluster (https://nmgn.mrc.ukri.org/clusters/cancer/), working closely with the Mary Lyon Centre at Harwell to develop and improve mouse models of human cancer. It is exciting to work within the multi-disciplinary network and capitalise on state-of-the-art expertise in Degron Technology, Home Cage Monitoring, and the microbiome to better understand diseases such as colorectal cancer, and to contribute to network-led initiatives training early career researchers and promoting responsible animal research (Sansom *et al*., 2024, *Cell Genom*).

Resources & News

Our lab is deeply committed to promoting Equity, Diversity, and Inclusion (EDI) and fostering a strong sense of community. This year, members of the team, led by Louise Mitchell (along with the School of Cancer Sciences VOICE Committee) have been actively involved in organising EDI activities, such as Ramadan Awareness, Neurodiversity in the Workplace, and a Diwali celebration. Jayanthi and Nimrit from the lab cooked up a storm, preparing gulab jamun for 100 people at our Diwali event! We were extremely proud of Louise being nominated for an EDI award and being asked to present these initiatives at a University of Glasgow EDI conference. The lab also hosted a masters student, Alifasya Baltimora, who undertook a 12-week research project investigating the role of CBFβ in breast cancer progression, working with Louise and PhD student Amy Lawlor.

This year, we invested in a VEVO Injection Mount, which will enhance our existing ultrasound system. This injection mount enables precise, minimally invasive delivery of cells/solutions to specific locations in murine models, eliminating the need for surgery. By providing high-resolution ultrasound guidance, the system improves cell transplantation accuracy, reduces animal stress, and shortens recovery times, supporting our commitment to the 3Rs (Replacement, Reduction, Refinement) and advancing the quality of our research.

BEATSON ADVANCED IMAGING RESOURCE

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[1]Joint Cancer Sciences Flow Cytometry Facility

Light microscopy and flow cytometry allow us to gather information about important regulatory mechanisms in tumours and the microenvironment. Using these techniques, we can simultaneously analyse large numbers of important molecules and cells with subcellular sensitivity and resolution in living samples whilst maintaining the context of the microenvironment, be that model substrate or living organism.

The Beatson Advanced Imaging Resource (BAIR) team works closely with the Institute's researchers to uncover and interrogate important molecular pathways in cancer. The BAIR is thus involved at some stage in nearly every study from researchers at the Institute that contains a light micrograph, or a flow cytometry plot or uses sorted cells for downstream analysis using one of the other advanced technologies. All of the beautiful fluorescence light microscopy images you see in this report were captured in BAIR. We are keen and able to assist from experimental design right through to the finished figures. We train scientists in all stages of modern cytometric and microscopical research, from advice and help with sample preparation, basic and advanced microscope and cytometer operation, and data acquisition through to quantitative image analysis and interpretation. At the start of a new project or application, we are enthusiastic to help researchers identify how our methods can be used to develop and test their hypotheses and help them to design experiments that make the most of our advanced instrumentation. We also identify and acquire new technology and methodology that allow our researchers to take the most elegant approaches.

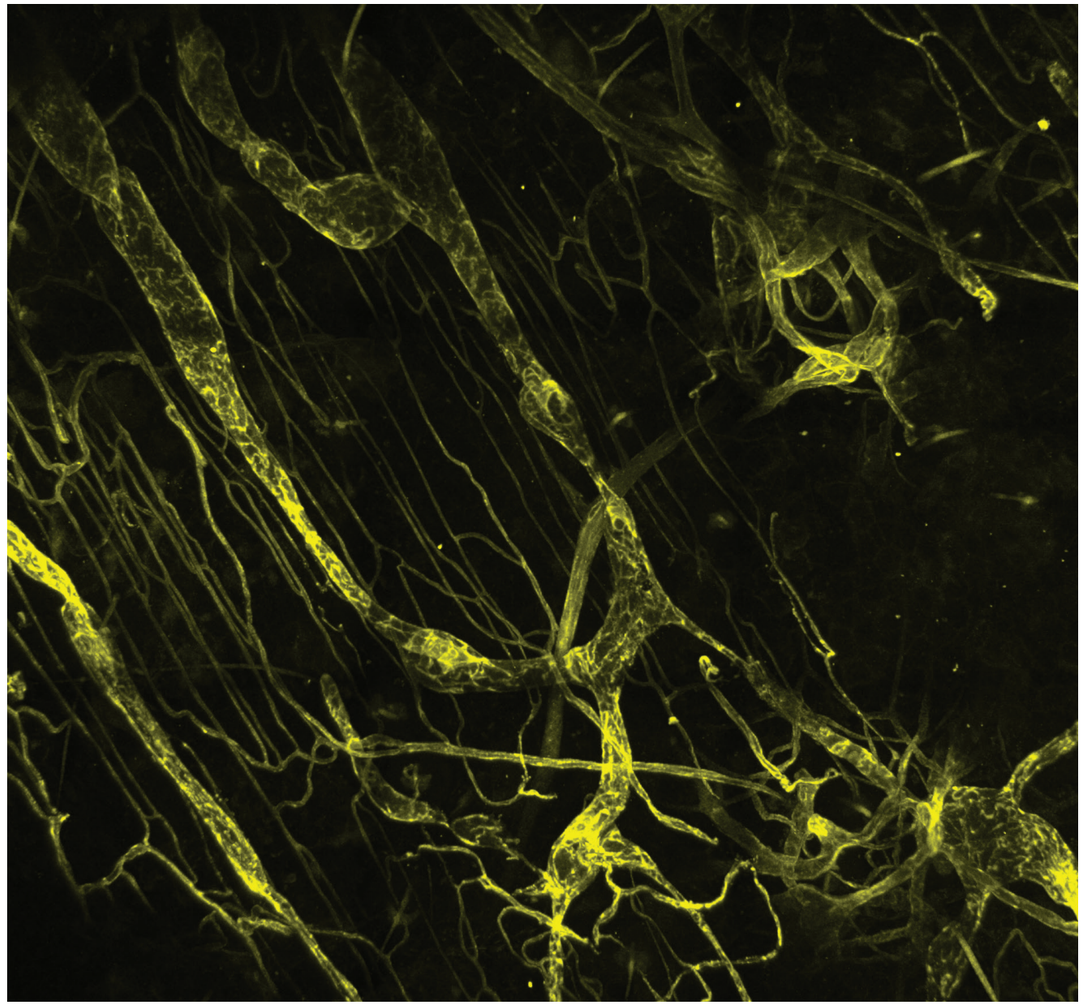
Imaging across different spatial and biological complexity scales

We have the expertise and instruments to:

- Perform automated liquid / multi-well plate handling and very high-throughput imaging experiments to analyse cell behaviour over thousands of experimental conditions via high-content imaging
- Image, spatially separate, and quantify up to eight markers simultaneously in thick tissue (3 and 4D) by combining fluorescently labelled antibodies and probes with label-free approaches (e.g. second harmonic generation to look at fibrillar collagen) using tissue clearing, multiphoton excitation and spectral imaging
- Image cell behaviour over several days in tissue culture incubators
- Address the physicochemical environment, molecular activity, and signal transduction of pathways below the diffraction limit at different spatiotemporal scales using FLIM, FRET and super-resolution imaging
- Monitor cell function in intact living organisms via advanced intravital microscopy
- Address multiplexed panels of up to 45 markers in liquid phase and dissociated tissue samples by flow cytometry and sort cell populations (with a smaller number of markers) for downstream analysis (with the joint Cancer Sciences Flow Cytometry Facility)

In this way, we underpin cancer research at the Institute, UoG School of Cancer Sciences and beyond by allowing our researchers to work up and down the biological complexity scale, taking the best and most important aspects of different models and patient samples and combining them into a larger more complete picture.

This year we were delighted to welcome Beatrice Bottura to the team and that Claire Mitchell and Tom Gilbey were both promoted to Principal Scientific Officer at the Institute, well done and well deserved. As noted in the Leukocyte Dynamics Group report, the BAIR team were instrumental in my own promotion to Senior Staff Scientist.



Expansion Microscopy of Drosophila Brain - *Nikki R. Paul and Jack Holcombe*

MOLECULAR TECHNOLOGY

Head
Graeme Clark

Senior Scientific Officers
Jillian Murray
Andrew Keith

Affiliate support scientists
Abbie McFarlane
Anna Shearer

The Molecular Technology Service offers a diverse range of services to individuals and research groups throughout the Institute. The main focus of the facility has centred around the continuing expansion of our Next Generation Sequencing (NGS) services, allied to which, we also offer a range of Single Cell services predominantly focussing on single cell RNAseq. We process samples for a variety of cancer associated projects, in both mouse and human derived materials. We offer a full end-to-end service, from initial study design & planning, through sample QC, full library preparation, sequencing and data return. Allied to these advanced techniques, we continue to offer a range of standard molecular tests covering, plasmid purifications, Sanger sequencing and mycoplasma screening.

The facility's core aim is to provide an efficient and adaptive service to research groups and researchers within the Institute. We have gradually established a core offering centred around NGS, which covers bulk RNAseq, total RNAseq, ChIPseq, RRBS, amplicon-based and whole exome preparations, and are continually striving to add more assays and tests which can be readily utilised by groups across the Institute. We routinely undertake early preparatory meetings with researchers prior to proceeding with NGS based projects and conduct these alongside colleagues from the Bioinformatics & Computational Biology core group. These joint meetings enable us to thoroughly understand the research goals of specific projects and give appropriate advice in order to meet both the experimental and analytic goals of the project.

A major advancement for the service during 2024 was the installation of an Illumina NextSeq 2000 benchtop sequencer. This new instrument was purchased to replace our aging NextSeq 500 instrument which had served well for around 10 years. The introduction of the NextSeq 2000 has given us increased flexibility and enhanced our sequencing capacity significantly. Previously, we were restricted to only a relatively small number of run variants (i.e. only 5 run variants over 2 flow-cell types) which could generate a maximum data output of 120Gbp per run, whereas with our new system we have a much greater number of potential run variants (i.e. 14 in total over 4 flow-cell types) at our disposal, and can now generate up to 600Gbp of data from a single run. Further to this increase in flexibility, the increased capacity has also meant that we can now sequence larger projects more efficiently and economically in-house than previously possible. We have also been able to multiplex projects using the same base assay, which has ultimately had the benefit of increasing turnaround times and reducing sequencing cost liability to the research groups. Up until late September 2024 we had been utilising a locally managed NovaSeq 6000 for larger scale sequencing (principally scRNAseq libraries), this access was removed for reasons outside of our control, and having the NextSeq 2000 in place meant we could now easily cope with sequencing most of these library types in-house, and in most cases, with cost savings associated.

In terms of sequencing content, over the period of 2024 we processed ~110 sequencing runs, covering all three sequencing platforms, with a combined output of ~18Tbp. As an example of our sample throughput, we performed full bulk RNAseq library preparations for more than 1000 samples throughout the year and have also regularly performed a range of other preparations, principally on an ad-hoc basis. We see this throughput increasing steadily year-on-year.

A further development through 2024 is the full integration of the single cell sequencing service into Molecular Technologies, and this is now representing one of our key service areas. The solutions on offer generally cover single cell gene expression analysis (10X Chromium), whereby the generated data can allow researchers to measure gene activity on a single cell basis, and aid in characterising tumour environments through identifying specific cell populations and cell types in diseased versus normal tissues. As with the NGS assays, we are continually striving to add more assays to our service repertoire and have been expanding services this year to include immune profiling capabilities, multiome assays, and gene expression flex assays. The gene expression flex assays represent an exciting area for further establishment, as these assays can potentially open the use of archival and low-quality samples which previously would have been incompatible with the technology (particularly archival FFPE tumour material). In terms of sequencing needs for the Single Cell service, previously we relied on outsourcing this element as the in-house options were insufficient and uneconomical to process such libraries. However, the introduction of our NextSeq 2000 has meant that we can now efficiently process all Single Cell libraries in-house, which has greatly sped up turnaround times and given us control of the complete end-to-end service. Similarly, we work closely with colleagues in the Bioinformatics core group (Y90), who support us with running primary analysis, which forms part of the data deliverables for service users, and is essential to maintain performance thresholds, and allows us to identify any potential sample-sample or run-run variability. Over the course of 2024, we processed and sequenced approximately 200 samples through the single cell service. We are also continuing to explore different technologies in this field, with the aim to offer the most appropriate assay tailored to samples/projects' specific needs and be vendor agnostic. It is likely this will require a degree of benchmarking, which is currently in early planning stages.

Finally, our facility is maintaining the more traditional aspects of our service provisions, which includes plasmid DNA purifications (mini/maxi-prep), collating and administering Sanger sequencing send-outs (including cell-line authentications), and performing Institute-wide mycoplasma testing. These functions still represent a significant portion of the facility's output with several thousand samples processed annually. We have been fortunate to have been able to maintain these services with the assistance of colleagues from the Lab Support team (Abbie McFarlane & Anna Shearer) which has enabled service provision continuation whilst allowing for developments in our core focus areas.

Figure 1. Example NextSeq 2000 primary data analysis, as viewed utilising Sequencing Analysis Viewer (SAV, Illumina). Represents key primary analysis data QC performed by MTS staff prior to full data analysis/data return.

DEEP PHENOTYPING ADVANCED TECHNOLOGY FACILITY

Head
John Le Quesne[1]
RCPath

John Le Quesne[1]
Leah Officer-Jones[1]
Silvia Martinelli[1]
Fiona Ballantyne[1]
Rachel Pennie[1]
Kara Luckett[1]
Cat Ficken[2]
Lucy Hillary[3]

[1]Le Quesne group funding (Mazumdar Shaw Chair Endowment)
[2]CRUK programme grant
[3]UoG integrated MSc placement

Our team design assays to answer scientific questions via the generation of data-rich images and bespoke image analysis methods to measure gene expression at multiple levels in intact tissues. We combine experience in the use of antibodies and RNA detection technologies to develop assays which probe biological areas of interest. This is achieved by working closely with our collaborators, which is essential to obtain the desired results from these challenging methods.

We routinely develop *in situ* assays to answer questions about the spatial context of the tumour microenvironment, including the immune system, plasticity of epithelial cells and fibroblasts, and cell-cell interactions.

Progress 2024
Key technologies

Multiplex Immunofluorescence

Using our Ventana Discovery Ultra autostainers in combination with the Akoya PhenoImager HT, we routinely detect up to 6 genes of interest on multiple tissue sections including tissue microarrays (TMAs), allowing us to answer complex questions about tumour biology in a high-throughput manner. We are leaders in this technology, and have collaborations with leading industry providers as well as numerous academic partners. We believe multiplex assays will form the basis of the next generation of clinical biomarkers, and are working towards translating our assays into a clinical setting.

Over 2024, we have worked with 13 research groups with this technology, and we have several manuscripts in preparation. We have developed panels for human prostate cancer (Leung, Campbell), human pancreatic ductal adenocarcinoma (Chang), human lung adenocarcinoma (Le Quesne, Bushell, MacVicar), human colorectal cancer (Edwards, Roxburgh), human breast cancer (McPherson), mouse models of hepatocellular carcinoma (Bird), cholangiocarcinoma (Braconi), and both human and mouse models of malignant mesothelioma (Chalmers, Murphy). A manuscript featuring our work from the Bird lab "Human-correlated genetic models identify precision therapy for liver cancer" has been accepted by Nature.

High Plex Immunofluorescence

Using our Akoya PhenoCycler Fusion, we can detect up to 100 proteins of interest within tissue spatial context. Our expertise with this platform has led to several large in-depth phenotyping collaborations, including our involvement in the SAMBAI Cancer Grand Challenge. This project is a multi-centre international effort to tackle cancer inequities in minority and socially deprived patient populations. In addition, we have collaborations with the Pearson lab at Cardiff University, focussing on fibroblast subtypes in human prostate cancer, and locally with the Chang, Inman and Le Quesne labs focussing on fibroblast subtyping and immune phenotypes in human malignancies.

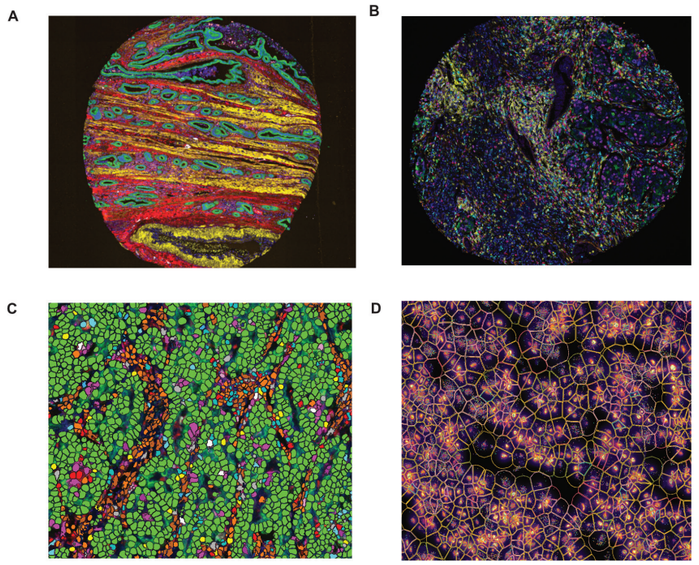
We have a methods paper detailing our in-depth validation protocol in process, and our custom PDAC panel has been presented at several meetings. This panel of 43 markers simultaneously detects expression of numerous markers of epithelial and fibroblast plasticity, new therapeutic targets, and highly detailed immunophenotypes. A murine version of this panel is in preparation.

Xenium

Our 10x Genomics Xenium platform enables detection of up to 5100 genes of interest in a single tissue section. The custom gene expression chemistry has been used by our collaborators to develop panels detecting multiple species simultaneously in parasitically infected tissues, and also to detect single nucleotide variants. Furthermore, the Xenium can be combined with PhenoCycler Fusion, enabling multi-omic detection of RNA and protein within the same tissue section. Of note, the Xenium is part of our SAMBAI cancer grand challenge work package, and data from our first mouse custom gene expression panel has been submitted to Nature by the Sansom lab.

Figure 1. Deep phenotyping imaging laboratory.

Figure 2. A) PhenoCycler Fusion assay applied to pancreatic ductal adenocarcinoma. B) Multiplex immunofluorescence assay applied to lung adenocarcinoma. C) Visiopharm cell segmentation applied to cholangiocarcinoma. D) Xenium gene expression panel applied to colorectal cancer.



Visiopharm

Visiopharm is our image analysis platform of choice. We have developed bespoke deep learning pipelines for several diseases enabling high throughput analysis of the images generated by our spatial platforms. We offer training for collaborators wanting to generate their own data, or we design custom workflows and run them as part of our deep phenotyping service. We are in the process of writing a manuscript for a novel deep learning classifier we have developed using the deep learning tools within Visiopharm, which accurately predicts cellular phenotypes, without the need for accurate cell boundary classification.

Outreach

The Deep Phenotyping team actively contribute to public engagement events, developing interactive displays and games to convey our research to the wider public. This year we took part in the CRUK Parliamentary reception, engaging with MSPs about our multiplex and AI applications, and the future integration of these technologies into a clinical setting. We have hosted several visits from potential philanthropic donors, Prostate Cancer UK and the Beatson Cancer Charity, and politicians including the Minister for Public Health and Women's Health, Jenni Minto. The Deep Phenotyping team took part in a "50 years of Cancer" donor event hosted by the School of Cancer Sciences in the University of Glasgow.

Our team provide training and learning opportunities for students of all ages. Two of our scientific officers took part in a careers workshop at the University of Glasgow, leading to a 1-year MSci internship. We have also hosted 2 students for work experience placements, taken part in the CRUK SI work experience week, and the CRUK SI high school open evening. In addition, we were involved in a Nanobiology student visit, hosting students from the Delft University of Technology and Erasmus Medical Centre, Rotterdam.

We hosted three MSc students in the Deep Phenotyping facility for their summer projects, one of which is now a scientific officer within our team. We are becoming increasingly involved in international training programmes due to our experience with both high plex assays and Visiopharm image analysis. This year we hosted a PhD student from the Instituto de Biología Molecular y Celular del Cáncer in Salamanca on a 3-month EACR/Worldwide Cancer Research internship focussed on the application of a multiplex immunofluorescence pipeline to murine models of prostate cancer. This was featured in EACRs Cancer Researcher magazine.

We host 6-monthly spatial technology events with industry sponsorship, increasing engagement with advanced spatial technologies within the institute. This year, we hosted one event sponsored by 10x Genomics, featuring talks on novel AI applications, spatial phenotyping, and multi-omic approaches alongside talks from Xenium experts. We also hosted an event sponsored by Biotechne featuring talks from experts in *in situ* hybridisation applications within Glasgow and from the CRUK Cambridge Institute alongside talks from Lunaphore and Biotechne.

TRANSLATIONAL MOLECULAR IMAGING

Head
David Lewis

PET Chemists
Gavin Brown[1]
Dmitry Soloviev[2]

Senior Image Analyst
Algernon Bloom[3]

[1]CRUK Scotland Centre
[2]University of Glasgow
[3]CRUK Radiation Centre of Excellence

Translational Molecular Imaging (TMI) develops novel imaging technologies and acts as a regional hub for molecular imaging research. Operating over three sites: the CRUK Scotland Institute, the West of Scotland PET Centre at Beatson Cancer Hospital and the Scotland Total-Body PET Facility, jointly managed with the University of Edinburgh. Our facilities house state-of-the-art PET radiochemistry and imaging equipment. Within the TMI, there is expertise in several key areas including PET chemistry, preclinical PET/MR imaging, clinical imaging and advanced image analysis. The TMI drives collaborative imaging research across our networks with a focus on developing and applying innovative imaging technologies, such as new PET radiotracers and MRI methodology for visualising cancer biology.

Projects in the TMI range from standard imaging studies where we facilitate access to imaging technology to much wider scale projects where the TMI acts as a collaborative partner in, for example the development of novel imaging agents or *in vivo* molecular phenotyping of new genetically engineered mouse models. The unique research environment at the CRUK Scotland Institute enables collaboration using its world-class cancer models to develop imaging biomarkers for new applications in tumour classification and personalised cancer therapy.

PET radiochemistry

We provide expertise and technology for the versatile development and labelling of a whole range of PET-labelled molecules. We have negotiated a Collaboration Agreement with the local Greater Glasgow and Clyde Health Board for open access to Glasgow's PETtrace 800 16.5MeV cyclotron, based at the PET Radiopharmaceutical Production Unit (RPU) in the West of Scotland PET Centre. We provide a range of radiolabelling collaborative opportunities around rapid carbon-11 and fluorine-18 labelling of novel PET radiotracers on our two automated multipurpose Synthra radiosynthesizers. These laboratories have the full range of radio-analytical equipment including a gamma-spectrometer, four HPLC instruments, gas-chromatography systems, radio-TLC scanner and pH-meters. Access to cold chemistry for precursor synthesis is by collaboration with the School of Chemistry (Sutherland). Since 2017, we have synthesized 18 PET radiotracers and we have developed five carbon-11 synthons (^{11}CO, $^{11}CO_2$, $H^{11}CN$, $[^{11}C]CH_3I$ and $[^{11}C]$methyltriflate) with one more in development ($[^{11}C]$formaldehyde). This provides one of the most versatile radiolabelling laboratories in the world for carbon-11 development.

We have continued to support the extensive imaging programmes in the TMI with radiotracers such as $[^{11}C]$acetate, $[^{18}F]$fluoro-ethyl-tyrosine (FET), $[^{18}F]$terafluoroborate (TFB), $[^{18}F]$fluorodeoxyglucose (FDG), $[^{11}C]$methionine, (4S)-4-(3-$[^{18}F]$Fluoropropyl)-L-glutamate (FSPG), $[^{11}C]$leucine and $[^{11}C]$nicotinamide. To support our collaborative partners at the Edinburgh Imaging Facility, we have enabled radiosynthesis and quality control methods for production of $[^{18}F]$fluoroproline and $[^{18}F]$LW233 for on-going preclinical studies. These tracers, which target collagen synthesis and translocator protein (TSPO) respectively, are now available for cancer imaging studies in Glasgow.

In 2024, in collaboration with the University of Edinburgh we helped establish the UKRI/MRC Scotland Total-body PET Facility. This is now a national PET imaging facility, one of only three total-body PET scanners in the UK. This successful award also granted us inaugural membership in the national PET imaging platform (NPIP) and as a result, the Translational Molecular Imaging Facility is engaging in collaboration on new national projects focusing on total-body PET development. Additionally, this grant is supporting three new positions in radiochemistry and image analysis.

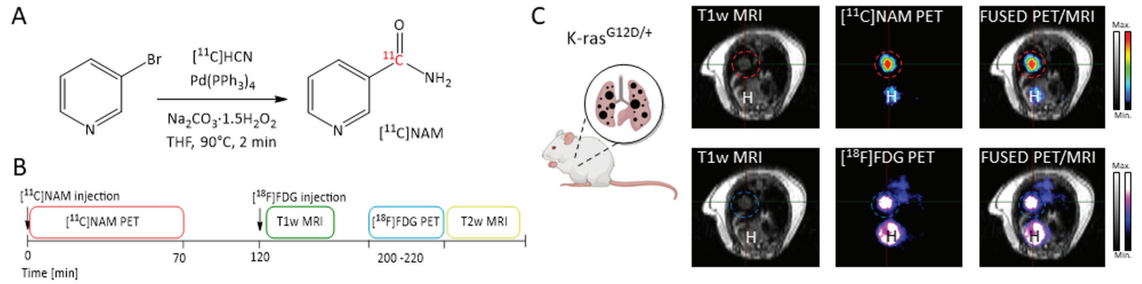


Figure 1. Tracing proliferating potential of lung tumours using $[^{11}C]$Nicotinamide. (A) Synthesis of [carboxyl-^{11}C] nicotinamide from 3-bromopyridine and hydrogen $[^{11}C]$cyanide by palladium catalysed reaction. (B) Dual-tracer sequential imaging protocol with $[^{11}C]$nicotinamide and $[^{18}F]$FDG. Dynamic PET was acquired between 0 and 70 min post-injection of 35 ± 5 MBq of $[^{11}C]$NAM per mouse and static PET was acquired between 80-100 min post-injection of 10 ± 2 MBq of $[^{18}F]$FDG/mouse with anatomical T1 and T2-weighted MRI. (C) Example of transversal T1-weighted MRI images, and PET images for $[^{11}C]$NAM (tumour circled red) at 30 min p.i. and $[^{18}F]$FDG (tumour circled blue) at 90 min p.i. in a K-ras$^{G12D/+}$ lung cancer model (n = 4 mice). H indicates $[^{18}F]$FDG PET signal from the heart.

Preclinical and translational imaging

In 2024, we supported research at our sister Institute at CRUK Cambridge to develop optimal timing and imaging of $[^{18}F]$FDG in tumours models (Hesketh *et al.*, 2024, *Mol Imaging Biol*) and optimising image co-registration methods (Lefebvre *et al.*, 2024). The latter was part of a series of UK-wide projects developed through the CRUK Radiation Centres of Excellence (RadNet) programme. We have also supported Thomas Bird's group to characterise mouse models of hepatocellular carcinoma (HCC) (Muller *et al.* 2025, in press). Building on collaborative work with Saverio Tardito, we continued development of $[^{11}C]$nicotinamide, vitamin B3, in this case as a biomarker of highly proliferative lung cancer (Figure 1).

BIOINFORMATICS AND DATA SCIENCE

Head
Crispin Miller

Scientific Computing Specialist
Naveed Khan

Bioinformaticians
Beto Bermudez Barrientos
Robin Shaw

Software Engineers
Mayank Sikarwar
Ifedayo Ojo

The variety of data generating platforms within the Institute make it possible to generate 'deep tissue phenotyping' data in which different modalities combine to provide a more holistic view of tumour tissue. The Unit provides support across the Institute for the analysis of this diversity of data. A major aspect of our work is to develop the data management strategies to deal with the high volumes of multimodal and imaging data.

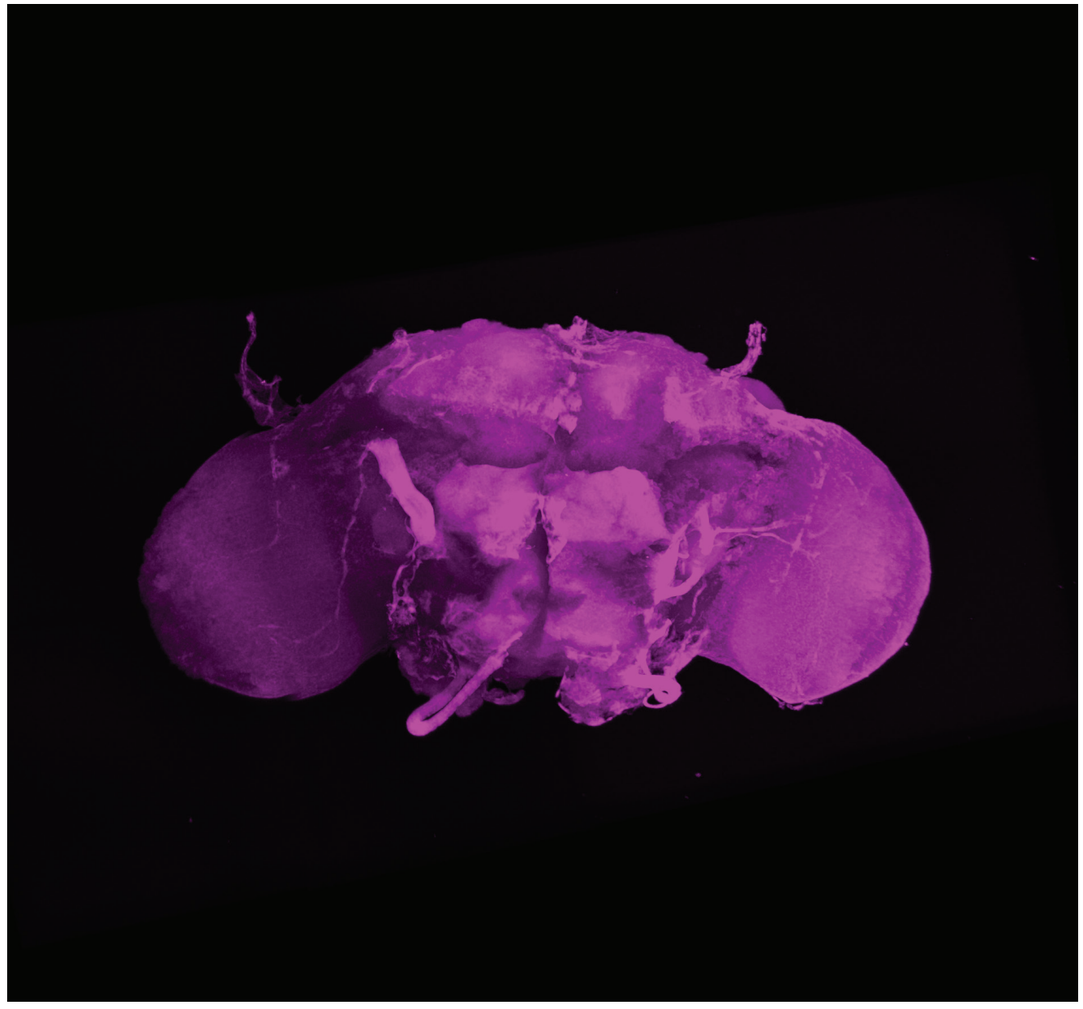
Our ultimate goal is to provide insights that enhance our understanding of cancer biology. The need for DNA and RNA sequencing analyses has continued to grow, and this has been accompanied by continued interest in using computational and machine learning approaches to interpret imaging and proteomics data. The Institute has access to a range of spatial transcriptomics platforms including Xenium and CosMX, and the data management and analysis demands of these platforms is becoming increasingly challenging, not least because of the volumes of data they produce. In collaboration with the Computational Biology Group, we are developing Next Flow workflows to support the systematic processing and management of spatial transcriptomics data.

Advances in technology are leading to a rapid increase in the size of the data we are analysing, leading to significant increase in our computing requirements. Naveed has commissioned a High Performance Computing (HPC) system that combines conventional processing with GPUs and a fast filesystem in order to support our data science, AI and deep learning needs. Naveed is also working closely with IT Services on the provision of Virtual Machines (VMs) to support non-HPC tasks. Robin and Naveed are also working together with IT services to standardise data processing workflows through our computing platforms and the different filesystems within the Institute.

Mayank and Ifedayo are working together to generate additional software to tools to support data sharing according to FAIR principles (that data should be **F**indable **A**ccessible **I**nteroperable and **R**eusable) and are developing NextFlow workflows to provide standardised analysis workflows for a range of modalities in addition to the spatial transcriptomics platforms described above. This is also exploiting synergies between the needs of the CRUK PREDICT-Meso accelerator and parallel needs across the Institute and the CRUK Scotland Centre. To this end Ifedayo is working with a team from the West of Scotland Safe Haven and NHS Greater Glasgow and Clyde.

Data analysis and modelling is performed using a variety of open-source software environments, programming languages and scripting tools, including Python, R, Bioconductor, Bash, PHP and Perl. We frequently make use of analytical routines that have been developed in-house, and/or in collaboration with our colleagues from the areas of mathematics, statistics, computer science and biology. We use a mixture of academic software tools for functional annotation, clustering, enrichment, ontology and pathway analysis, as well as commercial tools.

The unit also provides support and guidance to graduate students and postdocs in other research groups who are using computational approaches to analyse their data. This includes advice on R scripting (by appointment), experimental design, and data presentation. Our team also participates in delivering part of the Cancer Research & Precision Oncology MSc programme at the University of Glasgow.



Expansion Microscopy of Drosophila Brain - *Nikki R. Paul and Jack Holcombe*

HISTOLOGY

Head
Colin Nixon

Ashleigh Prosser
Barbara Cadden
Emma Paterson
Gemma Thomson
Mark Hughes
Rachael Whitelock
Saira Ghafoor
Shauna Currie Kerr
Sophie Mclaughlin
Vivienne Morrison

Histology performs processing of tissue and cellular material from the wide range of cancer models developed within the Institute. This allows material to be evaluated at a cellular level using an array of specialised histological techniques providing insight into the mechanisms of cancer and tissue structure.

The service offers processing for tissue samples fixed in different types of fixative dependent on subsequent/preferred analysis producing a paraffin embedded block. Once received, tissue samples are trimmed, appropriately processed and orientated into paraffin wax blocks to facilitate tissue sectioning and staining/analysis. The tissue samples are processed according to type and necessity using specialised processing cycles. We have four large capacity automated tissue processors allowing large scale consistent processing, but when required, specialised processing cycles can be designed. Other material such as agar plugs, cell pellets, drosophila, embryo's, organotypic assays and spheroids can be processed to produce a paraffin block allowing sectioning and investigation. All paraffin blocks sectioned are stained with haematoxylin and eosin providing general analysis of cell morphology and structure. After initial analysis, more specialised histological stains/techniques can be performed to investigate specific tissue structures.

Where fixation is not required or disadvantageous to tissue structure and analysis, the facility offers a frozen section resource. Cellular material, drosophila, embryos and tissue can be sectioned on a cryostat and stained using histological stains, immuno-histochemical/immunofluorescence staining methods or *in-situ* hybridisation techniques.

A comprehensive immunohistochemistry service is offered. The histology service has a large repertoire of previously validated antibodies that can be stained on our autostainers providing consistent high-quality staining. We continually look to expand the number of optimised antibodies to keep pace with the researchers' demands and up to date with relevant wider areas of interest. New antibodies can be provided for optimisation on our autostainers by researchers at any time. Immunohistochemical training can be provided in order that an individual scientist can understand the rationale and techniques available allowing them to perform the staining to an acceptable and consistent standard.

Where there is no antibody available for immunohistochemical analysis or a more specific conclusive technique is required, the service provides an *in-situ* hybridisation technique using a reagent system designed to visualise cellular RNA targets using bright-field or fluorescent microscopy. This technique can be performed for single, dual or multiple staining of targets on formalin-fixed paraffin-embedded sections, cellular material sections, cytospin preparations, drosophila or frozen tissue sections. The staining for this technique is performed on a Leica Bond Rx autostainer. Specific probes can be purchased or designed to exact specifications by the researcher, allowing the *in-situ* technique to be undertaken. If a probe must be designed, prior consultation with the histology service is required to make sure the correct type of probe is designed.

Another *in-situ* hybridisation option offered is BaseScope where this technique can be used to label and visualise much smaller targets, around 50 – 300nt. This technique can be used for RNA mutations, exon junctions/splice variants, circular RNA and point mutations.

Where possible, we can look to combine immunohistochemistry and *in-situ* hybridisation to stain targets using both techniques on the same histology section.

The service offers a wide range of specialised histological stains such as Alcian Blue (+/-PAS), Elastin Van Gieson, Gram, Grimelius, Martius Scarlet Blue, Picro-Sirius Red, Retic, Toluidine Blue and TUNEL staining.

Material for DNA/RNA investigation, immunofluorescence staining (single and multiplex), PCR analysis and spatial transcriptomics can be sectioned from both paraffin-embedded material and frozen tissue. Histology staff are available to discuss beforehand whether paraffin embedded, or frozen tissue would suit an investigation best.

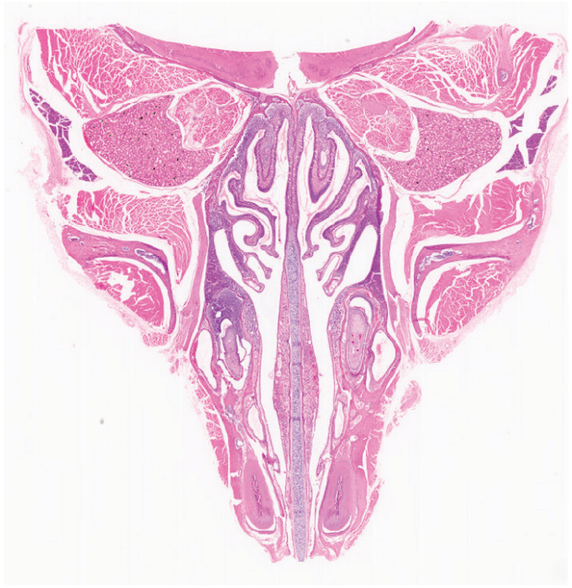
The histology service provides a slide scanning service using a fully automated large capacity Leica Aperio AT2 slide scanner which captures bright-field images. This allows high-quality digital images to be scanned, stored and if required, automated quantitative interpretation performed. For digital analysis, we offer access to Indica HALO™ image analysis software. This allows staining techniques to be scored using algorithms designed specifically for that staining result, using the researcher's input to designate which specific areas are to be scored. This produces accurate and reproducible scoring. The service provides full training regarding the software and modules available for the researcher to be able to use the image analysis software. Follow up support and assistance with the HALO software will be provided as required.

The Institute has a Leica LMD6500 laser microdissection system that allows subpopulations of tissue cells to be procured from histological prepared slides under microscopic visualisation. Tissue sections can be cut from both cryostat and paraffin blocks onto specialised slides, which can be stained appropriately allowing cellular material to be identified and separated to permit subsequent downstream analysis to be performed. Consultation regarding the downstream analysis is imperative prior to work beginning as this allows the correct protocols and procedures to be used to maximise the results obtained from the specific analysis required. Both DNA and RNA material can be retrieved from the tissue sections for downstream analysis.

If required, mouse tissue microarrays (TMA) can be constructed using paraffin-embedded tissue blocks to the researcher's requirements. We are also able to construct TMAs using material obtained from cell pellets. If a TMA is required, this can be discussed with histology staff on how to layout and orientate the tissue within the TMA. If the tissue or cell blocks supplied are suitable then a TMA can be built as agreed with a TMA map being created to allow the exact position of each tissue core to be known.

The service is also able to create multi-blocks specifically for use with CosMx, GeoMx or Xenium analysis. This can allow multiple pieces of tissue to be investigated on one section. These can be created where possible retrospectively from previously created paraffin blocks or fixed tissue waiting to be processed. Prior to any multiblock being created discussion and agreement on feasibility and layout must be discussed/agreed with histology staff.

H&E stain - mouse head



TRANSGENIC TECHNOLOGY

Head
Douglas Strathdee

Scientific Officers
Eve Anderson
Farah Naz Ghaffar

The Transgenic Technology Laboratory makes use of molecular genetic approaches to help understand gene function in the emergence and progression of cancers. We can accurately model changes in endogenous genes observed in human cancers by making use of technologies such as gene targeting or genome editing. By bringing together combinations of precise gene alterations we can generate sophisticated models of human cancers and help to understand how the combinations genetic changes contribute to the progression of the disease.

Embryonic stem (ES) cells have been valuable tools in helping with generating new models of cancer, as they have two key properties which facilitate this. Firstly, ES cells exhibit high levels of homologous recombination (HR), which allows us to precisely modify endogenous genes and insert mutations identical to those found in human cancers. Secondly ES cells will differentiate into a wide variety of cell types from different tissues. So once we have generated ES cells with the appropriate genetic alterations, we can then differentiate the ES cells into cells from the tissue of interest.

During the year, we have collaborated with other groups at the Institute to generate a wide variety of different types of genetically altered alleles. These included conditional knockouts, point mutations and inducible transgenes. Using this approach, we introduce mutations directly in the appropriate context of the endogenous gene, and this ensures that the changes we make directly imitate the mutations discovered in human cancers. Furthermore, we have been able to use these types of analyses to identify genetic modifiers that can play a role in the progression of the disease. Identifying such genetic modifiers not only enhances our understanding of the basic biology of the disease but in some cases can be potential therapeutic targets allowing the development of candidate disease treatments.

Making genome editing more effective for new allele generation

Increasingly, the adoption of genome editing by CRISPR-Cas9 technology has provided an attractive alternative to using ES cells as described above. Previously nucleases, such as zinc finger nucleases and TALENS were capable of accurate DNA modification, but were awkward to design and generate. As CRISPR-Cas9 is targeted by a short guide RNA (sgRNA), the design and application of this nuclease is much more straightforward. The application of the CRISPR-Cas9 system was further enhanced by the development of electroporation to deliver the reagents into zygotes for efficient genome modification. This allows direct introduction of CRISPR components along with donor template to initiate homology-directed repair (HDR) and directly produce correctly edited alleles.

Although these electroporation-based methods are very effective in introducing small deletions, insertions or substitutions at the site of DNA cleavage determined by the sgRNA, this method is restricted to short DNA modifications (typically <200 nucleotides) as the process relies on using single-stranded oligodeoxynucleotides (ssODNs) as repair templates. Microinjection of CRISPR-Cas9 reagents is an alternative to electroporation which can be used to generate knock-out mutations or in combination with ssODN or long single-stranded DNA (lssDNA) templates for the production of knock-in mutations. However pronuclear microinjection is technically challenging and is still limited by the synthesis limits of the lssDNA templates, as well as low efficiencies of accurate insertion of larger ssDNAs. So introducing larger and more complex DNA alterations, which can be accomplished using ES cells, cannot reliably be achieved by CRISPR-Cas9 methods

An alternative approach of HDR template delivery is to use recombinant adeno-associated virus (rAAV), which has emerged as a safe and effective gene delivery system with the inherent ability to transduce mammalian cells. Of note, rAAVs can also can stimulate gene targeting through promotion of homologous recombination. Naturally occurring serotypes of AAV can transduce cells and preimplantation embryos, and deliver the ssDNA genome to the nucleus with high efficiency. This approach was further improved by combining electroporation of CRISPR-Cas9 reagents as ribonucleoprotein with subsequent transduction using an rAAV encoding a donor template molecule which can achieve high targeting efficiencies.

In order to test if this approach would prove effective, we initially identified a suitable target locus. A fluorescent marker gene trap measure 4.4Kb of sequence was designed to target into exon 1 of a gene involved in human hepatocellular carcinoma (HCC; Fig. 1a). Prior to this experiment the introduction of sequence larger than 1kb by gene editing had been extremely challenging. An AAV1 virus was generated carrying the insertion sequence in two forms with the fluorescent marker in either the C-terminal or N-terminal orientation and was used in conjunction with four CRISPR guides located in the intronic sequence (Fig, 1b). Mouse embryos were generated by IVF and infected with either the C-terminal or the N-terminal rAAV for four hours prior to electroporation with the guides and Cas9 protein.

A total of 13 samples were obtained and screened for this project and, of these, seven were positive for insertion of the reporter gene, with six of these shown to be correctly inserted with the genome (Fig. 1c). Sequencing analysis of all six of these mice confirmed correct targeting of the entire transgene. Overall this experiment was shown to generate targeted alleles with nearly 50% knock-in efficiency. These data suggest that using an rAAV can work very efficiently for gene editing, and this method will allow larger and more complex genetic alterations to introduced into the genome.

Publications listed on page 130

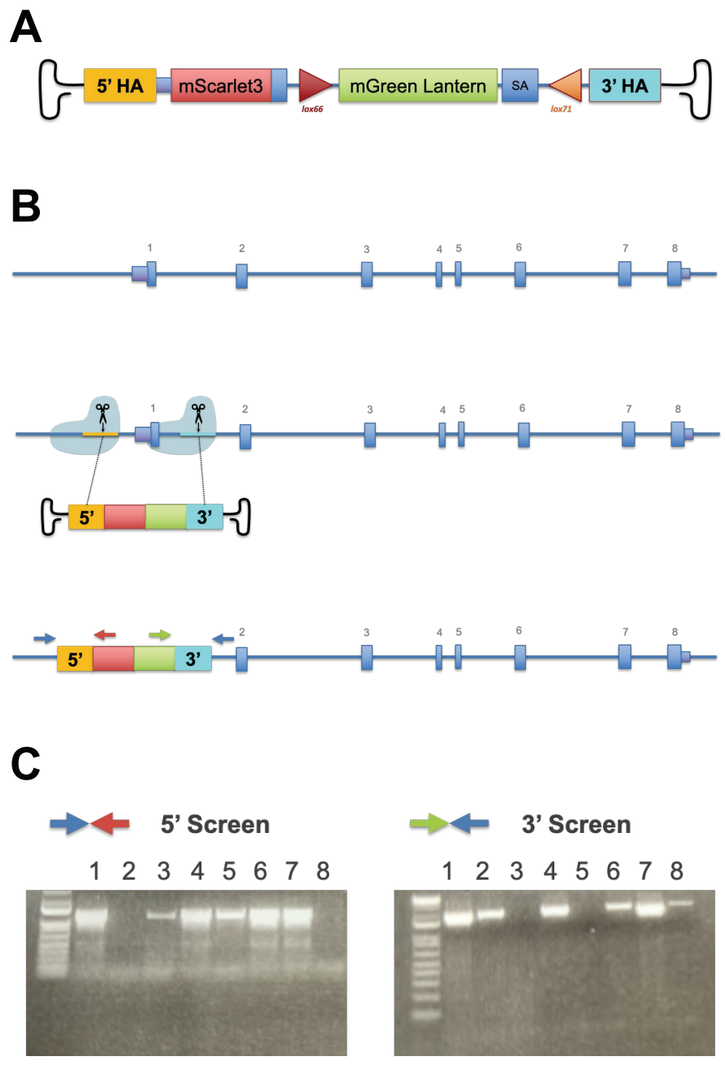


Figure 1. (A) Design of the rAAV1 used in a gene editing experiment. The AAV includes 5' and 3' homology arms along with mScarlet3 and mGreen Lantern fluorescent proteins (B) Inserting an rAAV1 into the target locus. Following cleavage of the DNA by the Cas9 RNP the rAAV genome is used as an HDR template inserting the fluorescent protein sequences into the gene of interest. Position of the screening primers is indicated on the modified allele. (C) Screening on the 5' (blue and red arrows) and 3'(green and blue arrows) sides of the homology arms. PCR screening from the endogenous gene sequence across the homology arms demonstrates that the rAAV1 sequences are inserted at the correct site.

METABOLOMICS

Head
David Sumpton

Scientific Officers
Alejandro Huerta Uribe
Engy Shokry

Metabolism is a centrepiece of cancer biology from its initiation, through its progression, to its response to treatment. The facility supports the Institute's research exploring the multiple roles of metabolism in cancer biology. We offer tailored support for the Institute's research projects, from experimental design to data analysis. Our well-established metabolomics platform uses state-of-the-art liquid-chromatography mass-spectrometry (LC-MS). Two Thermo Scientific Q Exactives instruments with high-resolution and accurate-mass are central for the targeted and untargeted analysis of the metabolome and lipidome of cells, tissues, and biological fluids. This platform is complemented by a Thermo Scientific Altis triple quad that broadens the sensitivity and specificity of the detection for specific metabolites of interest. In addition, an Agilent gas-chromatography mass-spectrometry (GC-MS) triple quad instrument provides complementary coverage to our LC-MS systems.

The facility's core aim is to provide access to state-of-the-art MS technology that is optimised for the detection of metabolites and lipids. We maintain and operate the instrumentation, providing both metabolite profiling and custom analysis when needed. We offer expertise and assistance in data analysis, data interpretation and experimental design. We run regular workshops to train users to carry out their own targeted data analysis. To learn as much as possible from the data generated, we also collaborate with users to make use of more complex untargeted analysis.

We work closely with many groups within the Institute who have interests in cancer metabolism and over the past year, we have continued to contribute to their research (see publications). A new and highly successful collaboration with Johan Vande Voorde (University of Glasgow) and Lars Vereecke (VIB Center for Inflammation Research, Belgium) led to the development of a method to measure Colibactin, using a byproduct of its synthesis as a proxy, N-myristoyl asparagine. Which in turn, allowed us to make a meaningful contribution to an important study demonstrating that Colibactin-driven colon cancer requires adhesin-mediated epithelial binding. Another highlight for us this year, was working with Saverio Tardito's group on the identification of lipids which contain monounsaturated fatty acids and their ability to act as anti-ferroptotic factors when secreted by triple-negative breast cancer cells into extracellular environment.

Over the last few years, biology has been undergoing a spatial revolution and a new generation of technologies are being adopted which has the potential to transform many areas of biological research and pathology. Until recently most omics approaches, could not capture any spatial information, a significant limitation given important biological processes are often driven by a spatial context. Spatial metabolomics is such an innovation, a research field focused on the *in situ* mapping of small molecules - metabolites, lipids and drugs in tissues and organs.

Next year, the facility will begin work on developing mass spectrometry imaging (MSI) as an approach to spatial metabolomics. This was made possible by the successful application and award of MRC Equip funding this year for a MRT (multi reflecting time-of-flight) mass spectrometer. This instrument can uniquely perform spatial metabolomics with two complementary techniques, Matrix Assisted Laser Desorption Ionisation (MALDI) and Desorption Electrospray Ionisation (DESI). These two methods together will allow us to measure the widest breadth of metabolites possible on the same instrument. We are eager to start developing this technique inhouse and are excited by the possibility of overlaying MSI data with those gathered from many other spatially resolved technologies available in the Institute.

During the summer, the lab also continued the long-standing association with Cold Spring Harbor labs, assisting in the organisation and practical instruction of the 2024 metabolomics course. The course runs for a period of two weeks, during which the students learn both the theory and application of different GC/LC-MS methodologies to measure metabolism and answer fundamental biological questions in their own research areas.

Publications listed on page 130

PROTEOMICS

Head
Sara Zanivan

Scientific Officers
Kelly Hodge
Sergio Lilla

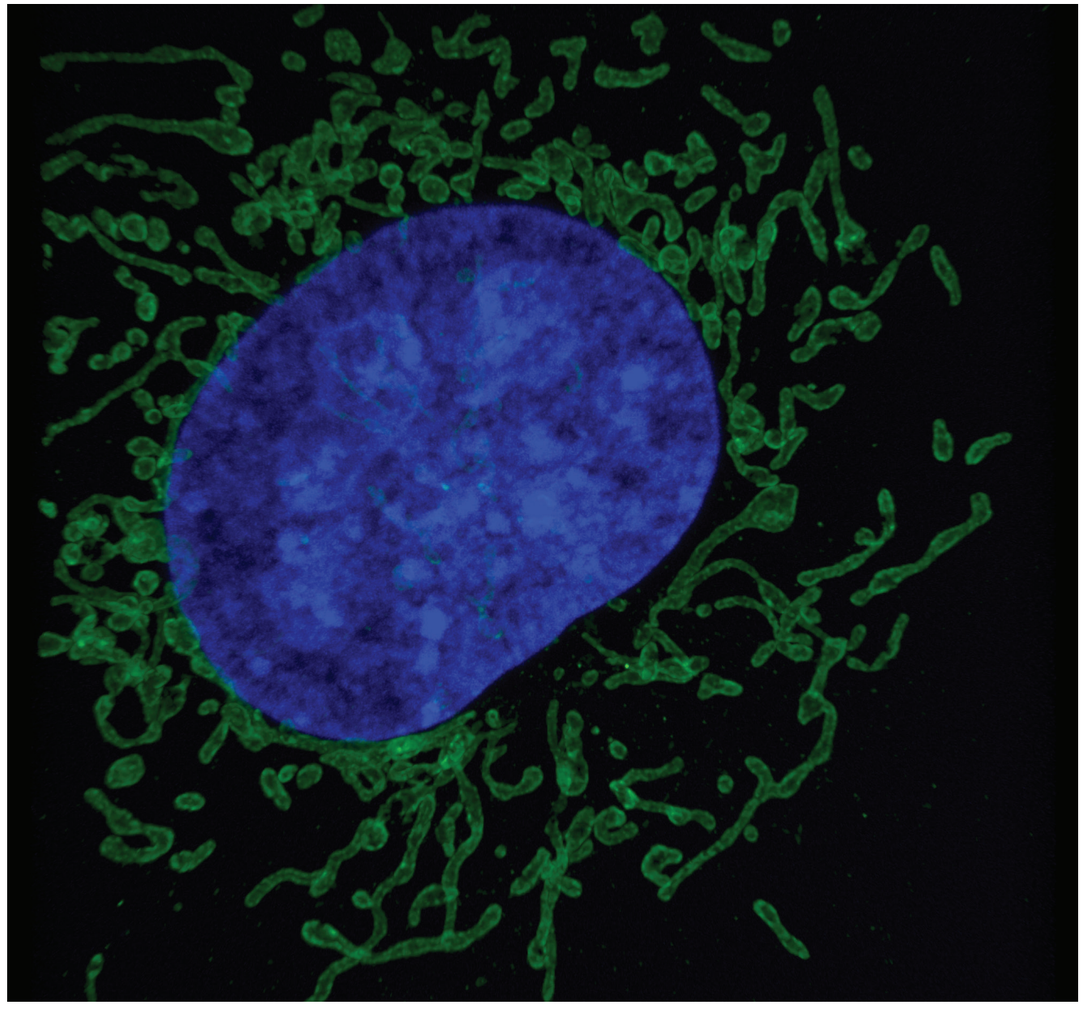
Proteins constitute half of the cell's (dry) mass and are key functional units that actively contribute to tumour initiation, progression and metastatic spread. Proteins are also used as blood markers to determine the wellness status of an individual. Mass spectrometry (MS)-based proteomics is fundamental to unravel the identity and function of each protein in the cell and body fluids. The Proteomics facility is working with cutting-edge MS proteomic technologies and innovative platforms for sample preparation and data analysis to answer fundamental questions of cancer biology, thus contributing to the progress of cancer research

The Proteomics team has an outstanding expertise in high-resolution, Orbitrap-based mass spectrometry (MS) proteomics, accurate quantification approaches and MS data analysis. We work in collaboration with research groups within and outside of the Institute, and we actively develop MS-based proteomic platforms to address a variety of questions to help scientists to increase their understanding of the mechanisms that regulate various aspects of cancer.
To achieve this, we are equipped with three nano liquid chromatography (nLC)-MS systems, including an Orbitrap Fusion-Lumos. All our instruments are coupled online to Easy-nLC systems, and high-resolution chromatography is achieved by packing our nano-columns in house.

We house a number of dedicated software packages, of which MaxQuant is most frequently used for highly accurate label-free or label-based quantitative analysis of data acquired in data-dependent acquisition mode and Spectronaut® for data acquired in data-independent acquisition mode. Moreover, we use Skyline for the analysis of PRM data. Finally, we use Perseus for data analysis and dissemination.

We have a competitive portfolio of techniques available, which span from single protein to sub-proteomes and global proteome analyses. We have strong expertise in quantitative analysis of secretomes (extracellular matrix, extracellular vesicles and conditioned media) and protein translation, including in approaches that allow us to study protein translation dynamics by tracing ^{13}C-labelled metabolites and amino acids into newly synthesised proteins (Schmidt *et al*, 2023, *Nucleic Acids Res*; Kay *et al*., 2022, *Nat Metab*). We are also experts in posttranslational modifications, particularly cysteine oxidation dynamics, for which we have developed SICyLIA, a method that enables us to quantify cysteine oxidation levels at global scale with no enrichment steps required (van der Reest, Lilla et al., 2018 Nat Commun) and that has been fundamental to answer different biological questions (Cao *et al*., 2020; *J Cell Sci Port et al*., 2018, *Cancer Discov*; Hernandez-Fernaud, Ruengeler *et al*., 2017, *Nat Commun*).

During 2024, we have worked with many of the groups at the Institute and significantly contributed to the success of their research (see publications). We are continuously striving to develop new methods to answer more complex biological questions using proteomics and to improve the methods currently in place enriching the quality of the data that the facility can provide.



Expansion Microscopy of SVEC cells stained for Mitochondira and Nucleus - *Nikki R. Paul and Rosalie Heilig*

LABORATORY OPERATIONS & PUBLICATIONS

LABORATORY OPERATIONS

Head of Operations
Scott Kelso

Operations cover several different functions with the remit to ensure the smooth running of the building, facilities, and support services, providing support to the research groups housed within the Institute, giving them the freedom to focus on delivering their world class research.

Our operational teams have continued to focus on delivering first class services to our researchers in the Institute. This has been delivered against a challenging backdrop of continuing high utility costs as well as ongoing inflationary pressures on routine consumables and equipment.

Our ability to mitigate these pressures is contingent on improvement activities and finding more and more opportunities to review existing contracts or to find more efficient or effective ways of working. Building on previous efforts in this area, we have looked at piloting schemes to centralise the purchase and issuing of common chemicals for groups to reduce wastage and ensure that we get best value for money, as well as further opportunities to reduce electricity consumption and liquid nitrogen usage. All these efforts not only help mitigate against rising costs, but also provide benefits against environmental sustainability targets.

Alongside the activity around ways of working, we have also continued to invest in equipment for the Institute, which is vital for day-to-day research work. Examples include the first phase of the replacement programme for our tissue culture hoods as well as increasing our primary data storage capacity which will help meet the challenges around increased data demands from new technology platforms.

The coming year will continue to pose challenges, particularly around capital spending requirements, to replace key pieces of equipment that are vital for routine operations as well as advancing the discovery science of the Institute. That said, our incredible teams are ready to meet these head on and deliver the best services possible to help our researchers deliver their world leading research activities.

Facilities Management & Maintenance

Alistair Wilson, **Andy Hosie**, Mark Deegan, Roy McCarthy

We manage the outsourced service provisions for catering, cleaning and janitorial services as well as providing maintenance support for the Institute's buildings, plant, and fabric. We manage minor project works, alterations and refurbishments and ensure that all statutory and regulatory issues with respect to buildings and systems are compliant with appropriate regulatory standards.

This year has seen us, in conjunction with the University of Glasgow, upgrade gas supplies to all our buildings from low to medium pressure. This has eliminated some of the costs associated with meterage standing charges and means that all gas and electricity supplies, are now managed for us through the University. We therefore benefit from the University's ability to bulk buy utilities via their National Procurement Framework which enables us to predict future utilities expenditure more accurately and protect the Institute more from market fluctuations in utility costs.

In addition, after a successful tender process, we welcomed a new catering provider to the Institute and saw a significant refresh to the café provision for our staff with new menus and improved services.

These activities have been completed successfully alongside the routine compliance and maintenance activities required to keep the Institute running smoothly for all our researchers.

Laboratory Management & Health and Safety

Euan Cameron, **John Kinsella**, Karen Thomas, James Dyball, Lauren McGowan

The Laboratory Management team ensure that the Institute's laboratories run as effectively as possible, performing vital support duties and planning operational improvements to allow research to occur efficiently and effectively.

Laboratory Management coordinates the servicing, maintenance and upgrades for communal Institute equipment, systems, and laboratory areas. The team works proactively to minimise equipment breakdowns, addressing those that do occur as quickly as possible. Additionally, the team maintains a comprehensive laboratory equipment database and asset register, using this to continually assess the status and capability of existing communal equipment, and prioritise new equipment purchases and replacements accordingly. The team also manage cryogenic sample storage provision, maintain sufficient supply of refrigerant gases, support researchers with troubleshooting and other queries and ensure safe, compliant disposal of laboratory waste including chemical, clinical and WEEE waste.

The Laboratory Management team works very closely with the Institute's Health & Safety Manager, John Kinsella, to ensure that all staff, students, and visitors work in a safe laboratory environment. Maintaining and improving health and safety standards within the Institute is an integral aspect of Laboratory Management's responsibilities: the team reviews health and safety processes regularly and identifies training needs for all staff. A primary role of the team is to provide advice, training, and information to all staff on matters relating to health and safety, either to ensure best practice or to effectively respond in the event of incidents or issues. This includes contributing to the creation of risk assessments and appropriate containment and control measures necessary for laboratory work involving biological, chemical, radiation, and genetic modification processes. Additionally, all staff and students attend a safety update once a year and new starts attend a series of safety and training inductions where fire safety is also managed in conjunction with the area fire officers. Lab Management also monitor all outgoing orders to ensure compliance with Institute safety procedures, particularly those relating to COSHH.

Laboratory Management needs to maintain strong relationships with relevant suppliers, to guarantee best prices and discounts for new equipment, maintenance, and servicing. The team works closely with the Laboratory Support Services team to control costs for purchases related to service contracts and laboratory consumables. In addition, assistance is given to researchers to enable smooth processing of their orders with relation to discounted pricing and to make sure that all orders comply with requirements related to import and relevant laboratory regulations. Additionally, when new equipment is purchased, the laboratory management team engages directly with sales and technical representatives from relevant companies, to organise required demonstrations and training for any new equipment installed.

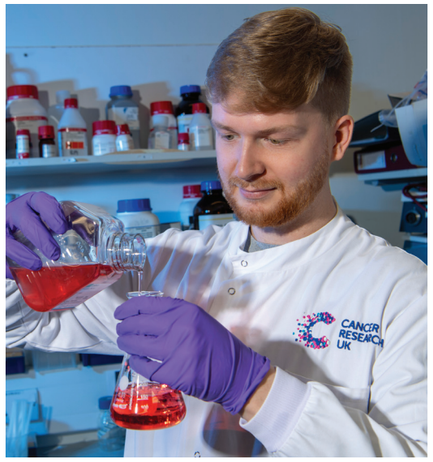
Laboratory Support Services

Angela Miller, Tracy Shields, Abbie McFarlane, Anna Shearer, Dilhani Kahawela, Jonny Sawers, Kirstie McPherson, Linda Bremner, Lisa Liu, Rory Mathie and Steph O'Brien.

Laboratory Support Services provides a vital service, supporting the research undertaken in the Institute. The team works closely with scientific officers and curators to ensure tissue culture suites are equipped with the consumables required to facilitate the work undertaken in these areas. Daily preparation of bacterial culture media and tissue culture solutions is essential, ensuring that our researchers have the supplies they require for carrying out their world-renowned research.

Essential laboratory equipment such as centrifuge rotors, water baths and pH meters are cleaned and calibrated by the team, preventing contamination, and allowing continual use of such equipment. The responsibilities of the team also include high turnover cleaning and sterilisation of laboratory glassware as well as collecting laboratory waste and ensuring the appropriate waste streams are rendered safe by autoclaving prior to disposal.

A sub team within Lab Support Services, called Specialised Lab Support focusses on the preparation of a repertoire of thirteen widely used buffers, *Drosophila* fly food and antibiotic containing agar plates for bacterial selection. The research demands have grown this year with the introduction of new labs requiring support, and the team continue to support and adapt to the requests of the researchers of CRUK SI with the dispensing of chemicals and new buffers for general use or in Ttissue culture.



Stores

Angela Miller, Alistair Horton, Laura McCartney and Michael McTaggart.

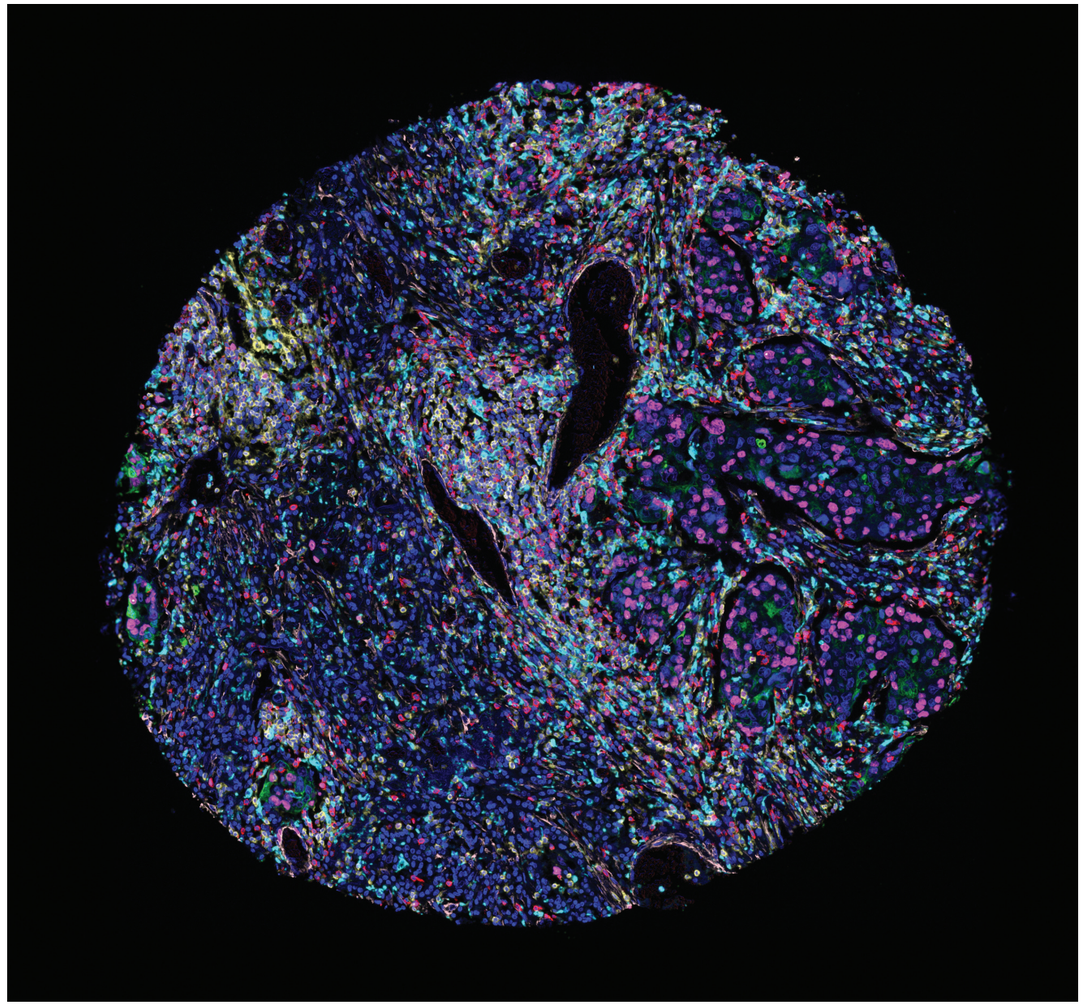
A wide range of stocks are kept of frequently used consumables from a variety of renowned scientific suppliers to ensure quality, high-use materials are always available. We maintain a good relationship with suppliers, which has allowed us to negotiate improved pricing and to reduce the overall value of stock held without compromising supply lines to the laboratories. This year, the Stores team have instigated various supply agreements to ensure that costs are kept as low as possible and to ensure that stores stock is readily available to researchers, with recent focus on cost savings between suppliers and contingency planning for several high-use tissue culture items.

Our Thermo Fisher Supply Centre established at the end of 2022, continues to hold consignment stock which is the property of the company until requested by the end user. In the last year, the number of items held on-site now stands at 83 research items reducing the numbers of external orders to this supplier. We are in constant communication with end users and the supplier to ensure that what we are holding on site is reflective of what is being ordered on a regular basis. The new items available are more in line with the current requirements of the researchers within the Institute. By introducing this concept, the items are replenished on a weekly basis in a consolidated order, eliminating packaging and dry ice whilst being more environmentally friendly in the long-term. This benefits the Institute, as it eliminates several items being purchased in bulk, in advance. By holding a set quantity, the supply centre can be replenished when required and this can be modified in line with research and project requirements.

In 2023, CRUK SI were also the first organisation to hold Peprotech items as consignment stock. The relationship with Peprotech is now established and we are communicating regularly about the possibility of adding new items in line with the current demand.

Stores items are withdrawn by researchers with automatic cost centre allocation and delivered to specific bays within the Institute at set times during the day. External orders are also received, processed, and delivered to the researchers, while outgoing samples or materials are processed by Stores for courier collection. The Stores team have increased their communication channels with the research groups since Stores has remained a closed service post Covid restrictions. Stores have implemented a substantial cost reduction for the Institute by transferring shipments of both UK and world-wide packages to an alternative courier, without impacting on the service provided. We continue to work closely with the research groups to review the services provided by Stores and improve what is offered to scientific staff. This includes negotiating samples from suppliers to enable the scientific staff to assess new or alternative products. This has resulted in considerable savings for the Institute, and, in the next year, stores will be undergoing some further changes, as stock items held will be reviewed and new kits and reagents brought in in conjunction with the changes in research needs.

Over this coming year, Stores plans to continue to make adaptations to the Stores list ensuring that the items held are being used regularly and we are optimising best use of our current space. There are ongoing meetings with external company sales representatives to ensure we have the stock we require for the research as well as alternative suppliers and products for when items are unavailable. We continue to focus on cost saving methods and we will be looking at more sustainable products in collaboration with suppliers and how to offer these whilst also ensuring best value for money when purchasing.



An 8-plex immunofluorescence panel applied to human lung adenocarcinoma - *Fiona Ballantyne, Leah Officer-Jones, Ian Powley, John Le Quesne*

PUBLICATIONS

Prof Imran Ahmad (page 12)
Models of Advanced Prostate Cancer

Primary Research Papers
Beijert IJ, Hagberg O, Gårdmark T, Holmberg L, Häggström C, Johnston A, Trail M, Hamid S, Dreyer BA, Padovani L, Garau R, Hasan R, Ahmad I, Hendry D, Compérat EM, Burger M, Rouprêt M, Gontero P, Ribal MJ, van der Kwast TH, Babjuk M, Sylvester RJ, Mariappan P, Liedberg F, van Rhijn BWG.
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Prof Tom Bird (page 14)
Liver Cancer, Disease and Regeneration

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Prof Karen Blyth (page 16)
In Vivo Cancer Biology

Primary Research Papers
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Prof Martin Bushell (page 20)
RNA Translational Control in Cancer

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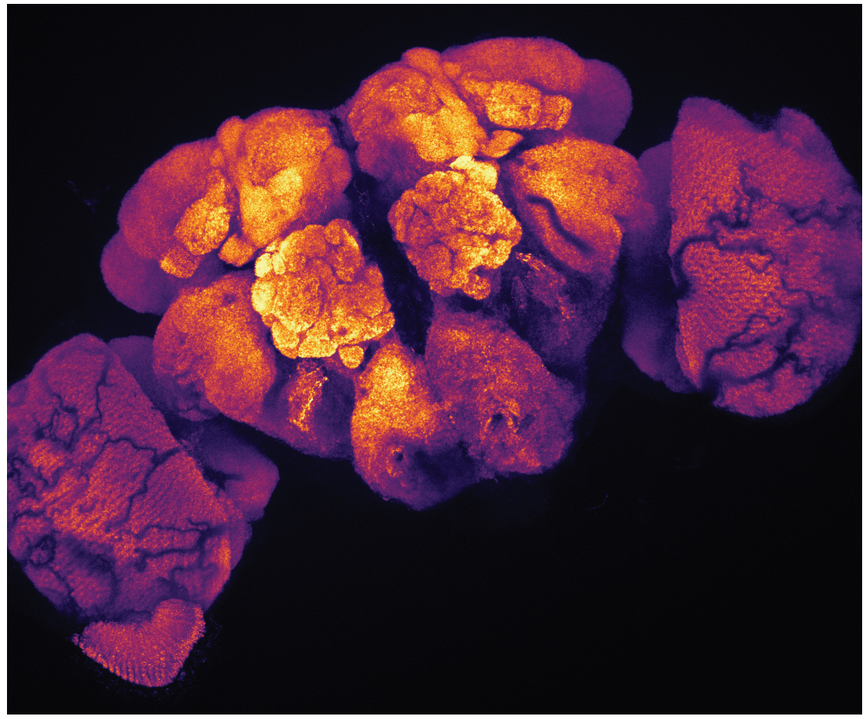
Image of Drosophila- *Jack Holcombe*



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Prof Ross Cagan (page 22)
Biology of Therapeutics

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Other Publications
Cong B, Cagan RL.
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Dr Leo Carlin (page 24)
Leukocyte Dynamics

Primary Research Papers
Derby SJ, Dutton L, Strathdee KE, Stevenson K, Koessinger A, Jackson M, Tian Y, Yu W, McLay K, Misquitta J, Alsharif S, Clarke CJ, Gilmour L, Thomason P, McGhee E, McGarrity-Cottrell CL, Vanderlinden A, Collis SJ, Rominyi O, Lemgruber L, Solecki G, Olson M, Winkler F, Carlin LM, Heiland DH, Inman GJ, Chalmers AJ, Norman JC, Carruthers R, Birch JL.
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Immune Cells and Metastasis

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Gene Regulation

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Prof Ram DasGupta (page 32)
Cancer Systems Biology and Tumour Evolution

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Phenotypic Plasticity in Colorectal Cancer

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Mitochondrial Oncogenetics

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Prof Danny Huang (page 44)

Ubiquitin Signalling

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Prof Gareth Inman (page 46)

Growth Factor Signalling and Squamous Cancers

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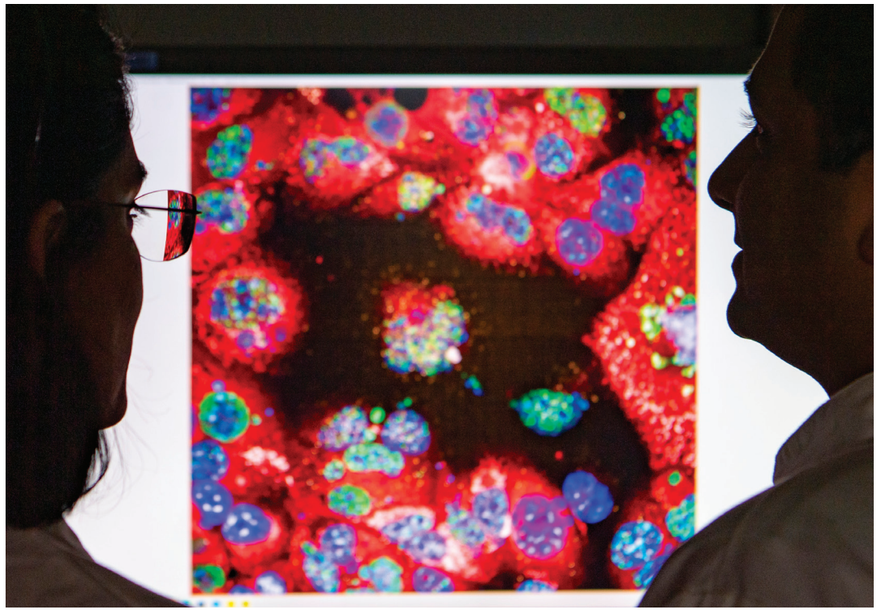

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Prof John Le Quesne
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Deep Phenotyping of Solid Tumours

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Prostate Cancer Biology

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Dr David Lewis (page 52)
Molecular Imaging

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Prof Daniel Murphy (page 62)

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Dr Maximiliano Portal (page 66)

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Prof Owen Sansom (page 74)

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Oncometabolism

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Dr Ke Yuan (page 84)

AI for Cancer Research

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Prof Sara Zanivan (page 86)

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Ackermann T, Shokry E, Deshmukh R, Anand J, Galbraith LCA, Mitchell L, Rodriguez-Blanco G, Villar VH, Sterken BA, Nixon C, Zanivan S, Blyth K, Sumpton D, Tardito S.
Breast cancer secretes anti-ferroptotic MUFAs and depends on selenoprotein synthesis for metastasis. *EMBO Mol Med.* 2024;16(11):2749-2774.

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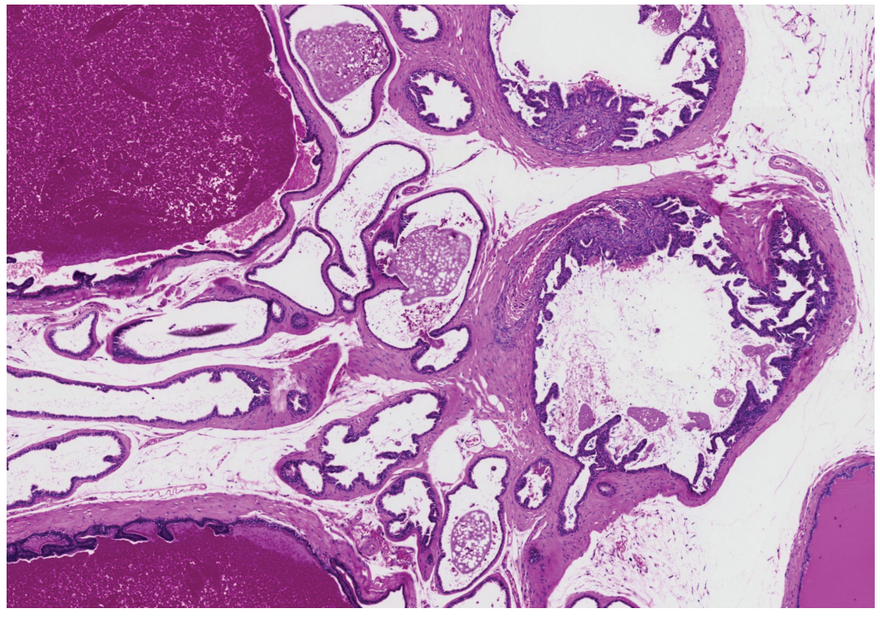
Prostate Staining - *Laura Martinez-Escardo*



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Douglas Strathdee (page 104)

Transgenic Technology

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David Sumpton (page 106)

Metabolomics

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Ackermann T, Shokry E, Deshmukh R, Anand J, Galbraith LCA, Mitchell L, Rodriguez-Blanco G, Villar VH, Sterken BA, Nixon C, Zanivan S, Blyth K, Sumpton D, Tardito S.
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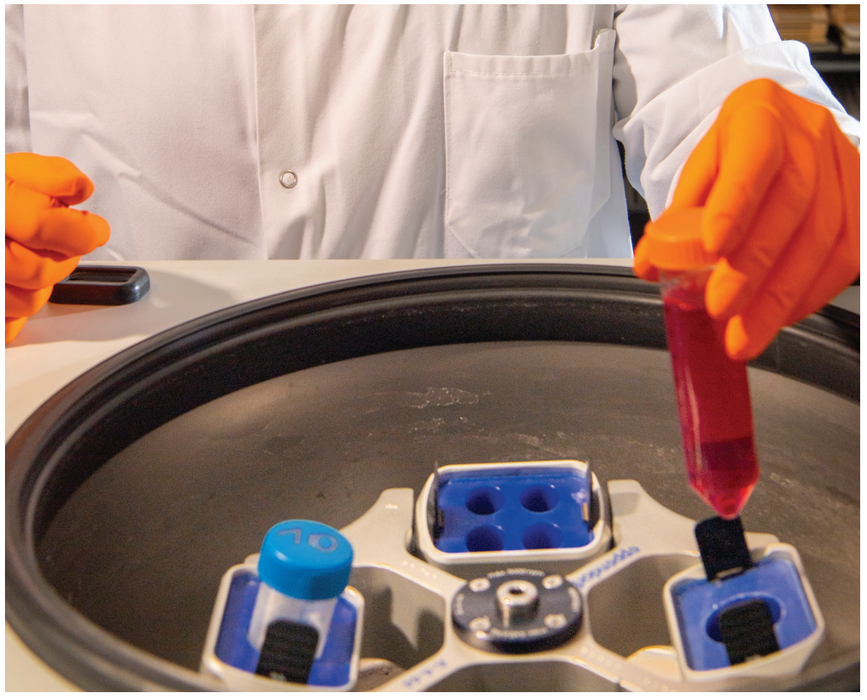

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THESES

Cartwright, Douglas (2024) Understanding the intercellular signalling that determines CAF heterogeneity in high grade serous ovarian carcinoma [PhD thesis, University of Glasgow, CRUK Scotland Institute]

Claydon, Sophie (2024) Identifying proteins that preferentially bind ATP- over ADP-F-actin [PhD thesis, University of Glasgow, CRUK Scotland Institute]

Curley, Emer (2024) ^{11}C-acetate as an imaging biomarker of radioresistance in mouse models of non-small cell lung cancer [PhD thesis, University of Glasgow, CRUK Scotland Institute]

Devlin, Ryan (2024) Acute Influenza Infection Drives a Transient Pro-Tumoral State in the Lungs [PhD thesis, University of Edinburgh, CRUK Scotland Institute]

Gillespie, Michael A. (2024) Development of a protocol to study novel radiotherapy-immunotherapy combinations in pre-clinical models of locally advanced rectal cancer [PhD thesis, University of Glasgow, CRUK Scotland Institute]

Li, Jia (2024) Investigating the role of DRAM1 in glucose metabolism [PhD thesis, University of Glasgow, CRUK Scotland Institute]

Mandrou, Elena (2024) Using FLIM-FRET to visualise self-generated gradients in cancer cells [PhD thesis, University of Glasgow, CRUK Scotland Institute]

Mearns, Hannah (2024) Investigation of biomarkers for the detection of pre-cancerous lesions associated with pancreatic ductal adenocarcinoma (PDAC) [PhD thesis, University of Glasgow, CRUK Scotland Institute]

Melissourgou-Syka, Lydia (2024) Investigating Radiotherapy in Combination with Immune Modulation in Preclinical Mouse Models of Rectal Cancer [PhD thesis, University of Glasgow, CRUK Scotland Institute]

Peters, Jasmine (2024) An investigation into the role of tumour cell Rab27a-dependent small extracellular vesicles in modulating the tumour immune microenvironment [PhD thesis, University of Glasgow, CRUK Scotland Institute]

Rolo, Sonia (2024) Investigating the role of Ajuba LIM domain protein in Pancreatic Ductal Adenocarcinoma [PhD thesis, University of Glasgow, CRUK Scotland Institute]

Smith, Craig (2024) Epidemiology and risk prediction modelling of head and neck cancer [PhD thesis, University of Glasgow, CRUK Scotland Institute]

Thakur, Teena (2024) Investigating Drug Resistance in RAS-driven models of Colon Cancer [PhD thesis, University of Glasgow, CRUK Scotland Institute]

White, Mark (2024) Pre-clinical modelling and treatment of Braf mutant colorectal cancer [PhD thesis, University of Glasgow, CRUK Scotland Institute]

Wicks, Alice (2024) Exploring CBL as an E3 ubiquitin ligase in proteolysis-targeting chimeras (PROTACs) [PhD thesis, University of Glasgow, CRUK Scotland Institute]

John Paul Career Award

All final year PhD students at the CRUK Scotland Institute are eligible for this award, named after Dr John Paul, the founding Director of the Institute. Candidates prepare a progress report on their work and give a talk to staff and other students.

The winners of this year's award were **Bianca Blochl** and **Jasmine Peters** from Maxi Portal and Jim Norman's groups respectively. Bianca's talk was titled "Exploring the interplay of non-genetic mechanisms and oncogene-induced transformation" and Jasmine's was titled *"Tumour cell Rab27a controls the production of immunomodulatory extracellular vesicles which enable T cell infiltration".*

CONFERENCES AND WORKSHOPS

High School Open Evening

13 March 2024

In March 2024, we held our annual High School Open Evening where local high schools are invited to come to the CRUK Scotland Institute to hear scientific talks and demonstrations as well as get a tour of the labs and the chance to network with some of our researchers.
This year, the scientific talks were given by Jasmine Peters, Fraser Edgar, Zoi Diamantopoulou and Derek Miller.

Delft University of Technology and Erasmus Medical Center Rotterdam Nanobiology Student visit

5 July 2024

In July, we hosted a group of 30 MSc and BSc Nanobiology students from the Netherlands. We had presentations from various members of the Institute to showcase some of the research that is going on as well as their career journey to the Institute. The students were also given a tour of BAIR and our spatial biology lab as well as some AI and computational biology demonstrations. Finally, the visiting students presented some of the projects they are working on, this session was well attended by Institute staff and students keen to hear more about the field of nanobiology.

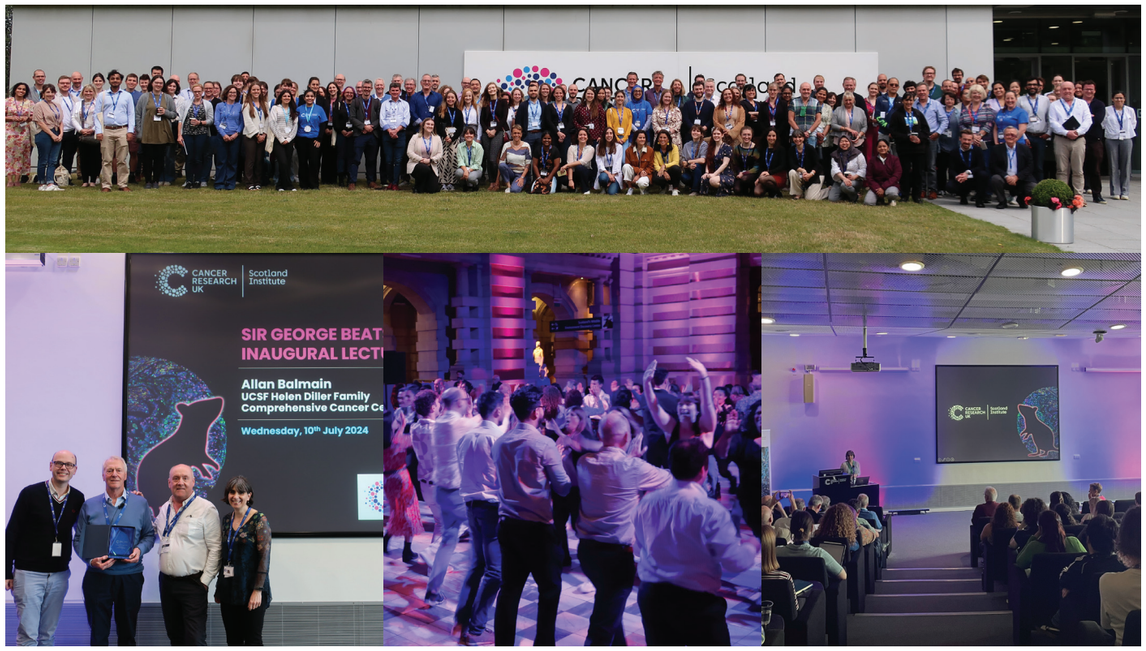
25th Beatson International Cancer Conference 2024 "Cancer Models: from Cages to Clinic"

8th–11th July 2024

Conference Committee: Kevin Ryan (Chair), Karen Blyth (Co-chair), Emma McLean (Conference Organiser), Imran Ahmad, Tom Bird, Leo Carlin, Seth Coffelt, Zoi Diamantopoulou, Jen Morton, Daniel Murphy, Ed Roberts, Owen Sansom

This year, the Cancer Research UK Scotland Institute celebrated its **25th Anniversary of the Beatson International Cancer Conference** with the topic of 'Cancer Models: from Cages to Clinic', welcoming over 165 delegates from across the world on the beautiful grounds of the Garscube Estate in Glasgow between Monday 8th – Thursday 11th July 2024.

Researchers using diverse genetically tractable mouse models of cancer gathered to discuss their latest unpublished work and developments in areas ranging from the development of sophisticated allele-specific and tissue-specific animal models to their use in the study of specific cancer types, the engagement of tumour with immune cells and the microenvironment, metastatic disease and therapeutic resistance. We had talks from 19 invited world-leading researchers, as well as selected short talks from 11 early career researchers and 60 posters presented over two bustling poster sessions and trade exhibition.



This special event showcased a fantastic programme including four excellent Keynote Speakers: **Karen Cichowski** (Brigham & Women's Hospital, Harvard Medical School), **Tyler Jacks** (Koch Institute for Integrative Cancer Research, MIT), **Ashani Weeraratna** (John Hopkins Bloomberg School of Public Health) and finally CRUK SI alumnus **Allan Balmain** (UCSF Helen Diller Family Comprehensive Cancer Centre), who presented the Inaugural Sir George Beatson Lecture in memory of the Institute's founding director.

To round off the conference, the Conference Dinner was held at the spectacular Kelvingrove Art Gallery and Museum, where attendees enjoyed a ceilidh and the chance to continue discussions and networking over dinner.

The conference was kindly sponsored by the CRUK Scotland Institute, Transnetyx (Gold sponsor), 10x Genomics (Silver sponsor), Thermo Fisher Scientific (Silver sponsor), Qiagen (Silver sponsor), The Company of Biologists, Disease Models and Mechanisms, Glasgow Convention Bureau, Molecular Oncology (FEBS), The MRC National Mouse Genetics Network and Worldwide Cancer Research.

We would also like to extend our congratulations to the well-deserved prize winners:

- Best Poster: **Mulham Najajreh** (German Cancer Research Center (DKFZ)).
- Best Poster: **Nuria Vaquero-Siguero** (The Heidlberg Institute for Stem Cell Technology and Experimental Medicine).
- Best Short Talk: **Alex Raven** (CRUK Scotland Institute).
- Best Student Talk: **Charlotte O'Riordan-Moore** (University of Sheffield).

The success of the event was only possible thanks to our generous sponsors, exhibitors and all of the delegates for the highly engaging discussions throughout the four days. We are now looking forward to the 2025 Beatson International Cancer Conference "Cancer Models: from Data to Discovery" which will be held 27th – 29th May 2025.

Work Experience Week

10 – 12 September 2024

In September 2024, we welcomed 16 students from 7 local high schools to the Institute, for our first post-pandemic, in-person work experience week. Researchers ran hands on activities for the students ranging from advanced microscopy to tissue culture. A highlight that came through in the feedback we gathered after the event was when students got the chance to sit down and meet some of our PhD students to ask them about their path into a PhD.

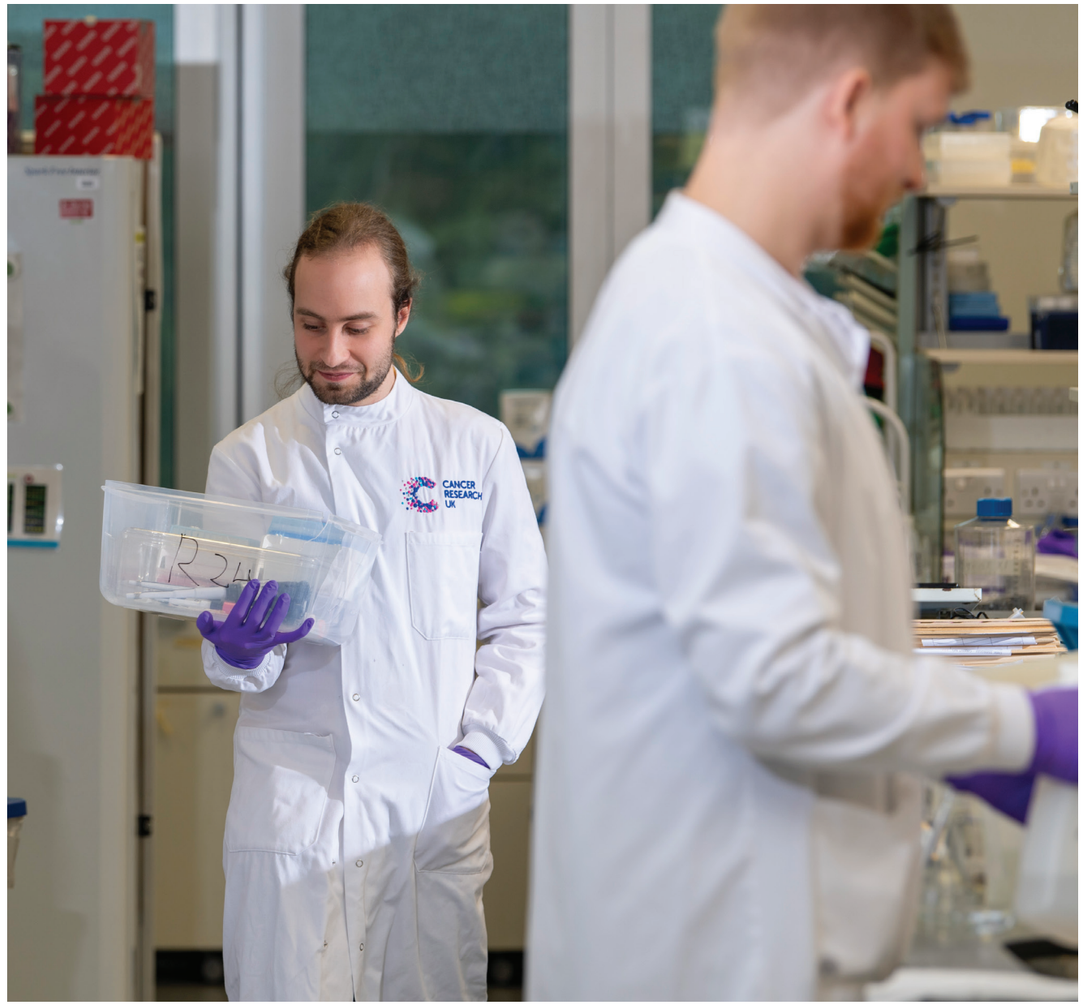
Betty MacGregor Memorial Lecture

28 November 2024

Organising Committee: Ed Roberts, Jen Morton, Vicky Cowling, Asma Alsheikh, Cassie Clarke, George Skalka, Rosalie Heilig, Tara Lorimer, Jasmine Peters, Alex Young, Sarah Williams
The Betty MacGregor Memorial Lecture is an annual lecture hosted by the CRUK Scotland Institute in honour of Janet "Betty" MacGregor (1920-2005). This event aims to highlight and celebrate the work of women in cancer research and an award is given each year to a woman who has made a huge impact in the field.

This year the Betty MacGregor Memorial Lecture was given by Ruth Plummer MBE FMedSci, Professor of Experimental Cancer Medicine (University of Newcastle) and oncologist specialising in melanoma, whose work was central to the introduction of PARP inhibitors. Ruth led trials testing a combination of rucaparib and temozolomide in patients with advanced solid tumours.

As part of the event, an early career researcher is invited to present their work. In 2024, Laura Greaves, a Professor of Molecular Pathology at the University of Newcastle, was invited to present her work on the role of mitochondrial DNA mutations in cancer. Laura has made important discoveries about how these mutations occur and subsequently expand with age and she now studies their functional consequences particularly in colorectal cancer development.



SEMINARS

The following seminars were held at the Cancer Research UK Scotland Institute during 2024.

January

Nick Jones, Swansea University, UK

February

Caroline Dive, CRUK Manchester Institute, UK

Alfredo Castello, University of Glasgow, UK

Peter Bankhead, University of Edinburgh, UK

Helen Pearson, Cardiff University, UK

March

Michelle Garrett, University of Kent, UK

Ralitsa Madsen, MRC Protein Phosphorylation and Ubiquitylation Unit, University of Dundee, UK

David Church, University of Oxford, UK

Lori Passmore, MRC Laboratory of Molecular Biology, University of Cambridge, UK

April

Kim Bak Jensen, University of Copenhagen, Denmark

TESSA HOLYOAKE MEMORIAL LECTURE
Alex Tonks, Cardiff University, UK

May

Kirsten McAulay, University of Dundee, UK

Manav Pathania, University of Cambridge, UK

Richard Gilbertson, CRUK Cambridge Institute, UK

Wafa Al-Jamal, Queen's University Belfast, Northern Ireland

June

Jiri Bartek, Karolinska Institutet, Sweden

Louise Fets, MRC Laboratory of Medical Sciences, UK

Marino Zerial, Max Planck Institute of Molecular Cell Biology and Genetics, Germany

David Bending, University of Birmingham, UK

July

Ed Reznik, Memorial Sloan Kettering Cancer Center, New York, USA

Aideen Ryan, University of Galway, Ireland

August

Elly Gaunt, University of Edinburgh, UK

Takis Panagiotis Karras, VIB-KU Leuven Center for Cancer Biology, Belgium

Liam Faller, Netherlands Cancer Institute, Netherlands

September

Jue Shi, Hong Kong Baptist University, Hong Kong

Jean-Emmanuel Sarry, Toulouse Cancer Research Center, France

Laura Donovan, UCL, London

October

Patrizia Romani, University of Padova, Italy

Lorraine O'Reilly, Walter and Eliza Hall Institute of Medical Research, Australia

Philip Quirke, University of Leeds, UK

Peter Hall, University of Edinburgh, UK

November

PROFESSORIAL LECTURE
Dave Bryant, CRUK Scotland Institute/University of Glasgow, UK

Carly Bliss, Cardiff University, UK

Alexander Anderson, Moffitt Cancer Center, Florida, USA

BETTY MACGREGOR MEMORIAL LECTURE
Ruth Plummer and Laura Greaves University of Newcastle, UK

December

Mirjana Efremova, Barts Cancer Institute, London, UK

Awen Gallimore, University of Cardiff, UK

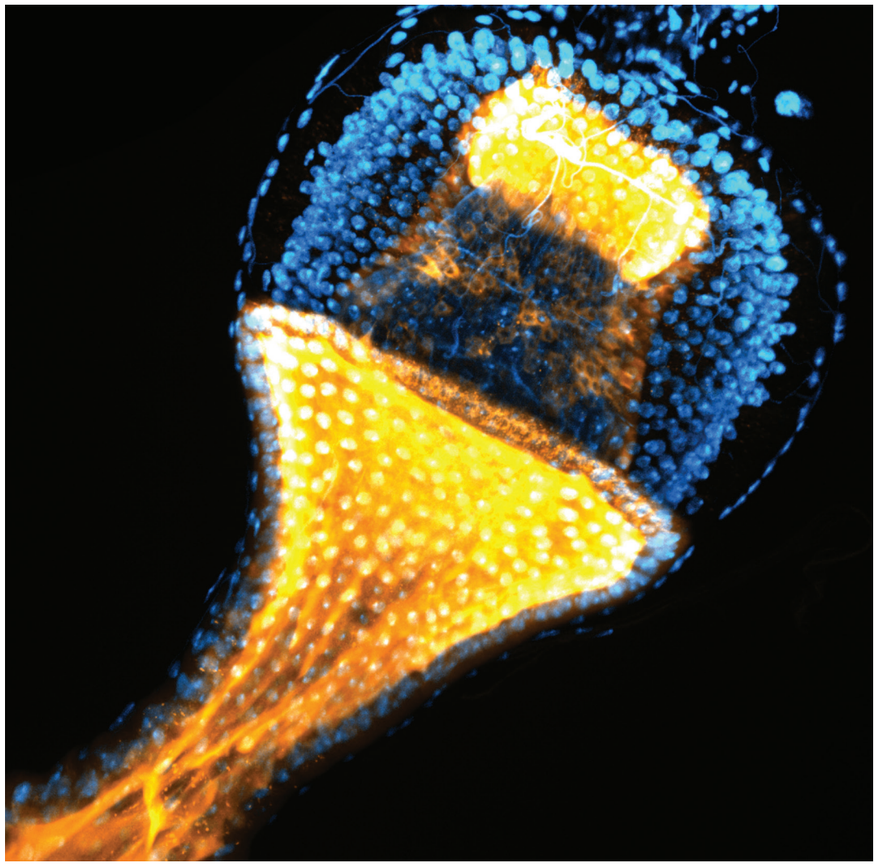


Image of Drosophila - *Jack Holcombe*

PHD STUDENTS, CLINICAL RESEARCH FELLOWS AND POSTDOCTORAL SCIENTISTS

The training and career development of early career researchers is an essential part of our mission to support cancer research of the highest standard. We aim to attract the best and brightest scientists and clinicians early in their careers to work with our established research teams, drawing on their experience and also sparking new ideas in an internationally diverse, stimulating and cutting-edge research environment.

As well as learning a wide range of practical and technical skills, these junior researchers are encouraged to develop their critical thinking, scientific rigor, present and discuss their work at internal seminars and external meetings, and publish their research findings. Early career researchers benefit from our tremendously collaborative environment and the opportunities we offer for scientific interaction and intellectual discourse through our international conference, workshops and seminars.

PhD Students and Clinical Research Fellows

The purpose of our PhD training programme is to give graduates and trainee clinicians who are starting in research an opportunity to work in state-of-the-art laboratories with leading researchers. This enables them to assess and develop their research talents to the full and to use their period of graduate study as a springboard for their future career path. Our four-year studentships (or three-year clinical research fellowships) are designed to give graduates (or clinical trainees) who show a strong aptitude and potential for research the opportunity to complete a substantial research project resulting in high quality publications. We also support an extra year post-PhD for publication ready projects. As well as developing their laboratory skills, students receive training in safe working practices, writing project reports, research integrity and other transferable skills. Training also involves learning to be an independent scientist and students are central to the intellectual life of the Institute, attending and giving seminars and actively contributing to scientific discussions. Students are also given the opportunity to present to national and international conferences to enhance their network of scientific contacts.

Our students are fully integrated with University of Glasgow graduate school (www.gla.ac.uk/colleges/mvls/graduateschool) and are allocated primary and secondary supervisors who are jointly responsible for supporting and monitoring their progress. The primary supervisor is responsible for developing the student's research abilities, providing all practical support required for the project and dealing with any administrative matters required in relation to the University or funding body. The secondary supervisor gives additional guidance by providing independent advice on any matters concerning the studentship. Students are also assigned two independent panel reviewers to assist them in reviewing their progress and advising them on their training and career development needs. The PhD training programme is overseen by a senior member of the Institute (Professor Stephen Tait). There is also a range of support available to help ensure the health and wellbeing of students.

Postdoctoral Scientists

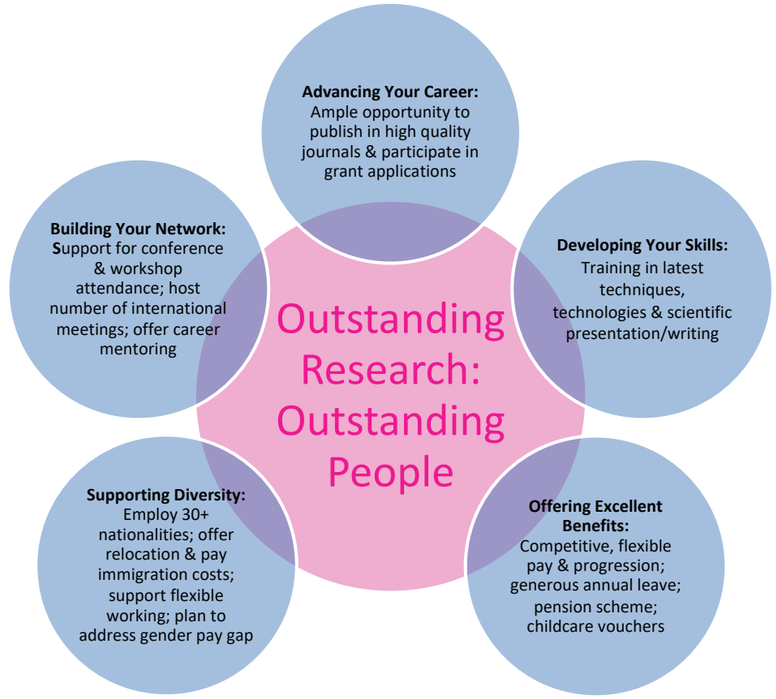
We see postdocs as pillars of the research and intellectual activities of their own groups and of the Institute as a whole. Our postdoctoral training, which is overseen by a senior member of the Institute (Professor Jennifer Morton), is designed to promote the development of outstanding and dedicated early career scientists. All postdocs participate in an internal seminar series and are offered feedback by group leaders following their mid-contract presentations. We hope that by the end of their time with us many of them will be ready to compete for an independent scientist position, however we recognise that a postdoctoral training position can lead to many different career paths. We have introduced a mentoring enabling scheme to help postdocs get the support and advice they need as they develop as scientists and make these important decisions about their career path. We also assist those making fellowship and small grant applications, either while at the Institute or as they make the transition to a new position elsewhere. In addition, our postdocs have developed their own support network through their postdoc forum, which covers topics ranging from research and technologies through to training and careers. They also organise regular scientific meetings and social events.

Postdocs are initially employed for three years but outstanding individuals who are developing into independent scientists may be given additional support and responsibility – such as more technical help or mentoring of a postgraduate student. At the discretion of their group leader, funding may also be extended for three more years. At the Institute, we are also committed to increasing the number of female scientists at the postdoctoral level and strongly encourage female applicants to apply for positions with us. We have introduced a highly attractive, innovative maternity policy, which includes providing a postdoc with support and funding so that their projects can continue during their maternity leave.

For further details on Studentships, Postdoctoral Fellowships and other posts currently available, see our website www.crukscotlandinstitute.ac.uk

www.glasgow.gov.uk and www.visitglasgow.com give general information about Glasgow and other useful links.

Postdoc opportunities at CRUK Scotland Institute



OPERATIONAL SERVICES

Finance

Gary Niven CA, Richard Spankie FCCA, Nicki Kolliatsas, Jo Russell, Jacqui Clare, Karen Connor, Sandra Watt, Helen Hannaway

The Finance team maintain stewardship of the Institutes financial resources and ensure that their use is aligned with strategic priorities. This includes management of the purchase-to-pay process, as well as provision of relevant financial/management information and support to all areas of the organisation. The team operate in partnership with budget holders to support best value decision making and ensure appropriate use of all Institute funds.

The Finance team operate in close partnership with the HR team to manage funding sources used for staff salaries/ student stipends. The Finance team also work closely with the Research Management team to provide support for grant applications, as well as management of grant awards. Furthermore, the Finance team maintain a very close working relationship with the University of Glasgow via the coordination and administration of shared/ common sources of funding.

2024 has seen a return to pre-pandemic levels of CRUK Core funding. During the year, the Finance team have focussed on actively managing the Institutes financial model to ensure that balanced and sustainable Core operating budgets are established. A 'continuous improvement' programme has recently been established within the finance function that will apply a data driven approach to review, refine and enhance the team's processes.

People & Culture Team

Sharon Gorman MCIPD, Elaine Marshall ACIPD, Jivaharini Nithianandam ACIPD, Selina Mungall ACIPD, Barbara Laing

Our vision is to be a People & Culture team that is professional, open, inclusive and collaborative. Our team provides support and advice across, Human Resources, Learning and Organisational Development, Equity Diversity & Inclusion and Health & Wellbeing.

In 2024, much of the team's focus was on creating a positive workplace culture where our staff are engaged and enabled to realise their full potential. We worked with staff to define and embed our values (Innovate, Respect, Integrity, Teamwork and Excellence) and leaders to embody these values. We commenced our management development programme to equip our managers with the skills and knowledge to create an environment where our employees feel valued, respected and motivated. We also commenced work to develop an annual employee engagement survey.

We continued work to review and improve our policies, implementing a Menopause policy and a Code of Professional Conduct. We promoted completion of our mandatory eLearning and maintained a focus on a quality appraisal conversation, setting of objectives and identifying personal development.

We have also remained committed to our Equality, Diversity and Inclusion agenda supported by our Board, Senior Management Team, EDI Advocates and University of Glasgow, VOICE Committee.

Administration

Andrena Fatkin (Senior Administrator), Catriona Entwistle (Receptionist/Administrator), Emma McLean (Events and Administration Officer), **Rebecca Gebbie** (EA to Director), Shona McCall (EA to Director), Laura Hughes (PA to Senior Management)

The Administration team provides an extensive range of admin support for both staff and visitors, often acting as the "glue" holding everyone together. The team plays an important role in maintaining internal links, and in relationships with Cancer Research UK, the University of Glasgow and many other organisations.

This includes operation of the Institute's reception and room booking service and onboarding of new starts (Catriona Entwistle); organisation of a range of Institute events, including our external seminar programme, annual conference, workshop and open evening (Emma McLean); and PA support to the Director (Rebecca Gebbie, Shona McCall) and other group leaders and senior managers (Andrena Fatkin, Laura Hughes), including preparing agendas, paperwork and minutes for meetings and organising travel, accommodation and hospitality for staff and visitors.

As always, it was a very busy year for the team, welcoming many new starts and visitors to the Institute, including for several important review meetings and our 25th Anniversary Beatson International Cancer Conference in July, as well as making sure our researchers were able to attend meetings and conferences elsewhere to talk about their work.

Research Management

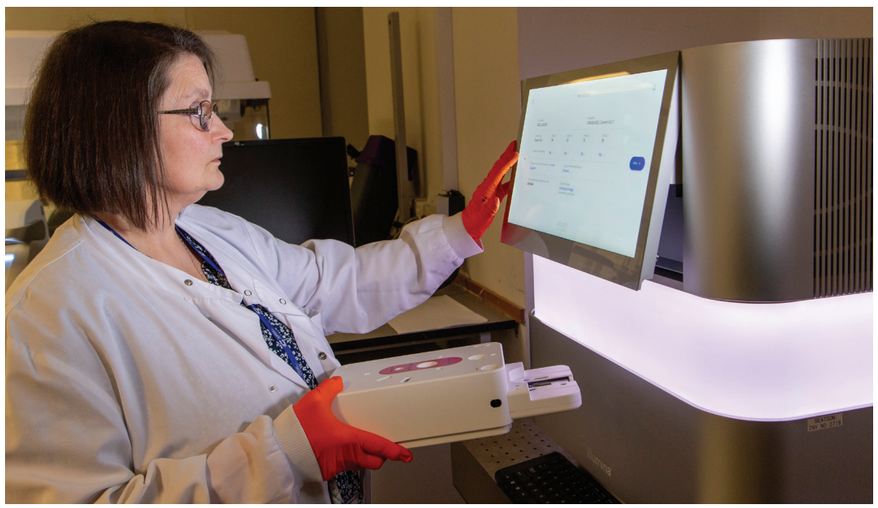
Jackie Beesley PhD, **Catherine Winchester PhD**, Afroditi Chatzi PhD, Fiona Paulin-Ali PhD, Linus Reichenbach PhD, Katharina Schraut PhD, Rebecca Sharland MSc

Members of the Research Management team are all scientifically trained and between them have considerable research and project management experience, enabling them to support researchers at the Institute in a variety of important ways.

This includes assisting with the many aspects of external grant applications (Afroditi Chatzi, who we welcomed as a new start in November); overseeing all aspects of the PhD student training programme and providing external communications for the Institute via its website, social media channels and annual reports (Rebecca Sharland); providing training and advising on good practice in research and checking manuscripts for research integrity prior to their submission (Catherine Winchester, who was promoted to Head of the Research Integrity Service this year cementing her leadership role in this space both internally and externally); and setting agendas and reporting on scientific meetings and reviews as part of helping support the Institute's scientific strategy.

Team members also provide project management support for significant network activities led from here: the CRUK Scotland Centre (Kate Schraut), the MRC National Mouse Genetics Network (NMGN, Linus Reichenbach) and the CRUK Colorectal Cancer (CRC) Accelerator, the next phase of which will launch in 2025 as CRC-STARS (Colorectal Cancer - Stratification of Therapies through Adaptive Responses (Fiona Paulin-Ali).

Highlights this year included Catherine's continued engagement with the research integrity community, acting as an advisor to UKCORI and speaking at conferences in Warwick and Athens; Kate's delivery of the Centre's Annual Meeting and Scientific Advisory Board, which met for the first time in August to assess the Centre's progress and future direction; Rebecca's role in our first recruitment round for the Black Leaders in Cancer PhD Scholarship Programme, where we were able to appoint five excellent candidates; Fiona's work towards the £5.6 million CRC-STARS award; and Linus's coordination of the NMGN's very successful mid-term review in November with the release of funds for the next 2.5 years. Supporting grant applications by group leaders is an important aspect of the team's work (led by Catherine and latterly Afroditi), but we were particularly delighted this year by how many of our early career researchers were successful with smaller grant applications (in total being awarded ~£0.57 million).



EQUALITY, DIVERSITY AND INCLUSION

The CRUK Scotland Institute is committed to promoting Equality, Diversity and Inclusion (EDI) within our community. Our EDI strategy was endorsed by our Board of Directors, Senior Management Team, EDI Advocates and the School of Cancer Sciences, University of Glasgow VOICE (Athena Swan) Committee.

Our current EDI vision is to create a diverse and inclusive culture that attracts and retains research and supports staff with a shared vision of collaborative world class cancer research.

Our aims are:

- Transparency, evidence and improvement (monitor, analyse and publish data to make improvements)
- EDI awareness and training
- Career support and development
- Equitable recruitment practices and opportunities, and
- Scientific engagement

We are in the process of reviewing our EDI Strategy, Vision and Aims.

To support female retention in science we have considered the language we use on recruitment adverts and maintained gender balanced interview panels and applicant shortlists. We have continued to promote our maternity and flexible working policies. We have reviewed grades to identify and address any gender pay gap issues and provided our female researchers with mentoring, coaching, and leadership and management development opportunities.

We emphasised the importance of a good quality appraisal conversation which jointly appraises the past years performance, sets clear objectives and jointly agrees personal development. This has enabled improved performance management. We continue to ensure there is good female representation on our seminar speaker series.

We have engaged our early career researchers to understand career development challenges and we introduced quarterly Postdoc drop-in sessions. We have also continued to support our Postdocs development through mentoring.

Jointly with the University of Glasgow VOICE Committee, we have delivered more events than the previous year, including LGBTQ Allyship event and Ramadan, Diwali and Lunar New Year celebratory events to raise awareness. These events have been driven by our staff.

We are actively working with a committee to recognise cancer researchers of colour and their significant contributions to cancer research. We are also delighted to be working with Black in Cancer and the CRUK Scotland Centre to host PhD candidates on the Black Leaders in Cancer PhD programme from 2025 onwards.

Going forward we intend on better understanding our workforce composition in relation to ethnicity and to report on our ethnicity pay gap. We are also exploring the opportunity to become a Disability Confident Employer and hope to achieve level 1 in 2025/26.

GENDER PAY GAP 2024

Addressing the Gender Pay Gap at The Cancer Research UK Scotland Institute

Creating a diverse and inclusive workplace culture where everyone can be themselves and realise their full potential is of great important to us at the CRUK Scotland Institute. Not only does it enable us to conduct cutting edge cancer research, but it encourages new ideas and creativity, which will help us achieve our objectives as an organisation.

In this report you will find:

- A summary of our gender pay gap
- A summary of the challenges, which contribute to our gender pay gap
- Our commitments and actions to narrowing our gender pay gap

What is the gender pay gap at The CRUK Scotland Institute?

To determine the gender pay gap, the Government requires companies to measure the average earnings of all male and female employees, regardless of role and working hours, and show the percentage difference between the two.

Figure 1 shows that compared to 2023, the mean hourly pay gap between females and males decreased by 2.16% and the median hourly pay gap decreased by 1.33%.

Figure 1: Pay Gender from April 2022 to April 2024
The figures shown here do not include Group Leaders who are employed by the University of Glasgow and who will feature in their Gender Pay Data.

Gender Pay (£/hour)			Gender pay differentials (%)		
(£/hour)	Female	Male	2024	2023	2022
Mean	18.89	20.86	9.44%	11.60%	11.44%
Median	19.11	20.32	5.95%	7.28%	12.24%

Gender pay gap vs equal pay

Gender pay gap is not the same as equal pay. The latter has been a legal requirement in the UK for nearly 50 years. At the CRUK Scotland Institute, we ensure our people are paid equally for equivalent work, subject to experience and individual contribution, and regardless of gender.

What is behind our gender pay gap?

Our gender pay gap has improved over the past year. The latest data from SRG (STEM recruitment agency) quotes a GPG for women in STEM of 19% from their 2024 ***survey in collaboration with New Scientist***. Our own data from a peer group survey quotes an average mean gender pay gap of 14.8% and median of gender pay gap 8.9%. Our mean difference between female and male salaries decreased to 9.44% and the median to 5.95%. This compares favourably with others in similar sectors and reflects our efforts in both recruitment and internal processes of salary review. We appreciate the need to remain focussed and vigilant on reducing this gap further.

In year 2023/24 we continue the trend of recruiting a high number of females across the institute with 62.17% of new starts female. There was a balance of male and female new starts in research roles. There is still however a disproportionate number of females recruited in lower grades. We acknowledge this imbalance impacts our gender pay gap and we continue our efforts to address this, whilst appreciating societal issues may be a factor influencing who applies for these roles.

In 2024, our workforce composition remained static at 38% male and 62% female. When we rank the pay of our staff into 4 quartiles, we can see that there is a majority of females in the first 3 quartiles which reflects the proportion of males and females across the workforce. It is encouraging that in the upper quartile we are close to parity, with females now representing over 48% of this group.

In 2024, 58% of promotions and 78% of advancements in grades (salary increases above our cost-of-living increase) were female.

GENDER PAY GAP (CONTINUED)

Table 2: Comparison of Quartiles 2022 to 2024

	M-2022	F-2022	M-2023	F-2023	M-2024	F-2024
Lower Quartile	30%	70%	32%	68%	32%	68%
Lower Middle Quartile	33%	67%	29%	71%	34%	66%
Upper Middle Quartile	45%	55%	39%	61%	33%	67%
Upper Quartile	54%	46%	53%	47%	51%	49%

What are we doing to close our gender pay gap?

The CRUK Scotland Institute is committed to reducing its gender pay gap through actions identified in our gender pay gap action plan.

Understanding the issues

The CRUK Scotland Institute operates in a sector that relies heavily on highly skilled scientific researchers and those wishing to train in this area. In the UK, the number of women now working as Science Professionals has dropped from 51.5% in 2022 to 43.7% in 2023 (WISE Campaign Report September 2023) presenting a challenge in recruiting from a decreasing pool of talent.

Almost, 63% of our postdocs are female and whilst this is encouraging, we recognise that we need to translate this higher percentage of female postdocs pursuing a scientific research career into more senior positions such as a Group Leader. At present only 31.57% of senior researchers are female, a figure we are actively looking to increase.

To improve our gender pay gap we have taken the following actions

- Adapted our recruitment practices to ensure more gender balanced interview panels and applicant shortlists. We have sought to ensure our language on adverts is inclusive. We have also continued to capture EDI data during recruitment.
- Engaged our early career researchers through Postdoc drop-in sessions to understand career challenges.
- Offered flexible work patterns.
- Provided maternity cover of up to 18 months to enable continuation of research careers.
- Reviewed grades to identify and address any gender pay gap issues.
- Promoted learning opportunities for women, including coaching, mentoring, leadership and management development.
- Ensured equal representation in our seminar series and at our scientific conferences.

We will aim for continuous improvement in these areas as well as introduce other actions to reduce our gender pay gap. This will include seeking to understand the career development challenges of our female staff. For postdocs, we will endeavour to support their transition to an independent research position. This will include support with fellowship applications, mentoring and funding to attend leadership development.

In summary

Whilst we acknowledge a gender pay gap and market challenges relating to recruitment of females in science, we are encouraged by the number of female researchers that have joined our Institute.

To retain women in science, we will continue to review our data, policies and processes to make improvements and promote development opportunities to support women to realise their full potential.

CRUK Scotland Institute is committed to improving equity. It is a fundamental aspect of encouraging equal opportunities for all. Through increased diversity we will be better able to conduct innovative and world-leading cancer research in support of Cancer Research UK's ambition of 3 in 4 people surviving their cancer by 2034.

THANKS FOR SUPPORTING US

The work of our various research groups would barely proceed without the substantial grant funding provided by Cancer Research UK to the CRUK Scotland Institute and the University of Glasgow, now amounting to £20 million per annum combined. We are also indebted to a number of other organisations that provide funding to our scientists, usually supporting projects in a particular sphere of special interest, or supporting the careers of talented junior scientists, enabling them to pursue their research interests within our laboratories. These organisations, whose funding we appreciate greatly, are listed below. The additional funding provided by these organisations makes possible much work that we otherwise could not be undertaking and has become integral and indispensable to our operations.

Cancer Research UK Scotland Institute

Tom Bird
University of Edinburgh, American Friends of Cancer Research, Trogenix, Precision Medicine Scotland, Tenovus,

Karen Blyth
MRC, The Urology Foundation

Martin Bushell
BBSRC

Kirsteen Campbell
Prostate Cancer Research, PCUK

Leo Carlin
British Society for Immunology, MRC

Vicky Cowling
ERC, MRC, Wellcome Trust

Zoi Diamantopoulou
UKRI

Payam Gammage
EPSRC, NIH, EMBO

Danny Huang
AstraZeneca, BBSRC

Gareth Inman
British Skin Foundation, DEBRA

David Lewis
NIH

Tom MacVicar
Medical Research Scotland, Genetics Society

Crispin Miller
NC3R's

Jennifer Morton
Pancreatic Cancer UK, MRC, Avacta

Jim Norman
Chief Scientist Office, MRC

Valeria Pavet
Trogenix, Oxford Drug Design

Ed Roberts
Beatson Cancer Charity, Cell Guidance Systems

Kevin Ryan
The Kay Kendall Leukaemia Fund

Owen Sansom
AstraZeneca, Boehringer Ingelheim, Chief Scientist Office, McNab, MRC, NHS Greater Glasgow & Clyde Health Board Endowment Fund, Novartis, Pancreatic Cancer UK, The Mark Foundation, Tenovus

Douglas Strathdee
Scenic Biotech

Saverio Tardito
University of Bergen

School of Cancer Sciences, University of Glasgow

Imran Ahmad
Beatson Cancer Charity, China Scholarship Council, Wellcome

David Bryant
UKRI, China Scholarship Council

Seth Coffelt
Breast Cancer Now, Indonesian Government Scholarship McNab, Medical Research Scotland, MRC, NHS Lanarkshire, Pancreatic Cancer UK, Worldwide Cancer Research

Julia Cordero
China Scholarship Council, The Royal Society, UKRI/ESPRC, Wellcome Trust

Fieke Froeling
CRUK NCI Cancer Grand Challenge, AstraZeneca, Pancreatic Cancer UK

John Le Quesne
Celgene, Jean Shanks Foundation

Daniel Murphy
Asthma + Lung UK, Merck, MRC

Colin Steele
Chief Scientist Office, McNab, UKRI, University of Edinburgh

Stephen Tait
Prostate Cancer Research, Swiss National Science Foundation

We do not purposefully solicit contributions to our work directly from the general public – we see this as the role of the cancer charities such as those that feature above. We are, however, fortunate to be in the minds of many local people and organisations that give generously of their time and effort to raise funds for good causes. We are also, more poignantly, in the minds of those who are suffering cancer, or who have lost loved ones to this disease. To those who give time and effort to raise funds on our behalf and to those who thoughtfully regard us as suitable beneficiaries of their generosity, thank you.

Bearsden Bowling Club
Mrs A M Bell
Better Points Ltd
Charities Aid Foundation
Charities Trust
Mr James Corbett
Mrs S Dempster
FirstMortgage.co.uk
Avril Haddow
Huber
Inverkeithing Senior Citizen Centre
Mrs Jacqueline Jackson
Mrs Margaret Johnstone
Fergus Leitch
Mrs Janet Lyke
The Mark Foundation for Cancer Research
Mrs Marion Marshall
Joseph McCarthy
Mrs Fiona McNeill and Family
Parse Biosciences
Mrs Patricia Simpson
St Bryce Kirk – *Donation raised by church at Mr William Cumming's retirement*
St John the Baptist Primary School
Stepps Bowling Club
Strathmore Funeral Directors – *in memory of the late Liz Murrie*
Mr John Teevan
C R Thompson
West of Scotland Women's Bowling Association
Olive Wilson
I Young
Just Giving
PayPal

PATRONS AND BOARD OF DIRECTORS

Patrons

His Grace the Duke of Hamilton
The Rt Hon. Lord Mackay of Clashfern
The Viscountess Weir

Board of Directors

The Cancer Research UK Scotland Institute is an autonomous charity, constituted as a company limited by guarantee, registered in Scotland. The Institute is governed by its Board of Directors who are the directors of the company and trustee of the charity. The Board is ultimately responsible for all aspects of the Institute, including its scientific strategy, operational policies, regulatory compliance and financial stewardship and accountability. On a day-to-day basis, many of these responsibilities are delegated to the Institute's Management Team.

Prof Sir John Iredale (Chair)
Professor of Experimental Medicine, University of Bristol

Ms Rosalie Chadwick
Partner, Pinsent Masons

Dr Iain Foulkes
Executive Director, Research and Innovation, Cancer Research UK and CEO, Cancer Research Horizons

Mr James Kergon
Senior Partner, KPMG Scotland

Prof Iain McInnes
Head of College of Medical, Veterinary and Life Sciences, University of Glasgow

Mr Adrian Walsh
Interim Finance Director
Cancer Research UK

Company Secretary
Mr Gary Niven
The Cancer Research UK Scotland Institute

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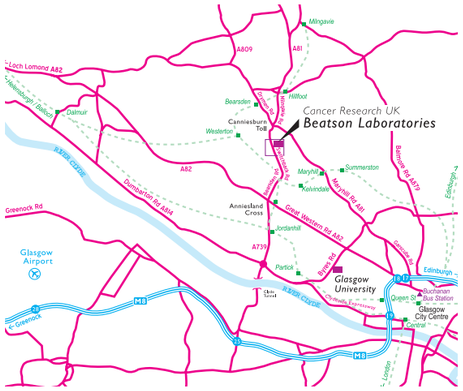
The Beatson Institute for Cancer Research [trading as the Cancer Research UK Scotland Institute] is a registered charity in Scotland (SC006106) and registered as a company limited by guarantee in Scotland (84170). Registered address: Cancer Research UK Beatson Institute, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD

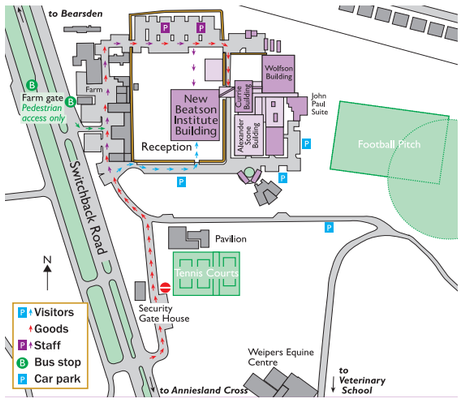
Tel +44(0) 141 330 3953

Electronic version of this report can be found at:
www.crukscotlandinstitute.ac.uk/about/annual-report

Cancer Research UK
Cancer Research UK is a registered charity in England and Wales (1089464), Scotland (SC041666) and the Isle of Man (1103). Registered address: 2 Redman Place, London, E20 1JQ.

Tel 0300 1231022
www.cruk.org





NOTES

NOTES

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Jacqueline Beesley

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