CANCER RESEARCH UK | Scotland Institute

SCIENTIFIC REPORT 2023

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COVER IMAGE

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SCIENTIFIC REPORT 2023

SCOTLAND INSTITUTE

University of Glasgow

CANCER RESEARCH UK | Scotland Institute

Cancer Research UK Scotland Institute *Image by Laura Ashman*

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DIRECTOR'S INTRODUCTION

Director of the Cancer Research UK Scotland Institute

Professor Owen Sansom

FRSE, FMedSci, FRCPS(Glasg)

2023 was an extremely important year for the Institute. The progress we have made with our key research themes over the last five years and our future plans were very positively reviewed during the Institute quinquennial review (QQR) in March. Overall, the panel of international experts felt the Institute was performing very well with world-class animal modelling and integrated state-of-the-art technologies led by excellent researchers. They were impressed by our science and very excited by our future vision and strategy (see page 6). Following the QQR and the confirmation of our funding of up to £123 million over the next seven years, the Institute rebranded as the "CRUK Scotland Institute"

We made several important strategic appointments in 2023. New research fellows, Dr Xiao Fu (previously at the Crick in London), who works on computational methods to map spatial features of the tumour microenvironment, Dr Kendle Maslowski (previously in Birmingham), who holds a CRUK Career Development Fellowship focused on bacterial cancer therapy and immune responses, and Dr Zoi Diamantopoulou (previously at ETH in Zurich), who studies how circadian rhythms might regulate metastasis, started between June and August this year. We also begun hosting Dr Maxi Portal (previously at CRUK Manchester Institute), who works on cell plasticity and epigenetics, for two years.

Senior Translational Scientist, Dr Valeria Pavet joined us in March with the aim of increasing our industry interactions, supporting spinouts, and improving the link between preclinical and clinical experiments. In addition, we appointed a new Head of People and Culture, Sharon Gorman, to replace Angela Stuart, our Head of HR, who retired after many years of excellent service in May. Sharon will be reviewing our EDI action plan and comparing it with best practice across the sector. For example, details of our gender pay gap and how we are tackling this can be found on page 139 of this report. I was also delighted this year to see so many of our researchers featured in news articles or participating in events for International Day of Women and Girls in Science, International Women's Day and LGBTQIA+ People in STEM Day and LGBTQIA+ People in STEM Day and in November, we hosted the first ever Betty MacGregor Memorial Showcase to highlight the contributions of women in cancer research (see page 131).

We had a couple of key departures in 2023. Professor Robert Insall, who has done some exciting work using computational cell biology to study metastasis, moved to University College London in October, while Dr Kristina Kirschner, who oversaw our single cell RNA sequencing facility, moved to the Mayo Clinic in the US.

We made good progress in targeting significant external funding this year. Professor Jen Morton was awarded an MRC Programme grant "Investigating complex crosstalk in the pancreatic cancer microenvironment" (£2 million over five years), while Dr Ed Roberts is co-leading a Prostate Cancer UK Transformational Impact Award "Investigating the tumour microenvironment of high-risk localised prostate cancer to identify actionable pathways involved in cancer" (£1.45 million over three years) with Professor Hing Leung (Glasgow). Dr David Lewis and Professor David Newby (Edinburgh) were awarded £12 million in MRC funding to establish a Scottish total-body PET facility, while Dr Leo Carlin received MRC funding (totalling £1 million) for a light sheet microscope and a project with Professors Tom Bird and Derek Mann (Newcastle) entitled "Neutrophils and cellular senescence: A vicious circle promoting age-related disease". Professor Tom Bird also received a Sir Jules Thorn Biomedical Research Award (£1 million over five years) and postdoc Dr Johan Vande Voorde will be taking up an independent group position in the School of Cancer Sciences in January with a CRUK Career Development Fellowship (£1.5 million over six years). Professors Seth Coffelt and Tom Bird and Dr Steph May were awarded CRUK Biology to Prevention grants, while through the CRUK Scotland Centre, several of us along with Professor Steve Pollard (Edinburgh) received an Innovate UK grant investigating the use of novel AAV viral vectors for gene therapy in colorectal cancer and hepatocellular carcinoma. Related to this, we received £2 million as part of a UKRI funded Engineering Biology Mission Hub being led by Professor Susan Rosser (Edinburgh), which will develop tools for synthetic biology approaches. In addition, I would like to congratulate postdoc Dr Susanti Susanti, who was a keynote speaker at the Cancer Research Horizons Innovation Summit in London this year, talking about the start-up she founded in Indonesia.

We were pleased to host visits by KJ Patel, CRUK's newly appointed Chief Scientist, and David Crosby, Head of Prevention and Early Detection Research at CRUK in early 2023. In addition, we held our first in-person open evening for three years with speakers Professors Kevin Ryan and Seth Coffelt and Drs Tom Drake and Holly Hall and several demos and lab tours provided by a group of 30 plus researchers.

Finally, it was announced in February that the number of people in Scotland diagnosed with cancer will rise by a quarter by 2040, taking the number of new cases to more than 42,000 for the first time. This is a sobering number, but it makes us even more determined to progress our shared vision of collaborative, world class cancer research.

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?

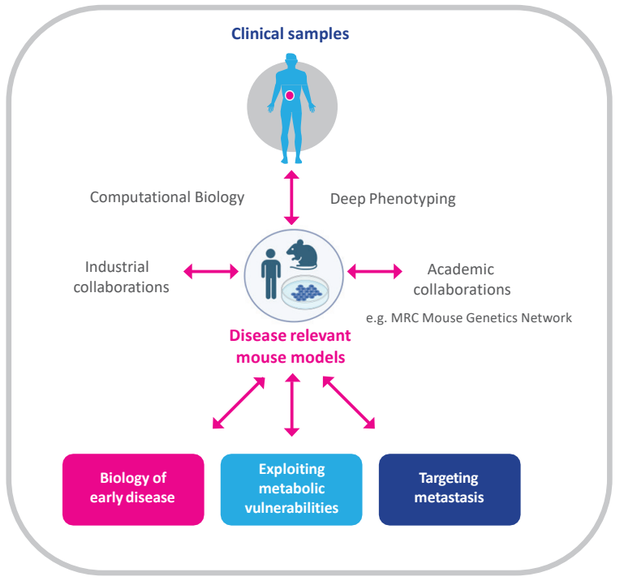
At the heart of our strategy is the generation and use of an unparalleled suite of complex *in vivo* and organoid models that accurately recapitulate critical events in the human disease - such as tumour initiation, growth and metastasis (Figure 1). To achieve this effectively, we have put particular 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 synthesis to block initiation and progression
4. Targeting energetic stress and tumour microenvironment following radiotherapy
5. Determining the impact of mitochondrial mutations in initiation and progression
6. Building a greater understanding of cancer immunology



RESEARCH HIGHLIGHTS

Bader AS, Bushell M. (2023)
iMUT-seq: high-resolution DSB-induced mutation profiling reveals prevalent homologous-recombination dependent mutagenesis.
Nat Commun. 14(1):8419.

Work by Martin Bushell and Aldo Bader describes iMUT-seq – a technique that profiles DNA double strand break (DSB)-induced mutations at a high sensitivity and single-nucleotide resolution around endogenous DSBs. This paper shows for the first time that scars introduced into the DNA sequence following DSBs are not just at the break site but also at a distance from the damage site. The team went on to determine the main mechanism by which these errors are introduced. Homologous-recombination (HR) repair, which was previously believed to be error free, is introducing errors at a distance from the break site and these errors are due to the polymerase used in this process.

Bailey P et al. (2023)
Driver gene combinations dictate cutaneous squamous cell carcinoma disease continuum progression.
Nat Commun. 14(1):5211.

This paper from Gareth Inman's lab provides a comprehensive overview of the molecular drivers and events that promote cutaneous squamous cell carcinoma (cSCC) disease progression from UV-induced precancerous actinic keratosis to malignant invasive cSCC and metastasis. The researchers performed cross-species experiments to reveal that the disease progresses in a continuum from a differentiated to a progenitor-like state. Knowing the events that promote and accompany disease progression provides potential therapeutic targets for cSCC.

Dzien P et al. (2023)
Positron emission tomography imaging of the sodium iodide symporter senses real-time energy stress in vivo.
Cancer Metab. 11(1):14.

David Lewis and colleagues' paper proposes positron emission tomography (PET) imaging of the sodium iodide symporter (NIS) as a rapid and sensitive in vivo test for metabolic treatments targeting energetic pathways. This new approach would make in vivo testing more practical, giving an immediate readout after a single dose, reducing drug costs, animal use, and the time associated with long therapeutic studies.

Farahmand P et al. (2023)
Asbestos accelerates disease onset in a genetic model of malignant pleural mesothelioma.
Front Toxicol. 5:1200650.

Using a genetic model of mesothelioma, CRUK Scotland Institute scientists, Pooyeh Farahmand and Daniel Murphy show that asbestos exposure, although not required for its development, speeds up cancer growth dramatically. On a cellular level, the presence of asbestos leads to increased infiltration of macrophages, however, no effects on survival are observed when these immune cells are targeted pharmacologically.

Grotehans N et al. (2023)
Ribonucleotide synthesis by NME6 fuels mitochondrial gene expression.
EMBO J. e113256.

Work by Tom MacVicar and colleagues reveals that the kinase NME6 drives mitochondrial gene expression. The researchers demonstrate that NME6 supplies pyrimidine ribonucleotides for mitochondrial transcription and ensures accumulation of mitochondrial transcripts and respiratory complexes. Additionally, loss of NME6 or its kinase activity impairs mitochondrial gene expression and OXPHOS function. This research highlights the importance of mitochondrial ribonucleotide metabolism for healthy mitochondrial function.

Koessinger D et al. (2023)
Glioblastoma extracellular vesicles influence glial cell hyaluronic acid deposition to promote invasiveness.
Neurooncol Adv. 5(1):vdad067.

Jim Norman's lab show that p53-mutant glioblastoma cells release podocalyxin-containing extracellular vesicles, which stimulate neighbouring brain cells to secrete factors that create an environment favourable to cell invasion. This investigation of the tumour microenvironment highlights a potentially druggable target for this cancer type, which has a particularly poor prognosis.

May S et al. (2023)
Absent expansion of AXIN2+ hepatocytes and altered physiology in Axin2CreERT2 mice challenges the role of pericentral hepatocytes in homeostatic liver regeneration.
J Hepatol. 78(5):1028-1036.

This paper from Stephanie May in Tom Bird's group, challenges the role of pericentral hepatocytes in liver homeostatic regeneration and highlights the importance of detailed preclinical model characterisation and the pitfalls of comparing across sexes and backgrounds of mice and the effects of genetic insertion into native loci.

Neilson L J et al. (2023)
Omentum-derived matrix enables the study of metastatic ovarian cancer and stromal cell functions in a physiologically relevant environment.
Matrix Biology Plus. 19-20(December 2023).

Sara Zanivan's group provide a step forward in the study of high-grade serous (HGS) omental metastasis with the proposal of a newly developed, clinically and physiologically relevant matrix: omentum gel (OmGel). OmGel is a matrix made from tumour-associated omental tissue of HGS ovarian cancer patients that has unprecedented similarity to the extracellular matrix of HGS omental tumours. OmGel has been shown to perform as well or better than the widely used Matrigel and did not induce additional phenotypic changes to ovarian cancer cells.

Nikolatou et al. (2023)
PTEN deficiency exposes a requirement for an ARF GTPase module for integrin-dependent invasion in ovarian cancer.
EMBO J. e113987.

This study from David Bryant and colleagues identifies ARF6 as a therapeutic vulnerability in PTEN-depleted high-grade serous ovarian carcinoma. PTEN depletion leads to PIP_3-rich invasive membrane protrusions into the extracellular matrix and ARF6 was found to be essential to this process. The expression of an ARF6 module (CYTH2-ARF6-AGAP1) was shown to be inversely associated with patient outcome, allowing for the prediction of clinical outcomes in ovarian cancer patients.

Pirillo C et al. (2023)
Cotransfer of antigen and contextual information harmonizes peripheral and lymph node conventional dendritic cell activation.
Sci Immunol. 8(85):eadg8249.

The Roberts group examines how the immune system can direct appropriate T cell responses when the lymph node is spatially separated from the site of immune challenge. They found that dendritic cells pass 'packets' of information, including antigens, as well as contextual cues, to lymph node resident cells so they can then respond appropriately. In cancer this means that the immune suppression occurring in the tumour gets transported and transferred to the lymph node, helping explain the poor T cell responses we see to cancer.

Roman-Fernandez A et al. (2023)
Spatial regulation of the glycocalyx component podocalyxin is a switch for prometastatic function. *Sci Adv*. 9(5):eabq1858.

This study shows that Podocalyxin (PODXL), a transmembrane protein that is part of the cell's outer coating, promotes invasive behaviour in prostate cancer cells. Delivery of PODXL to the cell surface, rather than expression per se, enables it to bind to GAL3 and therefore relays effects to the cell's anchoring system, resulting in increased metastatic spread in vivo – as such, intervening with the localisation of PODXL to the cell membrane may be an attractive therapeutic target for restricting metastasis.

Sandilands E et al. (2023)
The small GTPase ARF3 controls invasion modality and metastasis by regulating N-cadherin levels.
J Cell Biol. 222(4).

In a publication in the Journal of Cell Biology, the Bryant lab screened the ARFome – a group of ARF proteins involved in membrane trafficking – for their involvement in the regulation of cell shape and movement in prostate cancer. Using multi-day, live imaging of 3D cell culture in combination with machine learning approaches, they identified ARF3 as a key regulating switch between whether cells move collectively or invade by being guided by a leading cell.

Schmidt T et al. (2023)
eIF4A1-dependent mRNAs employ purine-rich 5'UTR sequences to activate localised eIF4A1-unwinding through eIF4A1-multimerisation to facilitate translation.
Nucleic Acids Res. 51(4):1859-1879.

Downstream of cancer-associated mutations such as KRAS and mTORC, eIF4A1 operates as part of the eIF4F translational initiation complex which forms the basis of oncogene-induced dysregulated translation. In Nucleic Acids Research, CRUK Scotland Institute scientists led by Tobias Schmidt and Martin Bushell, recently showed that the activation of eIF4A1 is controlled through RNA-sequence-dependent recruitment

and multimerisation of eIF4A1. This results in localised unwinding of repressive RNA structure and ultimately translation of eF4A1-dependent mRNAs in cells.

Suzuki T et al. (2023)
β-Catenin Drives Butyrophilin-like Molecule Loss and γδ T-cell Exclusion in Colon Cancer.
Cancer Immunol Res. 11(8):1137-1155.

Seth Coffelt's group show that bowel cancer downregulates factors called butyrophilin-like (BTNL) molecules, which usually attract cancer-killing immune cells to the gut. Targeting pathways regulating BTNL offers a potential avenue to re-sensitise the immune system to cancer cell detection and therefore stop colorectal cancer from growing.

Vande Voorde J et al. (2023)
Metabolic profiling stratifies colorectal cancer and reveals adenosylhomocysteinase as a therapeutic target.
Nature Metabolism.

Johan Vande Voorde and the Cancer Grand Challenges Rosetta team use genetically engineered mouse models and multimodal mass spectrometry-based metabolomics to study the impact of common genetic drivers of colorectal cancer on the metabolic landscape of the intestine. Their work reveals that loss of the gene APC in the mouse intestine drives expression of the enzyme adenosylhomocysteinase (AHCY) which is transcriptionally upregulated in human colorectal cancer. This work highlights AHCY as a potential drug target for the second most common cause of cancer-related deaths worldwide.

Whyte D et al. (2023)
NUAK1 governs centrosome replication in pancreatic cancer via MYPT1/PP1beta and GSK3beta-dependent regulation of PLK4.
Mol Oncol. 17(7):1212-1227.

Work from Daniel Murphy's lab with Jen Morton and Martin Bushell describes a novel role for NUAK1 in chromosome segregation and centrosome duplication during cell division. Although growing evidence has suggested NUAK1 as a potential vulnerability in cancer, particularly in conjunction with KRAS and MYC, this work raises questions about the application of anti-NUAK1 therapies.

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

RESEARCH GROUPS

www.crukscotlandinstitute.ac.uk/imran_ahmad

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)
Athul Kurian (MSc)
Vishaani Johnson Raja (MSc)

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.

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.

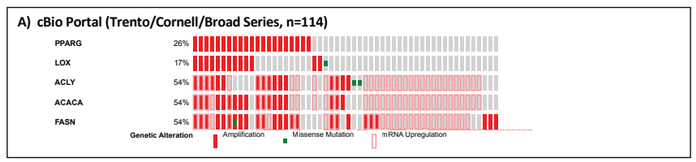


Figure 1. Data from cBio portal (www. cbioportal.org) demonstrating PPARγ gene amplification or its upregulated mRNA expression in 26% of clinical castrate-resistant prostate cancer specimens, with upregulation of one or more of the lipid synthesis genes (FASN, ACC, ACLY)

Figure 2.

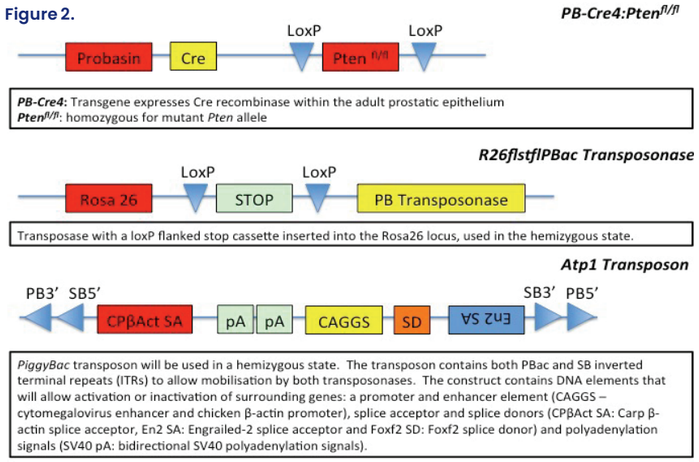


Figure 3.

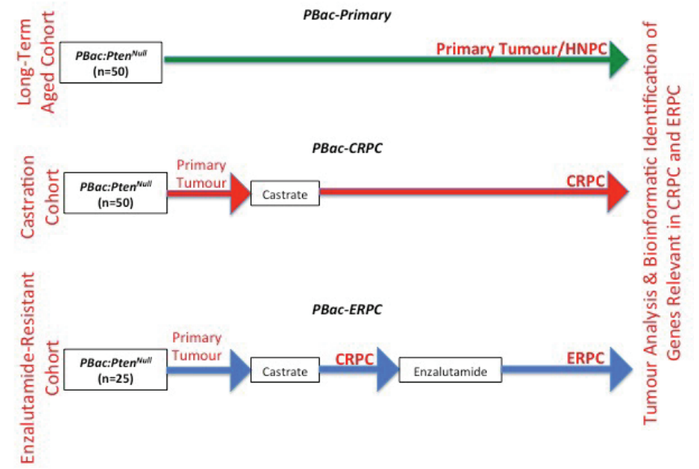
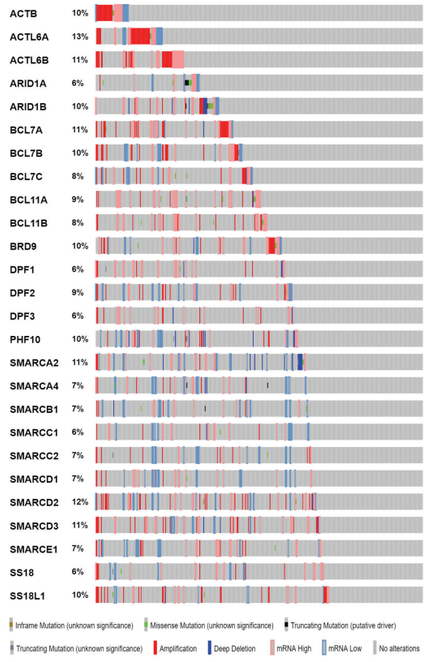


Figure 2. Genetic modifications of the PiggyBac mice.

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



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.

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, and successfully defended his thesis in 2023.

Role of MBPTS2 in prostate cancer

Membrane-bound transcription factor site-2 protease (*Mbtps2*), which was also been 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.

Publications listed on page 108

LIVER CANCER, DISEASE AND REGENERATION

Group Leader
Tom Bird

Professor of Hepatobiliary Cancer (University of Edinburgh)
Honorary Consultant Hepatologist
Senior Clinical Lecturer (University of Glasgow)

Principal Scientific Officer
Stephanie May

Research Scientists
Toshiyasu Suzuki
Fiona Chalmers
Anastasia Georgakopoulou
Megan Quince
Bashaer Alqarafi
Kyi Lai Yin Swe

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. We believe that understanding how, within an individual, specific mutational combinations drive a liver cancer 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 lead to cancer is central to precision prevention strategies for liver cancer development and may aid the early detection of disease. Similarly, understanding how specific combinations of mutations sustain cancer 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 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's aim is to create a network for HCC research and 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. 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.

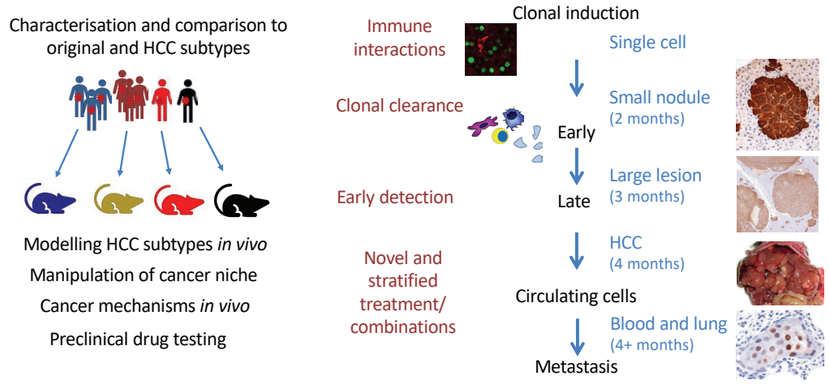
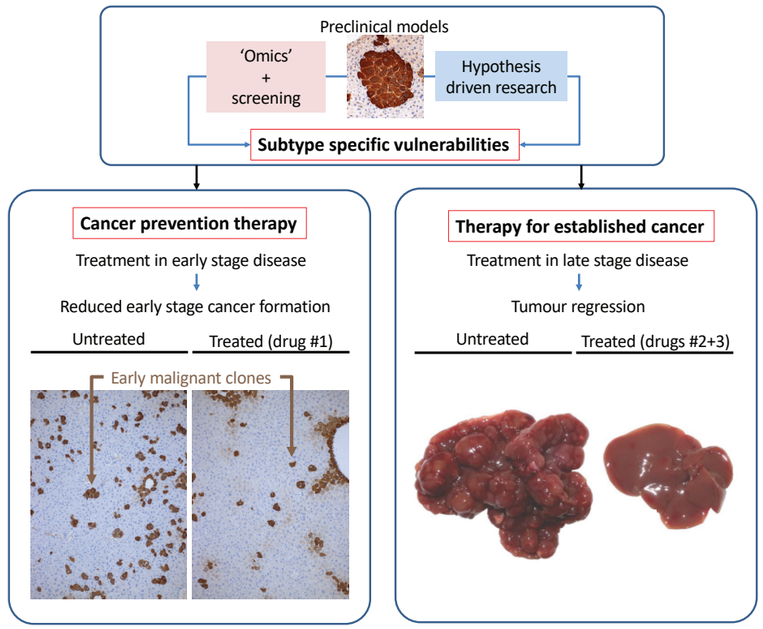


Figure 2. Cancer prevention in preclinical models by targeting early tumour clones. We are able to explore specific vulnerability of individual liver cancer subtypes. We have identified pathways which are specifically activated in early disease. When we apply therapies to early disease, we can reduce 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.



IN VIVO CANCER BIOLOGY

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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 complex and dichotomous role of the *RUNX* genes in breast cancer. Following her PhD defence, Dr Adiba Khan has continued to probe how CBFβ restricts WNT-driven mammary tumourigenesis using RNA sequencing to compare the transcriptome of *Cbfb* proficient and deficient mammary tumours. Together with Adiba, Masters student Merna Maung studied the concept that CBFβ regulates breast cancer stem cell activity. In addition to identifying key cellular pathways that RUNX/CBFβ regulate in breast cancer, we found that loss of the complex evokes changes to the tumour microenvironment where an important aspect of RUNX/CBFβ activity may be to orchestrate the immune microenvironment, a hypothesis that Amy Lawlor will investigate in her PhD.

Investigating the function of MCL-1 in tumour development and targeting the BCL-2 family to improve cancer therapy.

MCL-1 is a pro-survival member of the BCL-2 family that has typically been investigated in cancers of the blood where its overexpression facilitates tumour development and resistance to therapy. We were one of the first to show that MCL-1 is elevated in breast cancer where it is associated with poor prognosis. Targeting MCL-1 genetically, or with BH3-mimetic drugs, can restrict breast cancer growth in cell lines, and in mouse models of breast cancer (Campbell et al., *Cell Death Diff.* 2021). Building on this work, we are investigating the stage(s) of tumourigenesis where MCL-1 is critically required with the aim of identifying the point of maximum impact for drugs targeting MCL-1 to be used in the clinic. Interestingly, we have found a role for MCL-1 in breast cancer stem cells, thought to be responsible for tumour initiation, metastasis and treatment resistance (Figure 1). Dr Kirsteen Campbell and PhD student Matthew Winder are investigating the role and requirement for MCL-1 at all stages of tumourigenesis using *in vitro* and *in vivo* approaches. We hope that understanding the requirement for MCL-1 at early stages of tumour evolution may allow the development of cancer preventative treatment approaches.

There are several similarities between breast and prostate cancer, including high levels of MCL-1 expression. Advanced prostate cancer, where the tumour has spread to distant sites around the body, is a lethal diagnosis. MCL-1 seems preferentially increased in advanced prostate cancer and in metastases. Funded by Prostate Cancer Research, Dr Laura Martinez-Escardo is investigating the role of MCL-1 as a barrier to tumour cell elimination by prostate cancer therapies. Laura is using a combination of approaches including genetic targeting with CRISPR/Cas9 genome editing, inhibition of MCL-1 with BH3-mimetic drugs, and analysis of patient tumour tissue (Figure 2). MCL-1 as a valid target in prostate and breast cancer could expedite the use of MCL-1 inhibitors in these cancer types. We have also identified that pancreatic cancers with high levels of MCL-1, and a closely related protein called BCL-XL, have worst outcome. Pancreatic cancer is a difficult to treat cancer with devastatingly poor prognosis. Yasmin Hunter in the lab is targeting the BCL-2 family in pancreatic cancer cell lines to identify patient sub-groups that might respond to novel therapeutic treatments in a grant funded by a Pancreatic Cancer UK Research Innovation Award led by Kirsteen.

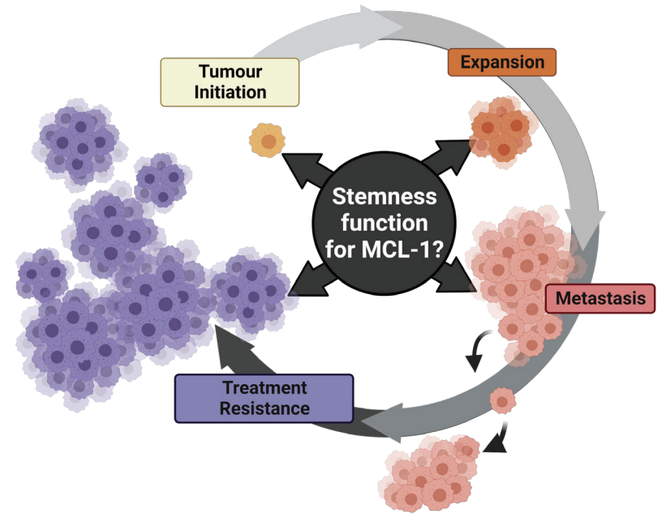

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Figure 1. Multiple stages where MCL-1 could play a key role in tumour development
Diagram to demonstrate the multiple stages of the tumourigenic process where MCL-1 could have a stemness function to enhance tumour initiation, expansion, metastasis, and treatment resistance.

Targeting the BCL-2 family to improve tumour cell elimination by radiotherapy.

As discussed, expression of pro-survival members of the BCL-2 family (e.g. MCL-1, BCL-2 and BCL-XL) are frequently upregulated in cancer. This elevated expression acts as a barrier to efficient cell death induction by cancer therapies including radiotherapy. Radiotherapy is one of the most widely used anti-cancer treatments with more than half of cancer patients receiving radiotherapy. In a CRUK RadNet funded project co-led by Kirsteen and Dr Joanna Birch, we have been investigating whether inhibition of pro-survival BCL-2 proteins can sensitise to radiotherapy and lead to more efficient cancer cell elimination in pancreatic cancer and glioblastoma using BH3-mimetic drugs. Masters students Eunbee Ko and Rinta Mathew have also been investigating radiosensitisation in prostate and breast cancer cells. We are excited to build on this work with the recent award of a CRUK Radiation Research Network Seed Fund grant where we will target the BCL-2 family in combination with radiotherapy in pancreatic cancer, glioblastoma, lung, breast and prostate cancer in a collaborative project with colleagues from University of Glasgow, CRUK Cambridge Institute, and Institute of Cancer Research.

MRC National Mouse Genetic Network (NMGN) Cancer Cluster

The lab is excited to co-lead 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 we are also 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.

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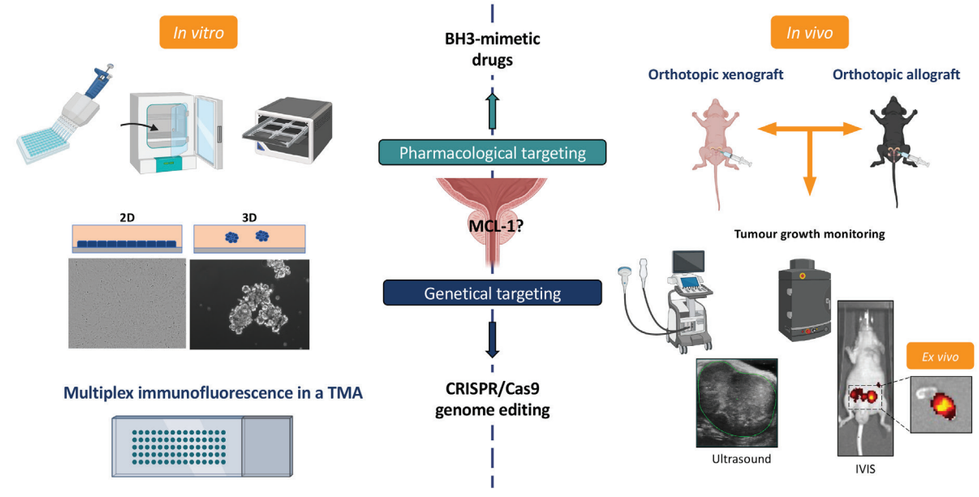

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Figure 2. Investigation of the role of MCL-1 in prostate cancer
The lab is using complementary approaches of genetic (e.g. CRISPR/Cas9 genome editing) and pharmacological (with BH3-mimetic drugs) targeting to study MCL-1 in prostate cancer. We use 2D and 3D cultures of prostate cancer cell lines, as well as orthotopic transplantation of fluorescently labelled human (xenograft) or murine (allograft) cancer cells into the mouse prostate, monitoring tumour growth and metastasis using ultrasound and fluorescent (IVIS) imaging. Multiplex immunofluorescence in a tissue microarray (TMA) of prostate cancer tumour samples is used to investigate associations between MCL-1, molecular pathways, and patient clinical data.

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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 sometime opposing roles in invasion, and show that there is specificity of signalling between family members. In two publications this year 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 (Sandiland *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.

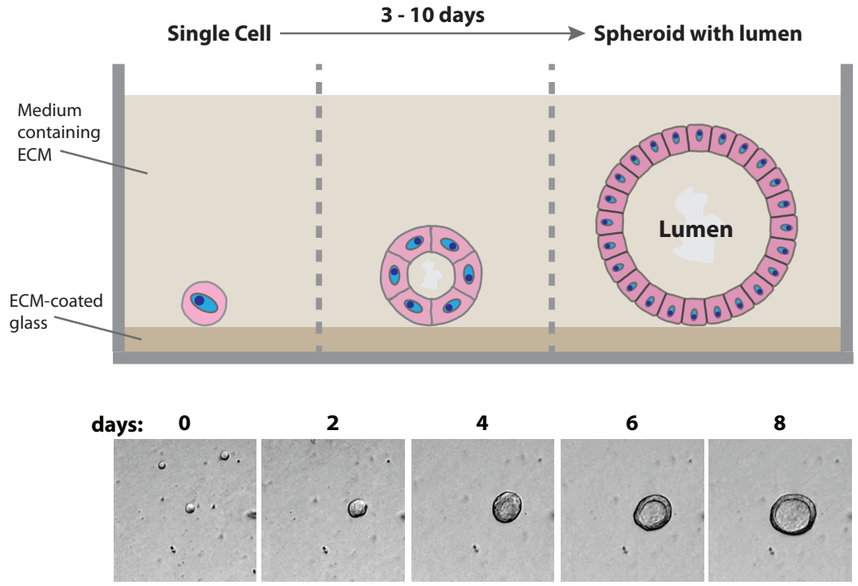

Figure 1. By culturing cells on glass-bottomed chambers coated with extracellular matrix (ECM), we direct the self-assembly of single cells into multicellular spheroid structures with a single, central lumen. This process occurs over 10 days, allowing us to study the dynamics of tissue formation.

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. We identified in PTEN-null prostate and ovarian tumours that the ARF6 GTPase is required for invasive activities in cancer. 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.

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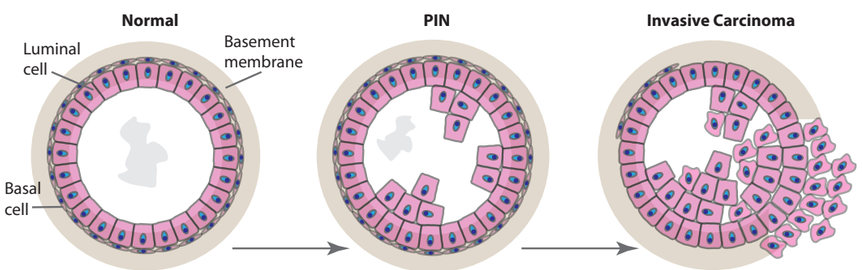

Figure 2. 3D cultures of cells to form cysts (also called spheroids or organoids) also allows us to model the loss of normal tissue architecture that occurs in cancer. For example, the progressive disrupted organisation of Normal, to Prostatic Intraepithelial Neoplasia (PIN), to Invasive Carcinoma typifies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

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, actually 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. 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 tolerated. However, in colorectal cancer (CRC) models, loss of eIF4A1 leads to reduced proliferation and increased survival, but the loss of eIF4A2 accelerates tumorigenesis 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 oncogenic Wnt signalling requires both the upregulation of Myc's transcriptional targets and the eIF4A1-dependent translation of these mRNAs. To test this, we 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 (Figure 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 *Apc*$^{-/-}$ *Kras*^{G12D} 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 (Figure 1B). This suggests 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. This interdependency of Myc and eIF4A1 to drive hyperproliferation (Figure 1C) 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 (**Figure 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 (**Figure 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 (**Figure 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 (**Figure 2C**). Utilising bioinformatics approaches we are currently investigating the mRNA features that may drive these distinct mRNA sensitivities towards the compounds. Preliminary results suggest that specific mRNA sequence motifs play a role in this process. 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. 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.

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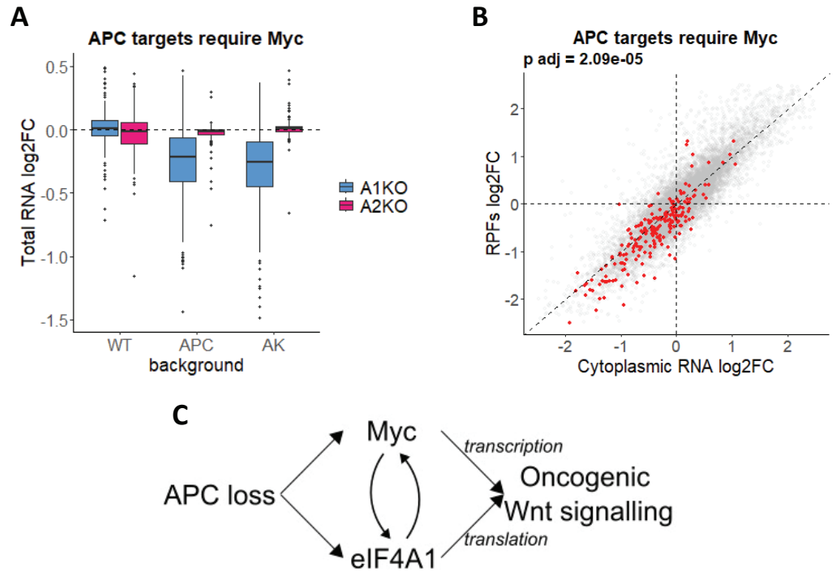

Figure 1. (A) Boxplot depicting changes in mRNA abundance of all Myc-targets (downstream of APC loss), within the small intestines of *Eif4a1*$^{fl/fl}$ (A1KO) or *Eif4a2*$^{fl/fl}$ (A2KO) mice in the stated genetic background. Loss of eIF4A1 but not eIF4A2 leads to the downregulation of the abundance 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 *Apc*$^{fl/fl}$ *Kras*^{G12D}/+ 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)** 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.

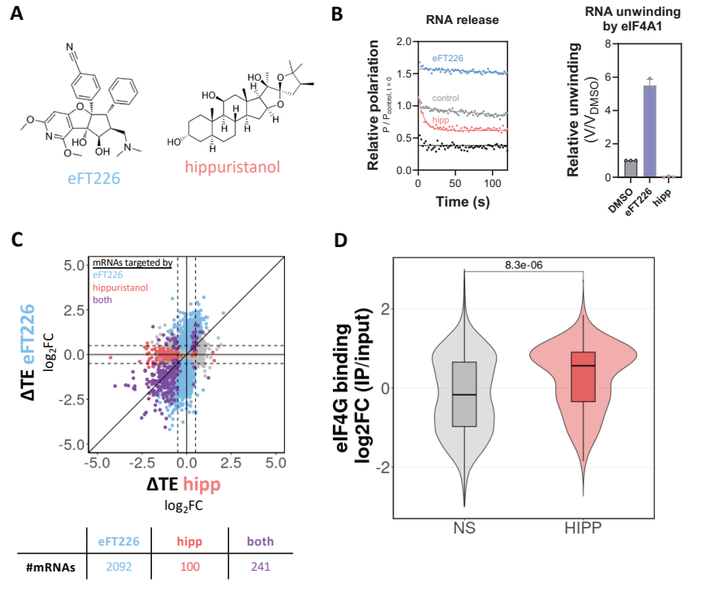

Figure 2. eFT226 and hippuristanol target distinct eIF4A1-dependent mRNAs.
(A) Chemical structures of eFT226 and hippuristanol. **(B)** RNA release of eIF4A1 bound to RNA in the absence (grey) or presence of eFT226 (blue) and hippuristanol (red). RNA in the absence of protein is shown in black. eFT226 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.

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, and human 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.

As part of an experimental fly-to-bedside clinical trial (*NCT02363647*), we recently reported a fly-based treatment of a CPCT patient with an advanced KRAS-mutant treatment-resistant colon adenocarcinoma. Building a patient-matched 9-hit 'personalised fly avatar', we identified a combination of trametinib plus zoledronate as effective in rescuing avatar viability (**Figure 1**) and a strong partial response in the patient (**Figure 1**) that exceeded 11 months.

Exploring our large set of patient-based colon cancer 'personalised fly avatar' lines, we found that increasing genetic complexity led to multiple avenues of drug resistance. For example, we have identified upregulation of detoxification pathways when specific cancer genes are paired. Blocking these emergent networks is sufficient to reveal a drug's full activity, leading to tumour shrinkage. We are taking both multi-drug and medicinal chemistry approaches to circumvent these resistance networks in flies and mouse/human organoids. Leaning into our biology tools, we are further connecting these resistance networks to fundamental biological processes such as 'cell competition', broadening our understanding of the relationships between complex mutation profiles, cell competition, and drug response.

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, which proved effective: 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 are now working to bring a new candidate lead therapeutic into clinical trials.

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 patients for clinical trials is challenging and, ideally, a trial would accept a broad cross-section of Rasopathy 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 as well as different levels of RAS activity as assessed with phosphorylated ERK (pERK), mirroring differences in RASopathy patients. Our models indicate these signalling differences have consequences: while several drugs worked against one or a few fly models, few drugs worked with multiple fly RASopathy models, emphasising the unique whole-body challenge presented by the RASopathies. We are currently working with Maria Kontarides to explore these compounds in mouse RASopathy models, as well as a drug company to help advance our most promising leads towards clinical trials.

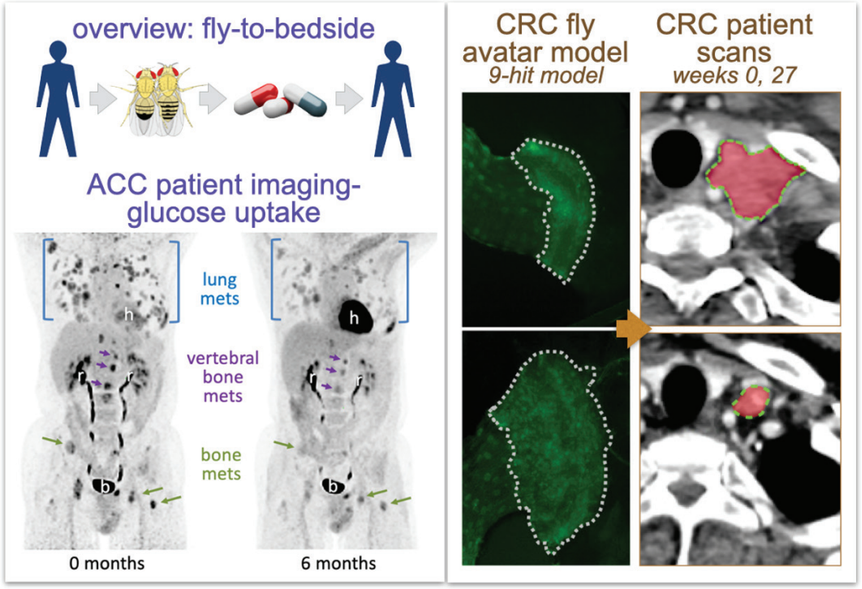

Figure 1. Our fly-to-bedside clinical trial, which led to successful treatment of adenoid cystic and colorectal cancer patients.

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 disease networks through 'rational polypharmacology', a whole animal version of Quantitative Structure/Activity Relationship (QSAR). We combine fly genetics with medicinal and computational chemistry, 'evolving' leads that are tuned for whole body efficacy (**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 RET-dependent thyroid and lung cancers, RAS-mutant colorectal cancer, hepatocellular carcinoma, and RASopathies. We are currently working with Lee Cronin's laboratory to further advance this technology through advanced automation, merging chemical evolution and 'chemputer' technologies.

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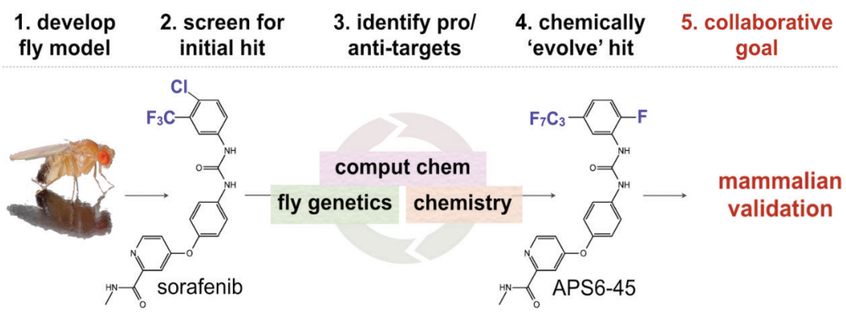

Figure 2. Platform to 'tune' therapeutic leads.

www.crukscotlandinstitute.ac.uk/leo_carlin

LEUKOCYTE DYNAMICS

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Leo Carlin

Fellow of the Royal Microscopical Society

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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 produce factors that 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. 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 a number of different cell types simultaneously to glean more information about the way that they interact and to uncover potential pathways to modify.

Neutrophil plasticity has only recently been appreciated but is consistent with tissue sensing features that seem to be general for immune cells of the myeloid lineages. Cellular cross-talk is often at the heart of how this plasticity is regulated in the tissues. Work led by Ximena Raffo-Iraolagoitia, in collaboration with the Murphy and Coffelt labs at the Institute, has identified important cross-talk between γδ T cells and macrophages in a lung cancer model. By taking a combined single cell transcriptomic / 3D multiplexed immunofluorescence approach (Figure 1), this work predicted interactions computationally from the transcriptomic data (Figure 1 A, B.) and then directly observed them by imaging across tissue compartments in the lung (Figure 1 D-E). We pre-printed this work in *BioRxiv* in 2023 (https://doi.org/10.1101/2023.09.14.557344).

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.

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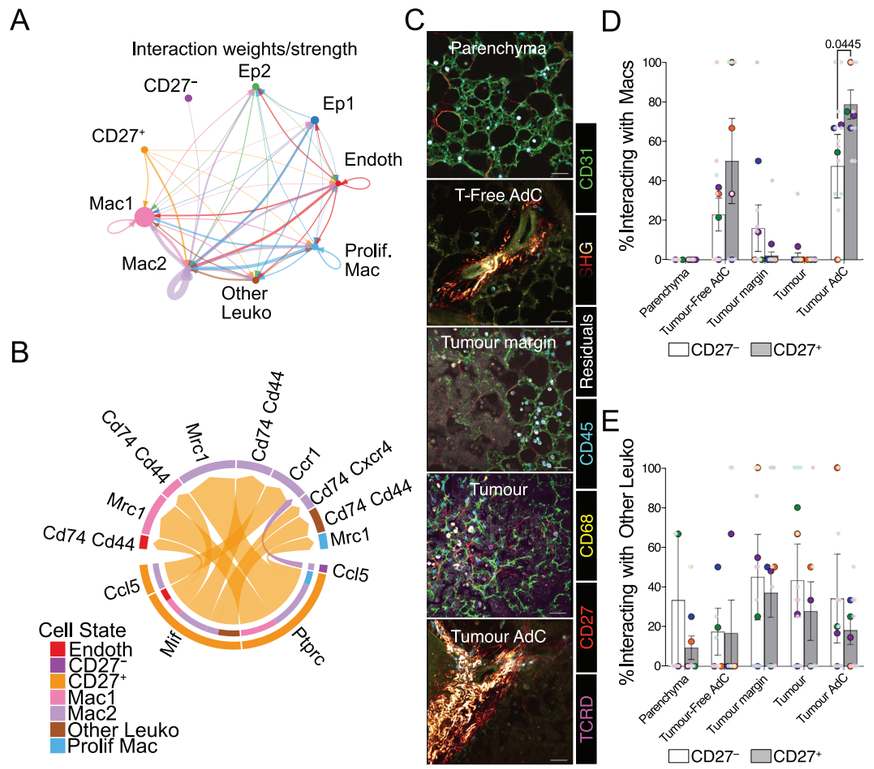

Figure 1. γδ T cell – macrophage interactions in a lung cancer model. ScRNA-seq data from microdissected SPC-Cre+ KM mouse tumours and naïve pulmonary γδ T cells (Edwards et al., 2023) were merged for CellChat analysis. (A) Aggregated cell-cell communication network visualised in a circle plot showing total interaction weights/strength between any two clusters. (B) Chord diagram showing all significant interactions from γδ T cells. (C) Representative fields of view of region types analysed in precision-cut lung slices; 50μm scale bars. (D,E) Quantification by confocal microscopy of CD27- (white) and CD27+ (grey) γδ T cells interacting with macrophages (CD45+CD68+, D) and other leukocytes (CD45+CD68-, E) grouped by different topologies in the tumour bearing lungs. Each colour represents a mouse, small dots for fields of view and big dots for means. Data are presented as mean ± SEM. Data were analysed by Mixed-effects Restricted Maximum Likelihood followed by uncorrected Fisher's Least Significant Difference tests. Taken from Figures 1, 2 in Raffo-Iraolagoitia et al. BioRxiv preprint 2023 https://doi.org/10.1101/2023.09.14.557344

IMMUNE CELLS AND METASTASIS

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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 2023, our lab contributed to three scientific papers, and we were asked to write two commentaries on the works of other researchers. Rob successfully completed his PhD. We said good-bye to Anna, and we welcomed Heather, Muhammad, Elizabeth, Cai, Amy and Yasmin.

Breast cancer

In previous years, we generated a single cell RNA sequencing (scRNAseq) dataset of γδ T cells isolated from the lungs of tumour-free and tumour-bearing mice. This analysis uncovered two new avenues of research in the lab. First, we found that subsets of IL-17A-producing γδ T cells express different co-inhibitory molecules on their surface. One subset (Vγ6$^+$ cells) express constitute levels of PD-1, while another subset (Vγ4$^+$ cells) upregulate TIM-3 in response to tumour-derived factors. Blocking either PD-1 or TIM-3 signaling in mammary tumour-bearing mice increases proliferation of Vγ6$^+$ or Vγ4$^+$ cells, respectively. This increase in Vγ6$^+$ or Vγ4$^+$ cell number counteracts T cell checkpoint inhibitor immunotherapy, as genetic deletion of γδ T cells sensitizes metastatic mammary cancer cells to anti-PD-1 or anti-TIM-3 and prevents lung metastasis. Second, the scRNAseq highlighted different subsets of IFNγ-producing γδ T cells, identifiable by the differential expression of Ly6C. These subsets have cancer-killing functions. We have found that Ly6C$^+$ γδ T cells are maintained by the cytokine, IL-27, which amplifies their cancer-killing ability. In adoptive transfer experiments, Ly6C$^+$ γδ T cells delay mammary tumour growth, while Ly6C— γδ T cells do not. Future efforts will focus on the endogenous role of these cells in breast cancer progression.

Colorectal cancer

We have continued our collaboration with Owen Sansom and Adrian Hayday (Francis Crick Institute) to investigate the role of γδ T cells in mouse models of bowel cancer. We are particularly interested in the gut-resident γδ T cell population that express the Vγ7 chain T cell receptor chain and their role in cancer progression. We have found that these cells counteract intestinal adenoma formation and kill transformed enterocytes in mice. When tumours develop, however, these cells are largely excluded from the tumour microenvironment. We have found that Butyrophilin-like 1 (BTNL1), a molecule expressed on gut epithelial cells required for survival of Vγ7 cells, is absent from tumours in the bowel. This observation has led to an examination into the mechanism of BTNL1 loss. We have found that deletion of the tumour suppressor *Apc* induces the down-regulation of *Btnl1* mRNA using organoids derived from our mouse models. This down-regulation of *Btnl1* is accompanied by decreased expression by gut-specific transcription factors, such as HNF4A and HNF4G. Interestingly, inhibition of β-catenin in mouse models reverses the down-regulation of *Hnf4a*, *Hnf4g*, and *Btnl1* in tumours, which is associated with higher numbers of γδ T cells in the tumour micronenvironment.

Liver cancer

Together with Tom Bird's lab, we have started to address the role of γδ T cells in hepatocellular carcinoma. We have discovered that γδ T cells promote cancer progression in mouse models driven by oncogenic β-catenin and MYC. We performed scRNAseq on these cells to gain an in-depth perspective of how their phenotype changes in the presence of a tumour (Figure 1).

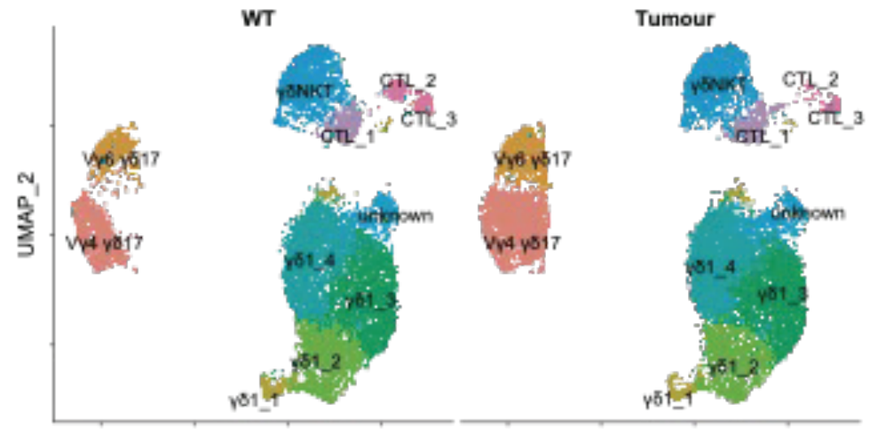

Figure 1. Diversity of gamma delta T cells in normal liver and tumour-bearing livers. scRNAseq analysis of liver gamma delta T cells by Chromium 10X technology.

Data were analysed by Sarina Raven's lab (Hannover, Germany).

This analysis has provided important clues about their behaviour and function.

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.

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

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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.

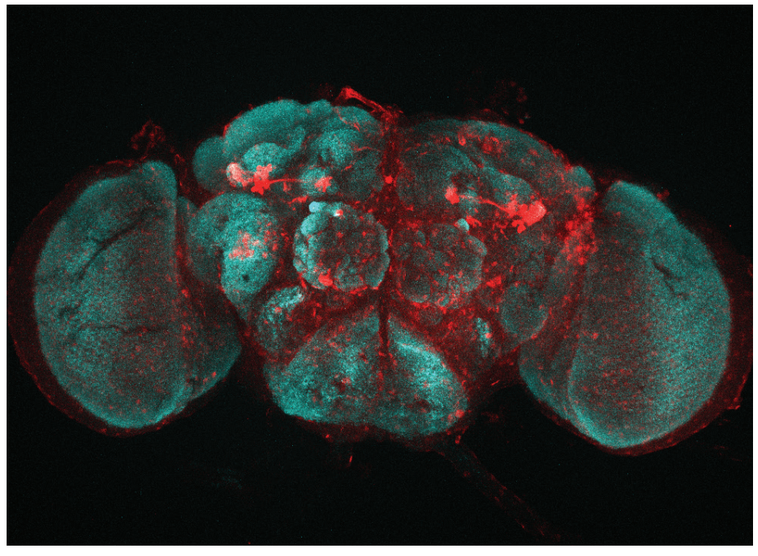

Figure 1: Gut/brain crosstalk in intestinal disease. Adult brain from a *Drosophila* model of intestinal cancer stained with a reporter of sleep need (BRP reporter; cyan) and showing activation of JAK/Stat signaling in sleep regulatory neurons (Stat-GFP; green).

Image credit: Jack Holcombe.

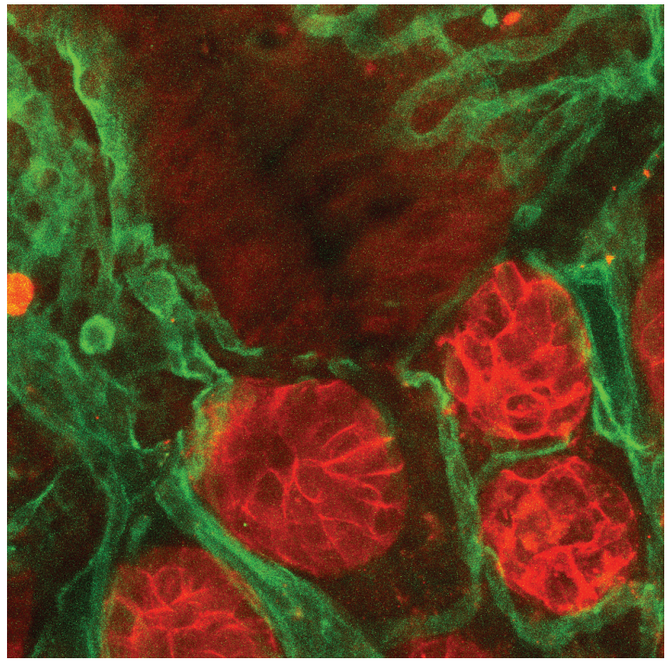

Figure 2: Gut/vasculature interactions in the intestine. Confocal image of the small intestinal crypts (red) surrounded by blood vasculature (green).

Image credit: Jade Phillips

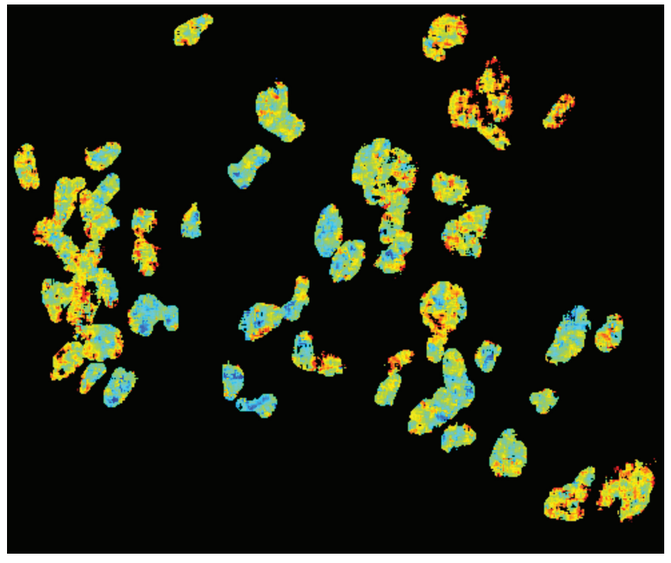

Figure 3: Metabolic adaptations of intestinal stem cells in health and disease. Oxidative phosphorylation FRET sensor in *Drosophila* adult intestinal stem cells (cyan and yellow).

Image credit: Yuanliagzi Tian

GENE REGULATION

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Precise and responsive gene regulation directs development, immune responses and organ function. In cancer cells, gene expression mechanisms become deregulated which can result in 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 pathway and how this impacts on gene expression and cell function, in health and disease. 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 is key to understanding their role in tumour initiation and progression. The development of therapeutic targeting approaches requires an understanding of RNA capping enzyme structure and interaction with ligands. We investigate the biochemical functions of the RNA capping enzymes and how they are regulated by cellular signalling pathways through post-translational modifications and co-factors.

We are interested in the mechanisms by which growth factors influence gene expression by regulation of the capping enzymes. Growth factors co-ordinate cellular responses by activating kinase cascades which drive co-ordinated responses in their target proteins. The RNA capping enzymes activity, function and expression are regulated by several major kinases. This year we have focussed on the impact of CK2 phosphorylation of the capping enzyme CMTR1 which induces an intramolecular interaction and promotes interaction with RNA pol II. CMTR1 phosphorylation increases methylate its substrate caps and is required for cell proliferation. In collaboration with Ed Roberts and Ed Hutchinson we have demonstrated that CMTR1 phosphorylation is critical in interferon responses and influenza infection.
We continue to collaborate with Owen Sansom to investigate the role of the RNA capping enzymes in 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.

How do the RNA capping enzymes influence T cell function?

T cells are key cells of the adaptive response to infections and cancer. When T cells interact with cognate antigens, gene expression and cellular metabolism increase massively, permitting rapid proliferation and the production of cell populations required to target infection and cancer. We investigate how the RNA capping enzymes are upregulated during T cell activation, direct cell proliferation and differentiation. The different RNA capping enzymes have distinct roles in gene expression during T cell activation, and as a consequence, have distinct roles in T cell function and fate decisions. Recently, we discovered that the RNA cap methyltransferase, RNMT is upregulated during T cell activation, resulting in upregulation of mRNAs and snoRNAs involved in ribosomal protein and RNA production and processing. As a result, RNMT upregulation increases ribosome production during T cell activation, a process critical to produce effector populations. We are now investigating the role of RNMT and CMTR1 during T cell activation, specifically defining their role in cell fate decisions. We are collaborating with Ed Roberts to understand the role of the RNA capping enzymes in T cell responses to cancer, focusing on T cell proliferation, differentiation and memory cell production.

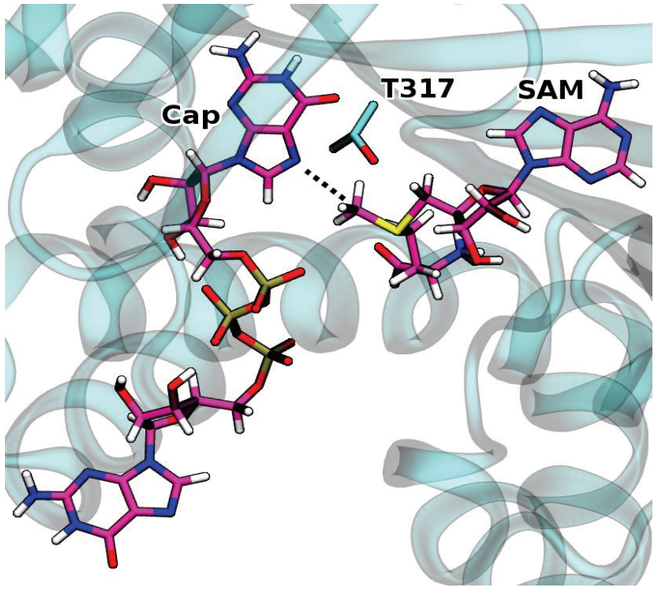

Figure 1. Configuration of RNA cap substrate and methyl donor, S-adenosyl methionine, in the human RNMT active site.

Image credit: Marcus Bage and Andrei Pisliakov, University of Dundee.

How do the RNA capping enzymes co-ordinate gene regulation programmes during differentiation?

Distinct regulation of the RNA capping enzymes during differentiation allows the co-ordinated regulation of key genes associated with cell identity. For example, RNMT repression is required for loss of pluripotency genes during differentiation and CMTR1 upregulation is required for histones and ribosomal protein genes, and associated DNA replication and protein synthesis. These findings are relevant to development but also have parallels in tumour initiation and progression, during reprogramming of gene expression. This year we have focussed on understanding the interaction of the RNA capping enzymes with their RNA substrates as a mechanism to understand gene specificity.

Are the RNA capping enzymes viable therapeutic targets?

The RNA cap methyltransferases have influential roles in gene expression and cell proliferation. We investigate whether inhibiting these enzymes can have selective roles in inhibiting the growth and proliferation of cancer cells. We aim to identify cancer genotypes which sensitise cells to inhibition of RNA capping, in collaboration with Owen Samson and Ed Roberts, see above. We have initiated a collaboration with Cancer Research Horizons to investigate targeting RNA cap metabolism in colorectal cancer models. We continue to 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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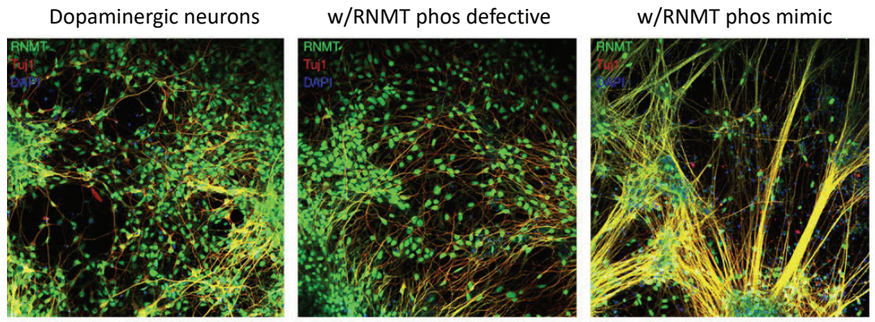

Figure 2. Dopaminergic neurons derived from induced pluripotent stem cells. Cells engineered to express phospho-defective RNMT (mid panel) and phospho-mimic RNMT (right panel).

Image credit: Rajaei Almohammed, CRUK Scotland Institute.

METASTASIS AND CIRCADIAN RHYTHM

Group Leader
Zoi Diamantopoulou

Senior Scientific Officer
Amelie Juin

Research in our laboratory focuses on understanding 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 administered them at specific times of the day?

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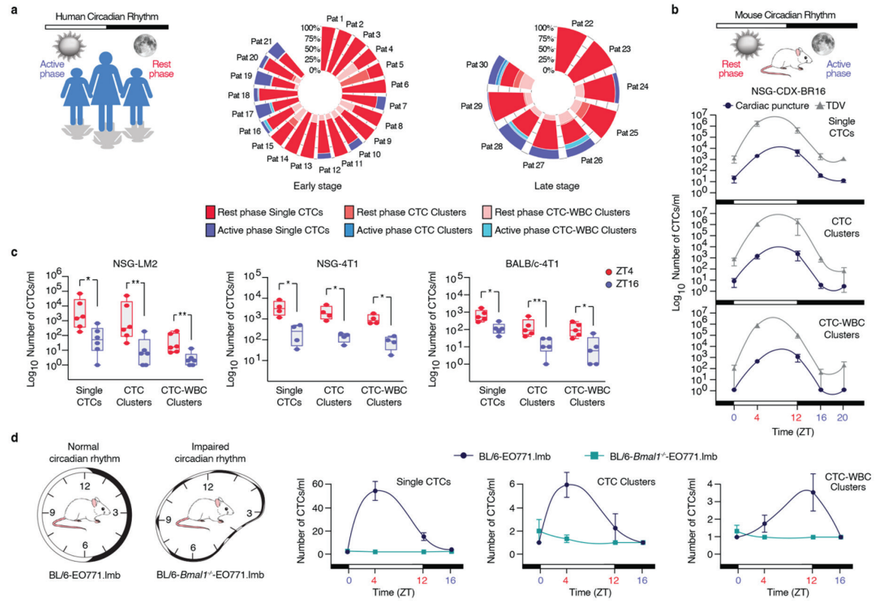

Figure 1. CTCs intravasate during the rest phase of the circadian rhythm. (A) Graphical representation of the human circadian rhythm. The white and black bars represent environmental light (active period) and dark conditions (rest period), respectively (left). The radial histograms show the percent of 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-Bmal1$^{-/-}$-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.

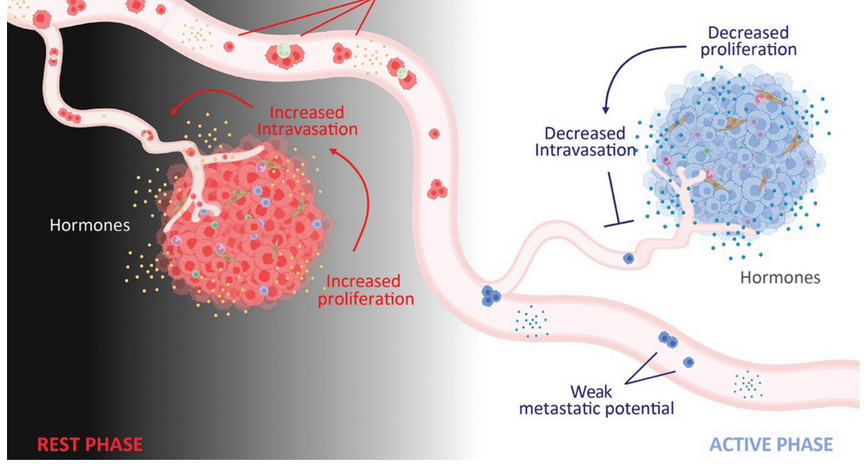

Figure 2. Proposed mechanism for the regulation of metastasis by the circadian rhythm.

PANCREATIC CANCER EVOLUTION AND THERAPEUTIC DEVELOPMENT

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[1]CRUK Precision-Panc and Pancreatic Cancer UK
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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 its 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.

With a median survival of less than a year after diagnosis, pancreatic cancer is a cancer of unmet need that is fatal for most patients. To date, there has been little improvement in these poor outcomes, with very few effective therapies available. We do, however, see exceptional tumour responses, where patients derive significant benefits and have better outcomes. Thus, there is an urgent need to personalise our patient care and better identify the right treatment for each patient.

In an era of precision medicine, one of the challenges for therapeutic development for pancreatic cancer is its heterogeneity and large cellular plasticity. Research within the field has shown two biologically different and prognostically important transcriptomic subtypes, or lineages: a relatively better "classical" and a poorer prognostic "squamous/basal-like" subtype (Figure 1A). Recent single-cell analyses have demonstrated the coexistence of squamous and classical lineages within a single tumour, and the presence of "hybrid" cells that co-express markers of both. These data suggest that molecular subtypes of pancreatic cancer exist as a continuum, with a classical tumour that has more indolent biology on one end, a highly aggressive squamous/basal-like tumour on the other, and a range of cellular states in between (Fig. 1B).

The cell-to-cell differences that drive this cellular plasticity are determined by a complex interplay of multiple genetic and non-genetic factors (Fig. 1B). Our research aims to better understand the dynamics and evolution of pancreatic cancer progression with the overall goal to develop novel, biomarker directed therapies. To do so, we use routine clinical health care data and patient samples for in-depth analysis, preclinical patient-derived models for functional studies and, in collaboration with the School of Computing Science, methods of deep learning techniques and artificial intelligence. 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.

Within the UK, the Precision-Panc consortium has been established to accelerate therapeutic development for pancreatic cancer and overcome challenges of delivering precision medicine for this disease. By means of a "Master Protocol", patients provide their informed consent for biopsy and molecular profiling with subsequent enrolment into multiple PRIMUS clinical trials. Within the Precision-Panc consortium, different studies are in development we have started a national molecular tumour board to enable a more personalised approach and possible treatment within second line or early phase clinical trials. Overall, the goal is to provide a clear pathway for translation of preclinical discoveries into scientifically driven clinical trials and allows reverse translation of clinical observations into the laboratory to keep advancing our knowledge and refine therapeutic approaches.

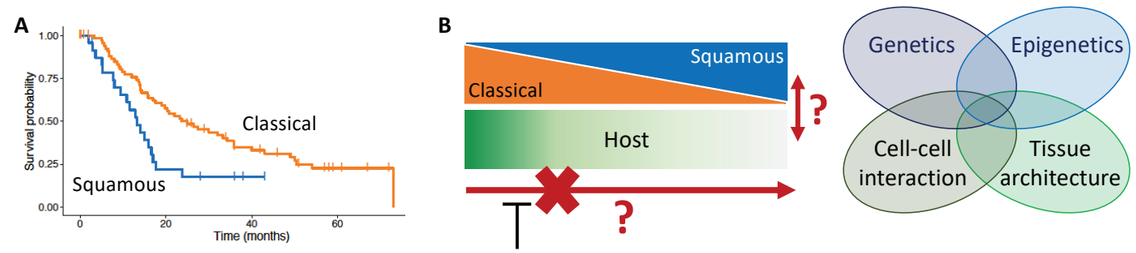

Figure 1. A) Two consensus subgroups, or lineages, of pancreatic cancer (PC) are a "classical" and a poorer prognostic "squamous/basal-like" subtype. Overall survival by subtype is shown. **B)** Recent research is indicating subtypes of PC exist as a continuum, with co-evolution of tumour and host cells driving the progression from a classical tumour into a highly aggressive squamous/basal-like tumour. By investigating and integrating key determinants of cellular state, our research aims to identify the key steps involved in PC progression, and how to therapeutically target these.

We are currently working as part of Team SAMBAI, which in 2023, was selected to receive a Cancer Grand Challenges award of up to £20m over five years to build an unprecedented resource, which will comprise a comprehensive measurement of social, environmental, genetic and biological factors that can be used to help define the causes of disparate outcomes in the selected populations. The team will focus on prostate, breast and pancreatic cancers spanning diverse cohorts of African descent from regions of Africa, the UK and the US.

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INTEGRATIVE MODELLING

Group Leader
Xiao Fu

Research Scientist
Jayathilake Pahala Gedara

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 cancer treatment.

The Integrative Modelling lab was established in August 2023. Since then, we have been developing collaborative interactions and recruiting team members to join the lab in 2024. In our lab, we are interested in developing computational approaches to investigate the 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 evolutionary dynamics

Our first strand of research evolves from our previous research in modelling tumour evolution, 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 tumour snapshots 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 windows of opportunity for therapeutic intervention.

In the context of pancreatic cancer, Jayathilake, a postdoc who will join the group at the beginning of 2024, will develop computational models to investigate the co-evolution of tumour, stroma, and the immune compartment, to improve our understanding of therapy resistance mechanisms. Initial stages of the model development will be integrated with previously conducted pre-clinical mice experiments that evaluated the efficacy of drugs or drug combinations, in collaboration with Jen Morton's lab. The established model will then be used to explore treatment strategies for overcoming therapy resistance, with an aim to inform future experiments for validation.

Another postdoc expected to start in our lab in 2024 will develop computational models to investigate growth patterns and microenvironments of colorectal cancer liver metastasis. The computational modelling will establish mechanistic insights into the dynamic integration of biological processes governing the distinct histopathological growth patterns, encapsulated or replacement growth, associated with better or worse patient outcomes, respectively. In integration with pre-clinical mouse modelling work in Owen Sansom's lab, these computational models will have the potential to inform 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".

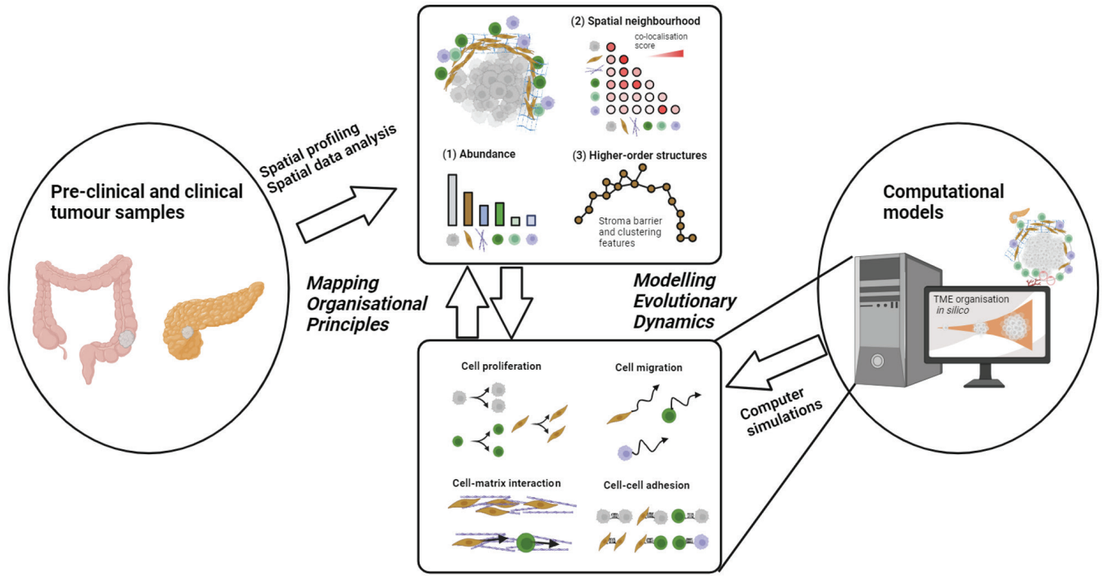

Figure 1. A framework for integrating computational approaches with pre-clinical and clinical work to investigate the evolutionary dynamics and organisational principles of the tumour microenvironment.

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. We are interested in developing innovative data analysis tools to discover spatial biomarkers within the cellular ecosystems, in collaboration with John LeQuesne's lab and Nigel Jamieson's lab in the School of Cancer Sciences, University of Glasgow. We are currently exploring opportunities to recruit a PhD student to focus on this research area.

The second level of mapping will be achieved through the integration of computational modelling and spatial data analysis. Computer simulations of the mathematical and computational models will result in diverse dynamic co-evolutionary trajectories of the tumour and its microenvironment *in silico*.

Linkage of these simulated tumour snapshots with the static 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, the postdocs starting in 2024 will develop data analysis and statistical inference tools, 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 to inform clinical and pre-clinical experimental research. In the coming year, we look forward to welcoming our first group members to the ***Integrative Modelling*** lab and taking our research ideas forward together.

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www.crukscotlandinstitute.ac.uk/payam_gammage

MITOCHONDRIAL ONCOGENETICS

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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.

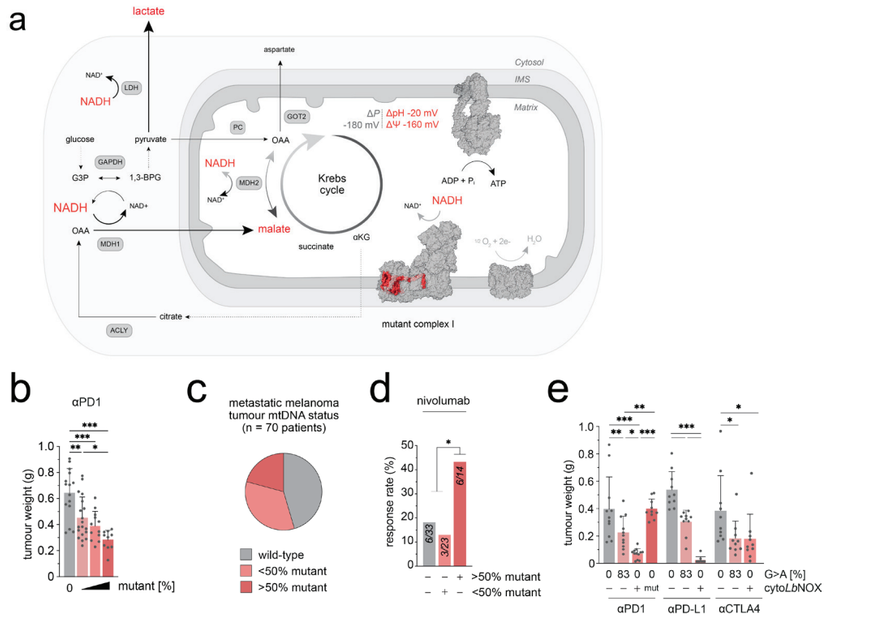

Figure 1. (a) Summary of the metabolic impacts in cancer cells resulting from truncating mutation in Mt-Nd5 of complex I. Redox imbalance due to diminished levels of complex I result in a saturated malate-aspartate shuttle and subsequent excess fermentation of pyruvate to lactate, coupled to loss of 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.

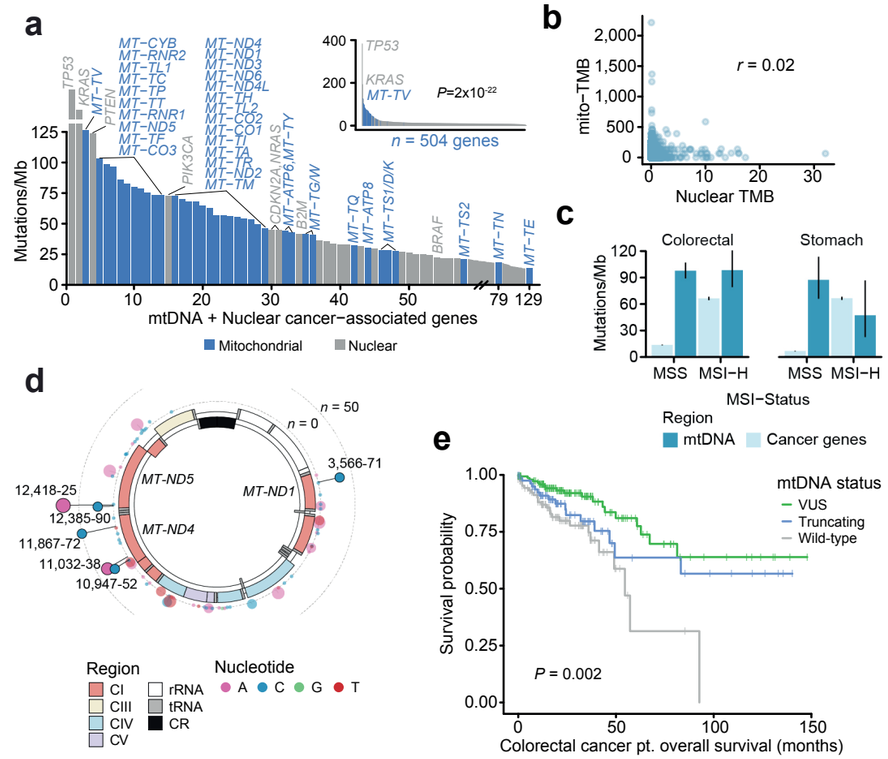

Figure 2. (a) Mutation rates (mutations/Mb) of individual mtDNA-encoded genes (blue) and nuclear-encoded cancer-associated genes (grey). Inset plot: mutation rates among 504 genes with mtDNA genes highlighted. Outer plot: closeup of the inset plot in the region containing all 37 mtDNA genes; commonly 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, *Nature Metabolism*). 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, *Nature Metabolism*).

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.

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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 Mg^{2+} 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

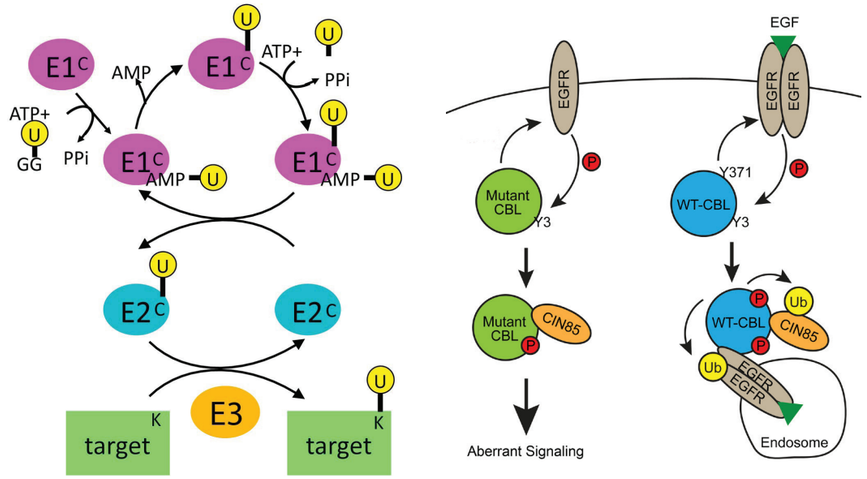
CBL proteins (CBLs) are RING E3s that negatively regulate receptor tyrosine kinases, tyrosine kinases and other proteins by promoting their ubiquitination and degradation by the proteasome or lysosome. Mutations in CBL have been observed in human patients with myeloproliferative diseases. Investigating 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.

Mechanism of K48-linked polyUb chain synthesis

We are interested in understanding the basis for polyUb chain formation. The K48-linked polyUb chain is an important signal that targets protein substrates for proteasomal degradation. While the enzymes that assemble K48-linked polyUb chain are known, the mechanism of Ub chain synthesis remains elusive. We studied one of the E2 enzymes, UBE2K, that selectively catalyses K48-linked polyUb chain formation. To visualise this reaction, we chemically trapped UBE2K covalently linked to donor Ub and acceptor K48-linked di-Ub, where the C-terminus of donor Ub was linked to UBE2K's active site cysteine and K48 of the acceptor di-Ub was linked to an UBE2K active site residue. We then determined the crystal structure of this cross-linked UBE2K complex and a RING E3 and validated that the structure approximated the transition state of the K48-linked Ub chain synthesis. The structure revealed several key features: (1) UBE2K active site residues and the C-terminal Ub-associated (UBA) domain bind the acceptor Ub and orient its K48 toward the UBE2K–Ub active site for catalysis, (2) the UBA domain contains multiple Ub-binding surfaces that serve to bring UBE2K to Ub-primed substrate to overcome weak acceptor Ub affinity and accelerate Ub chain extension, and (3) UBA domain exhibits a preference for K63-linked polyUb chain as the acceptor and promotes branched K48-K63 polyUb chain formation (Nakasone *et al.*, 2022, *Nature Chemical Biology*). Our ongoing effort is exploring varying mechanisms in polyUb chain formation.

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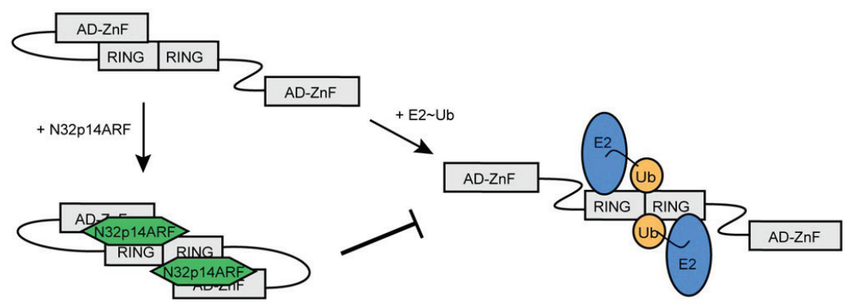
Figure 3. Regulation MDM2 E3 activity by p14ARF



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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.

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 (plural). 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 (Harwood *et al*., 2016, *Acta Derm Venereol*). 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, 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 1). Utilising genetically engineered mouse models (in collaboration with Owen Sansom and Karen Blyth) we revealed that driver gene combinations rather than overall genetic complexity drive cSCC disease progression (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. Excitingly, initial transcriptomic analysis has enabled us to generate a 20 gene expression signature that predicts metastasis (Wang *et al*., 2023, *J Am Acad Derm*).

In collaboration with the Glasgow Head and Neck Cancer group (GLAHNC) we are seeking to understand the molecular basis of chemo-radiotherapy resistance, disease recurrence, lymph node metastasis and distant metastatic spread of HNSCC. Our efforts are initially focusing on molecular profiling of clinically annotated patient samples from local site-specific cohorts and clinical trials coupled with the development of pre-clinical experimental models.

TGFβ signalling in squamous cell carcinomas

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,

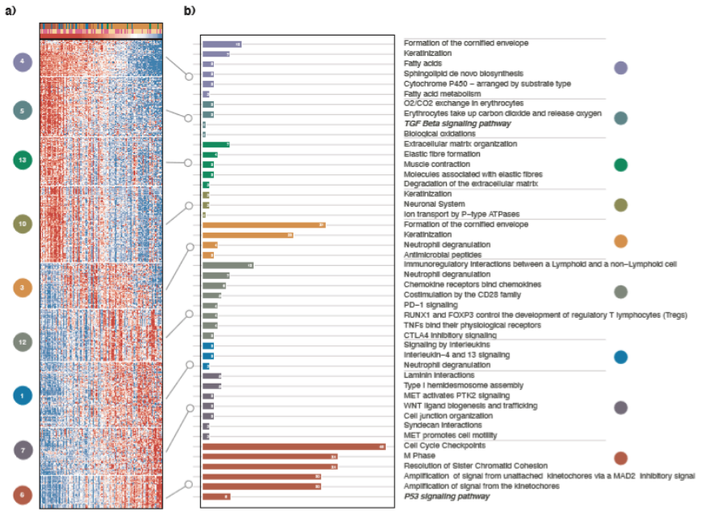

Figure 1. Pathways and processes associated with disease progression of the cSCC disease continuum. (A) Heatmap of gene expression levels of genes in 9 core co-expressed gene clusters ordered by differentiated to progenitor like status (left to right). **(B)** GSEA analysis showing significantly enriched molecular pathways and/or processes in the 9 core co-expressed gene clusters (Adapted from Bailey *et al*., 2023).

and regulate expression of hundreds of target genes. 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 many of the remaining ~70% of tumours show overexpression of TGFβ1 and TGFBR1 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 major 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. Our efforts are focusing on both understanding the molecular processes by which TGFβ signalling acts to drive proliferation, migration and invasion in these tumours and on identifying novel therapeutic susceptibilities of these aggressive cSCCs.

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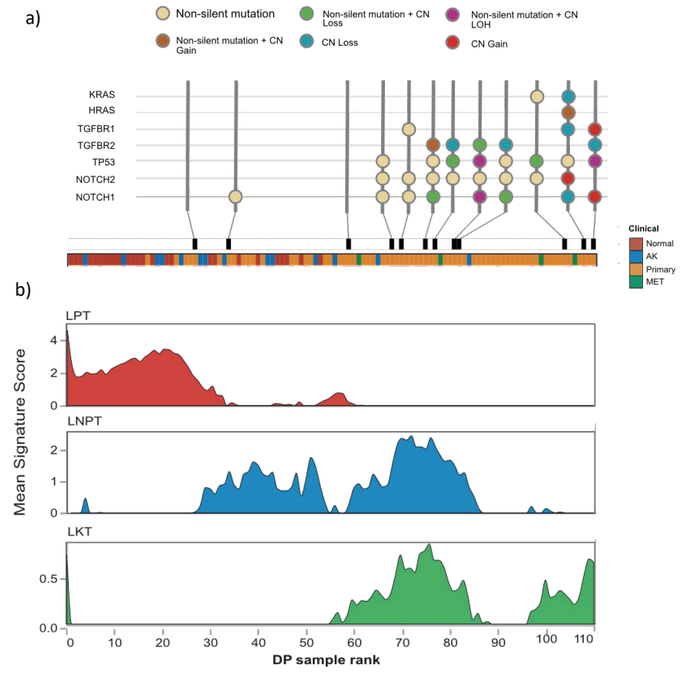

Figure 2. Driver gene combinations dictate cSCC disease progression. (A) Oncoprint analysis of selected driver genes in human samples profiled by WES ordered by differentiated to progenitor like (DP) status (left to right). **(B)** Bulk RNASeq of genetically engineered mouse models (GEMM) of cSCC was used to generate genotype specific signature genes for enrichment analysis. Area charts show mean GEMM signature score for human cohort samples ordered by DP status. Genes significantly enriched in a specific mouse genotype were used as signature genes for enrichment analysis. Single sample gene set enrichment analysis was employed to determine signature enrichment in bulk human cSCC. L= *Igr5Cre*, p= *Trp53*$^{Fl/Fl}$, T= *Tgfbr2*$^{Fl/FL}$, N= *Notch1FL/FL*, K= *LSL-KRas*^{G12D}. (Adapted from Bailey *et al*., 2023).

STEM CELL AGEING & CANCER

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The aim of our research is to understand how ageing influences stem cell behaviour, the stem cell niche and cancer outgrowth. We also consider the influence of the ageing tumour microenvironment and the effects of senescence, induced by either ageing or DNA damage inducing cancer therapies on the tumour niche. We aim to use this knowledge for early detection of cancers and to identify and test new clinical therapies to prevent or treat cancer at an early stage.

Age is the single biggest factor underlying the onset of many haematological malignancies, with myeloid disease being especially prominent. The onset of myeloid bias in the haematopoietic stem and progenitor cell (HSPC) compartment with increasing age is well documented and leads to malfunction of the immune system but might also be a factor for predisposition to myeloid cancers. Clonal hemopoiesis of indeterminate potential (CHIP) is characterised by mutations in leukaemia driver genes in healthy aged individuals. Several groups reported that CHIP is driven by somatic mutations in HSPCs in *DNMT3A*, *TET2*, and *JAK2* genes, mutations previously described as drivers of myeloid malignancies. CHIP is associated with an increased risk for haematological cancer and all-cause mortality, whereby age is a major risk factor. In addition, patients who are carrying CHIP mutations and are undergoing chemo- or radiation therapy for solid tumours, are at an increased risk of developing secondary leukaemia.

Myeloid malignancies such as acute myeloid leukaemia (AML), myelodysplastic syndrome (MDS) or myeloproliferative neoplasms (MPNs) result from mutations in HSPCs. In myeloid cancers a single mutation can often account for disease. For instance, the *JAK2V617F* mutation is sufficient for the development of myeloproliferative disease (Clark *et al.*, 1987). Such mutations can increase stem cell fitness, leading to growth advantages over neighbouring cells and eventually cancer. Larger clones are more likely to acquire additional mutations that increase fitness, predisposing cells further towards malignancy. Therefore, studying HSPC ageing is essential for gaining insights into mechanisms underlying the transformation of aged HSPCs into cancer stem cells.

Senescent cells accumulate during ageing, upon the exposure to DNA damage, the hyperproliferation of an oncogene or other events compromising a cell's integrity. Senescence is a tumour suppressor pathway where the p53 and p16/Rb pathways are engaged to permanently force exit from the cell cycle. A prominent feature of primary senescence is the senescence-associated secretory phenotype (SASP) (Acosta *et al.*, 2008). Through the secretion of factors like extracellular matrix proteases and signalling proteins such as interleukins and chemokines, senescent cells modulate tissue organisation and recruit immune cells, mediating their own clearance. In addition, SASP factors can act in a paracrine fashion to induce secondary senescence in surrounding cells and tissues (Nelson *et al.*, 2012). Secondary senescence is thought to act as a sentinel mechanism enhancing immune surveillance and to act as a fail-safe programme minimising the retention of damaged cells in the vicinity of primary senescent cells. Our work has shown that senescent cells also spread by inducing senescence more directly, through cell-cell contact (juxtracrine) (Teo *et al.*, 2019). However, whether secondary senescence is indeed part of a fail-safe mechanism or has other implications remains unknown (reviewed in Kirschner *et al.*, 2020).

Longitudinal profiling of clonal haemopoiesis mutations

The Lothian Birth Cohort (LBC) of 1921 (n=550) and 1936 (n=1091) are two independent, longitudinal studies of ageing. Participants have been followed up every ~3 years, for five waves, from the age of 70 (LBC1936) and 79 (LBC1921) years. They provide one of the most comprehensive assessments of later-life ageing anywhere in the world.

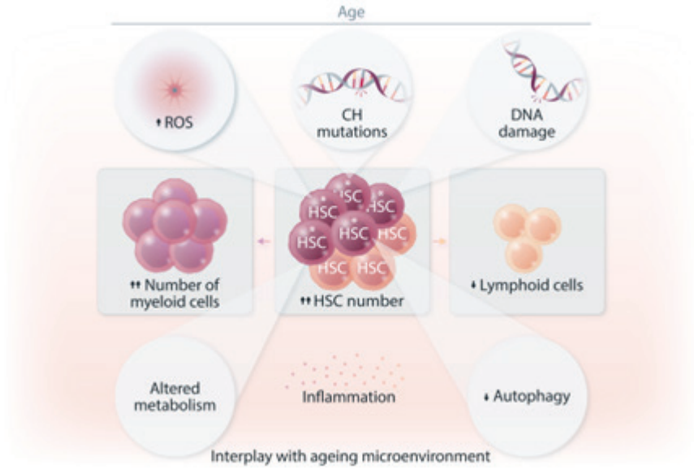

Figure 1. Ageing related changes in the hematopoietic compartment.
With ageing, phenotypic hematopoietic stem cells (HSCs) increase in numbers alongside an increase in myeloid cells and a decrease in lymphoid cells. Aged HSCs accumulate DNA damage which can lead to clonal hematopoiesis (CH) of mutations over time. Other features of ageing HSCs include an increase in reactive oxygen species (ROS), alterations in their metabolism and decreased levels of autophagy. The interplay with the ageing microenvironment leads to local inflammation.

We have previously shown an association between an increase in biological age acceleration and the presence of CHIP in the LBCs, as well as finding transcriptional differences between young and old HSCs carrying the *Jak2V617F* mutation (Robertson *et al.*, 2019, Kirschner *et al.*, 2017).

We set out to quantify the fitness effects of CHIP drivers over a 12-year timespan in older age, using longitudinal error-corrected sequencing data from the LBCs. We developed a new filtering method to extract fitness effects from longitudinal data, and thus quantified the growth potential of variants within each individual, while taking into account individual mutational context. We showed that gene-specific fitness differences could outweigh inter-individual variation and therefore could form the basis for personalised clinical management (Robertson *et al.* 2022). We believe that fitness is a better predictor of outcomes than clone size. We are increasing our longitudinal cohort size to enable us to associate stem cell fitness with outcomes, such as all-cause mortality. We are also linking differences in stem cell fitness to transcriptional changes longitudinally in the LBCs.

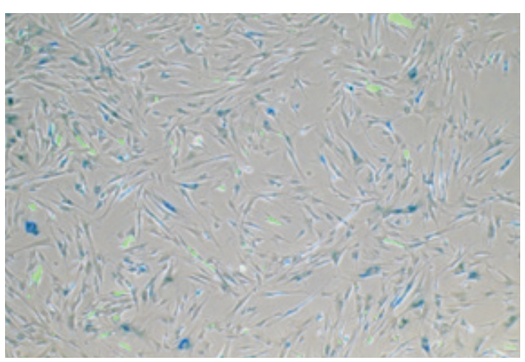

Figure 2. Microscopy image of primary and secondary senescent cells *in vitro*.
Primary senescent cells are translucent, secondary senescent cells display GFP. All cells were stained for the senescence marker senescence associated beta galactosidase, showing blue staining in primary and secondary senescent cells.

Single cell approaches to investigate senescence heterogeneity in the tumour microenvironment

The roles of secondary senescence remain elusive since its discovery. Secondary senescence is thought to enhance immune surveillance initiated by the primary senescent cell and to act as a fail-safe mechanism to minimise the chances of retaining damaged cells in the vicinity of primary senescent cells. However, this concept has thus far not been formally studied.

Previously, it was assumed that primary and secondary senescence phenotypes are identical. However, we were the first to show that each form of senescence is transcriptionally distinct (Teo *et al.*, 2019, *Cell Reports*). We found that Notch mediated secondary senescence blunts SASP, typically seen at high levels in primary senescence. Moreover, upregulation of collagens on the transcriptional level in secondary senescence contrasts with a well reported downregulation in primary senescence (Teo *et al.*, 2019, *Cell Reports*), hinting at functional differences in heterogeneous senescence populations. Fibrillar Collagen deposition is a characteristic of fibrosis, creating a pro-tumorigenic micro-environment. We are now combining single cell omics approaches with advanced mouse models to assess consequences of senescence heterogeneity in the tumour microenvironment, in the context of leukaemia and liver cancer.

Elucidating senescence heterogeneity is an important concept in the context of senolytics, a novel group of drugs, specifically targeting senescent cells. These drugs have shown great promise in rejuvenation approaches in a wide variety of organs but have not been exploited in pre-neoplastic disease setting and tumour prevention.

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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:

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.

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.

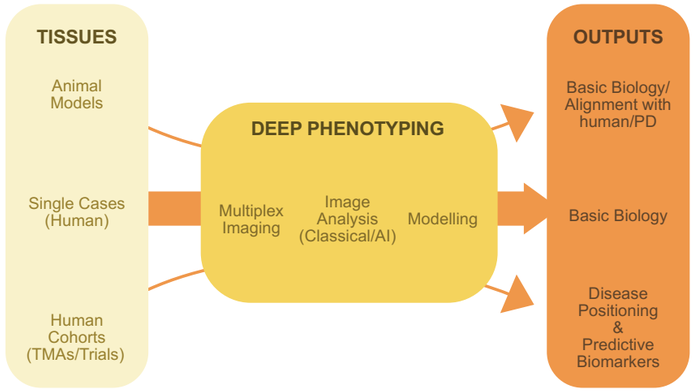

Figure 1.
Workflow schematic of deep phenotyping methods. The basic pipeline (centre) is applied to a range of tissue types to achieve answers to diverse scientific questions.

Application of machine learning to tumour microscopy

Machine learning and artificial intelligence offer 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.

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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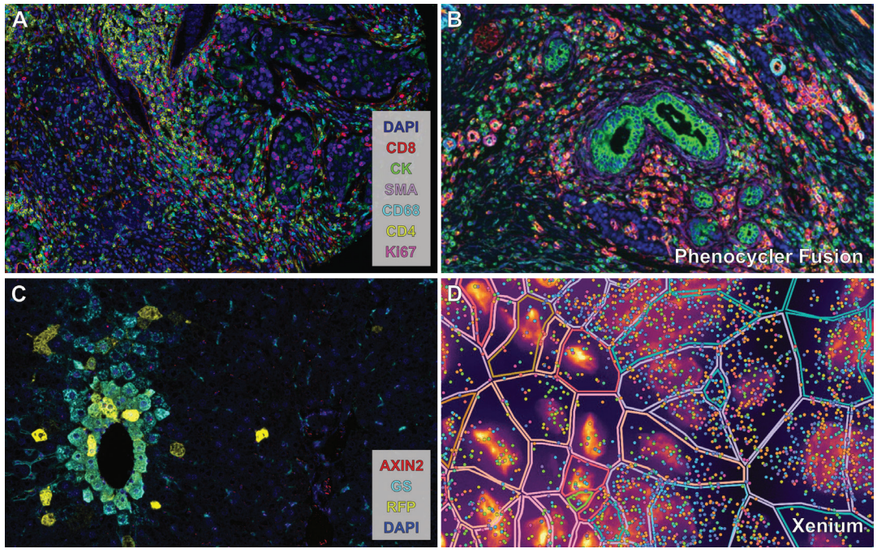

Figure 2.
Example multiplex images. **A** Spectrally unmixed multiplex staining of eIF4A1, eIF4A2 and P-ERK in archival human lung adenocarcinoma tissue. **B** AKOYA Phenocycler FUSION image of multiple protein markers on human tissue sections using this technology we can image >50 markers per section; **C** Spectrally unmixed co-ISH IHC of AXIN 2 mRNA with IF markers for red fluorescent protein and glutamine synthase in transgenic mouse liver. **D** Spatially resolved gene expression analysis using the 10x Genomics Xenium analyser, each dot represents detection of an mRNA molecule, with colours donating different transcripts overlaid on DAPI stained FFPE tissue and cellular masks.

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 has a uniquely comprehensive cross-disciplinary strategy encompassing preclinical laboratory model systems, analysis of clinically obtained samples and timely clinical trials based on our research findings. Our research objective is to identity, characterise and validate key aberrant cellular signalling events to inform and facilitate the development of novel therapies.

Recent research from our group highlighted the importance of aberrant lipid and cholesterol metabolism as drivers of treatment resistance. Our ongoing research focusses on the underlying biology to develop novel treatment strategies and accompanying biomarkers.

Aberrant tumoral cholesterol uptake as driver of treatment resistance in prostate cancer

Research in our laboratory builds on the important and clinically relevant finding that combined inactivation or deficiency of the tumour suppressor genes *PTEN* and *Sprouty2* (*SPRY2*) is sufficient to drive aggressive prostate cancer (Patel *et al*, 2013). We further identified a subtype of prostate cancer resistant to hormonal (androgen) deprivation therapy, or castration resistant prostate cancer (CRPC), because of *de novo* testosterone synthesis by treatment resistant cancer cells. This androgen self-sufficient form of CRPC depends on cholesterol bioavailability, and SCARB1 (Scavenger Receptor Class B Member 1) mediates tumoral cholesterol uptake to fuel androgen biosynthesis as a resistance mechanism (Patel *et al*, 2018). Intriguingly, our data points to critical interactions between cancer cells under treatment pressure and the host, with the host adipose tissue mobilised to support cholesterol synthesis in the liver. Cancer cells with enhanced SCARB1 expression are then able to uptake more cholesterol from their cancer microenvironment. Once inside the tumour cells, cholesterol is metabolised to form androgens required for CRPC.

We carried out strategic experiments to disrupt three distinct steps related to tumoral cholesterol uptake as driver of CRPC, namely (1) Neutralising IL6 antibody to inhibit the mobilisation of host adipose tissue, (2) Statins to suppress synthesis of cholesterol by the liver, and (3) ITX5061, a small molecule inhibitor of SCARB1, to directly block cholesterol uptake by tumour cells. All three approaches were able to re-sensitise the cancer cells to androgen deprivation therapy because of diminished ability of cancer cells to produce testosterone as a resistance mechanism (Patel *et al*, 2018).

To test the clinical relevance of our observation on tumoral cholesterol uptake, we designed a proof-of-concept clinical study called the SPECTRE trial, which is a 6-week long single-arm Phase II treatment trial combining atorvastatin and androgen deprivation therapy in patients with CRPC. As expected, all 12 recruited patients experienced substantial falls in serum cholesterol levels following statin treatment. While all patients had comparable pre-study PSA velocities, 6 of 12 patients showed decreased PSA velocities following statin treatment, suggestive of stabilised disease following statins treatment (Rushworth *et al*, 2023). Unbiased mass spectrometry-based metabolomics analysis on serial weekly blood samples identified tryptophan to be the dominant metabolite associated with patient response to statin. Our data from the SPECTRE study provides the first evidence of statin mediated effects on CRPC and early sign of disease stabilisation. Our data also highlights the possibility of altered tryptophan metabolism as a potential biomarker for tumour response to statins.

In collaboration with Karen Blyth, Head of the *In Vivo* Cancer Biology Lab, our ongoing research aims to investigate the consequence of genetically deleting the *Scarb1* gene in relevant prostate cancer genetically engineered mouse models. We are also keen to explore the impact of modulation of tumoral cholesterol uptake on the tumour micro-environment, including tumour infiltrating immune cells.

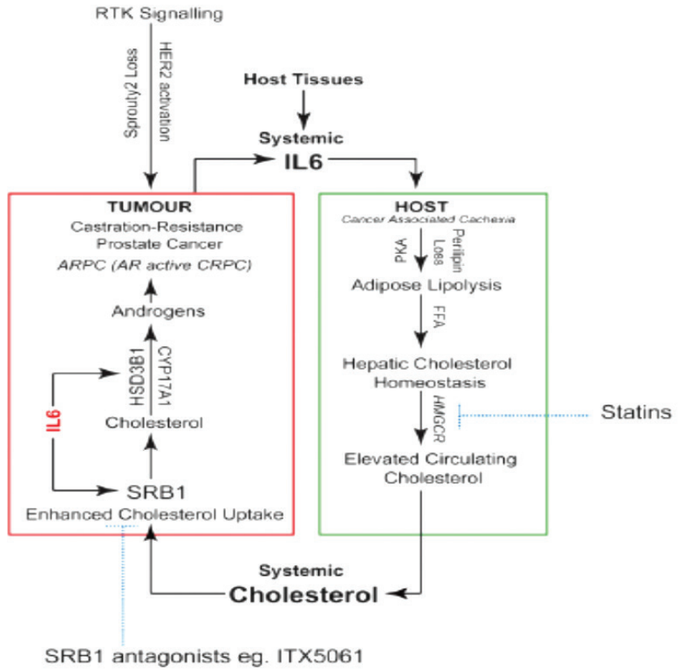

Figure 1. Scheme of tumour-host interactions in supporting enhanced tumoral cholesterol uptake as a driver of castration resistant prostate cancer (Patel *et al*, 2018)

The role of PSMA related metabolites in the progression and treatment resistance in prostate cancer

Mark Salji in the group has recently pioneered multi-omic (RNA sequencing, proteomics, and metabolomics) analyses of three isogenic pairs of human hormone naïve and castration resistant orthografts. Untargeted metabolomics revealed N-acetylaspartate (NAA) and N-acetylaspartylglutamate (NAAG) commonly accumulating in CRPC across three independent matched models. In addition, proteomics analysis showed upregulation of related enzymes, namely N-Acetylated Alpha-Linked Acidic Dipeptidases (FOLH1/NAALADL2; also commonly referred to as Prostate-Specific Membrane Antigen/PSMA) (Salji *et al*, 2022). Of note, PSMA is a highly relevant clinical marker in routine prostate cancer PET imaging to detect metastatic and/or recurrent disease. Here, our findings are pointing to a new research direction in understanding how PSMA enzyme activity may promote progression and development of treatment resistant prostate cancer.

As part of his Prostate Cancer Foundation Young Investigator Award, Mark's ongoing focus is on developing a pipeline for non-disruptive MRI analysis and explant culture of resected prostate tumours as a platform to apply novel assays for NAA and NAAG using cutting edge MR Spectroscopy and metabolic tracing methodologies. Overall, his research aims are to determine the importance of NAA/NAAG as potential biomarkers of recurrent prostate cancer as well as investigating the molecular mechanism of how NAA/NAAG mediate prostate cancer progression and CRPC (Figure 2).

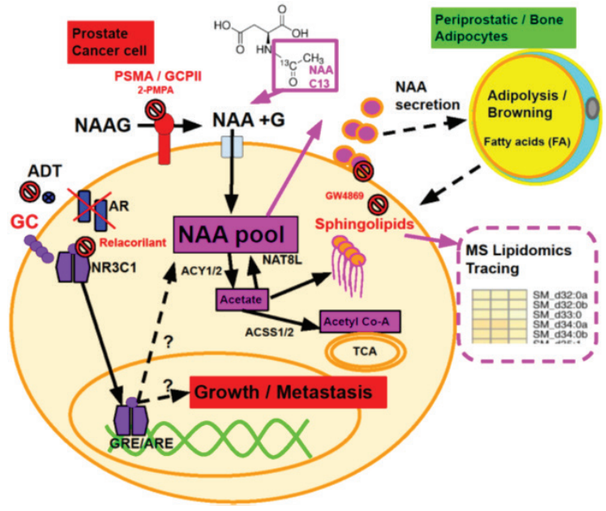

Figure 2. Scheme highlighting working hypothesis of NAA/NAAG as mediators of progressive prostate cancer.

Concluding comment

Our ongoing research continues to highlight the importance of key aspects of cancer metabolism in driving prostate cancer progression and treatment resistance. Our focusses in cholesterol and NAA/NAAG metabolism will help develop future treatment strategies in addition to previous focus on agents directly targeting the androgen receptor, thus increasing the likelihood of successfully improving patient outcomes.

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MOLECULAR IMAGING

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Our lab develops new ways to visualise cancer – we create novel molecular tracers that image metabolic reprogramming, a hallmark of cancer, and use state-of-the-art methods such as PET/MRI to non-invasively detect and characterise tumour development. This year, we have been developing technologies to image metabolic responses to cancer treatment. Our goal is to develop a better understanding of how cancer drugs work, identifying when those drugs succeed or fail, and supporting the use of more effective therapies.

The primary focus of our work is to develop new methods to non-invasively image cancer metabolism and then apply these techniques to investigate the causes and consequences of metabolic heterogeneity in high-fidelity mouse models of cancer. Our research has two main themes, first we develop and validate novel technologies such as new metabolic radiotracers and new 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 we can use to guide therapy in individual patients.

Visualising metabolic heterogeneity and plasticity in lung cancer

Metabolic heterogeneity presents both a challenge and an opportunity to imaging. Due to heterogeneity, it is unlikely that a single imaging test will detect cancer in all cases. However, if we could develop a complementary panel of PET tracers and develop a better understanding of how PET imaging signatures relate to underlying metabolic and molecular features of cancer, we could potentially identify metabolic differences between or within patients and use this information to stratify treatment.

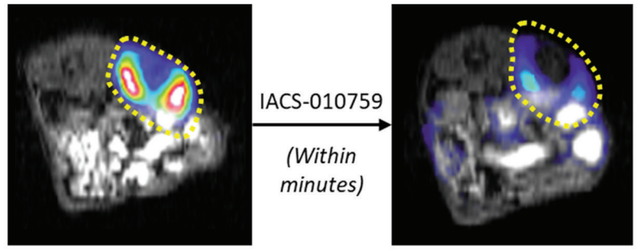
Lung cancer has large regional variations in glucose uptake, hypoxia and blood flow; regions of high and low perfusion within the same lung tumour have striking differences in metabolism. To understand the significance of these imaging signatures we need to relate them to the underlying genetics and metabolism of tumour sub-regions.

To address these challenges, we have developed a dual tracer approach – combining [^{11}C]acetate as a tool for imaging fatty acid synthesis and [^{18}F]FDG, a surrogate of glucose uptake – to visualise and deconvolve regional tumour metabolism. Using dual-isotope positron emission tomography, we imaged the LSL-Kras$^{G12D/+}$ p53$^{fl/fl}$ mouse model of lung adenocarcinoma and found that tumours arising from the same genetic lesions and in the same tissue-of-origin produced two spatially heterogeneous metabolic subtypes. One subtype was characterised by high uptake of the radiolabelled tracer [^{18}F]FDG and the other by high [^{11}C]acetate uptake. Evident on dual-isotope autoradiographs, these tumour sub-regions appeared to demonstrate reciprocal metabolic phenotypes within the same mouse.

To investigate the molecular mechanisms underlying these imaging subtypes we developed a dual-isotope tracking method, DIOPTRA, and traced [^{11}C]acetate and [^{18}F]FDG within the same lesions *ex vivo*. Unbiased molecular profiling of these regions showed distinct transcriptional, proteomic and metabolic signatures. Regions with higher glucose consumption were more proliferative with activation of cell cycle genes, Myc targets and the unfolded protein response. While regions of high acetate uptake showed signatures of fatty acid metabolism, reactive oxygen species, tricarboxylic acid (TCA) cycle and oxidative phosphorylation.

To establish metabolic pathway activity in each subtype we compared PET imaging to metabolic pathway flux measurements using stable isotope tracing with [U–^{13}C]glucose and [U–^{13}C]acetate. FDG-avid tumours utilised glucose for synthesis of serine and glycine and used acetate to replenish the TCA cycle intermediates. In contrast, acetate-avid tumours used glucose for TCA anaplerosis and glutamine biosynthesis while using acetate for synthesis of palmitate, suggesting marked differences in metabolic pathway activation in the two subtypes.

This is the first example of using non-invasive radionuclide imaging to identify cancer subtypes within lung adenocarcinoma. As this imaging is eminently applicable to the clinic, we aim to develop these imaging signatures to identify subtype-specific cancer vulnerabilities.



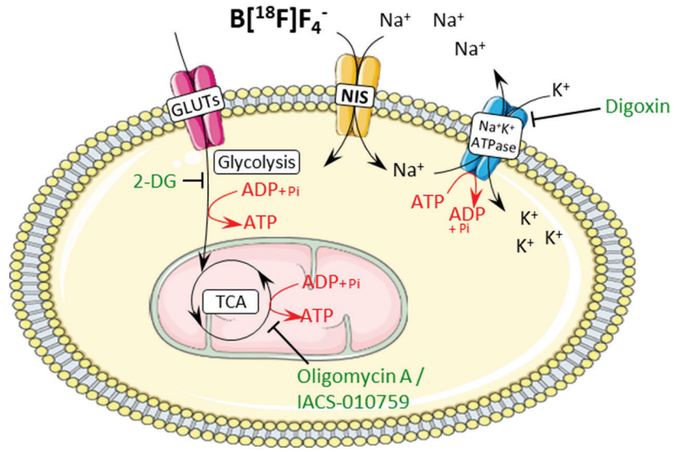


Figure 1 Metabolic sensing of energy charge *in vivo*
Rapid decrease in [^{18}F] tetrafluoroborate PET uptake within minutes of administration of drugs (2-DG, oligomycin and IACS-010759) targeting ATP production.

Imaging energy stress in real-time by in vivo PET imaging of the sodium iodide symporter

Despite recent advances in our understanding of tumour metabolism over the last several years, relatively few metabolic cancer treatments have been successfully translated. Predicting how well drugs targeting metabolism will work in the clinic is a real problem. Testing drugs solely in cell culture models, although relatively straightforward, does not provide a good indication of how well that drug will work in animal models or patients. Recent efforts to make more physiologically relevant cell culture media are an important step, but there is still not a substitute for *in vivo* testing. However, *in vivo* experiments are long and complex which limits throughput. Here we set out to make an *in vivo* system allowing a rapid and non-invasive readout of drug efficacy for metabolic treatments. We used a positron emission tomography (PET) imaging reporter gene, sodium iodide symporter, previously used for cell tracking, and exploited the fact that NIS-mediated PET uptake is coupled to the sodium gradient maintained in an ATP-dependent fashion by Na^+/K^+ ATPase activity. We showed that targeting metabolic pathways that lead to energy stress also led to decreases in NIS-mediated radiotracer uptake (Figure 1). Importantly, this happened very quickly, and using PET imaging, we could sensitively detect drug effects within minutes of their administration, suggesting NIS could act as a rapid *in vivo* sensor of energy stress.

This tool can be used by any laboratory with access to PET imaging and it could be easily adapted to SPECT imaging using sodium pertechnetate, which would eliminate the need for radiochemistry facilities. Radionuclide imaging is very sensitive and quantitative, meaning that NIS can be used in any *in vivo* system without issues like penetration depth or tissue pigmentation that affect optical reporters. In parallel, we are developing a number of *in vivo* tools, including a palette of lentiviral vectors carrying NIS and a germline reporter mouse carrying a Cre-inducible NIS, so that the approach presented here can be used more widely, facilitating its further dissemination. This tool can be a useful means to obtain a rapid indication of the efficacy of drugs that target energy pathways *in vivo*.

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MITOCHONDRIAL REPROGRAMMING IN CANCER

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[1]CRUK Career Development Fellowship
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Tumours must overcome numerous metabolic challenges in order to thrive in nutrient-deprived microenvironments and 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.

Metabolite transporters sit in the impermeable inner mitochondrial membrane and couple the metabolic reactions of the cytosol with the mitochondrial matrix. These transporters represent crucial sites of cellular metabolic control that help govern tumour growth and survival under different environmental conditions. Our recent focus has been on the transport and metabolism of nucleotides within mitochondria, which are essential building blocks for the mitochondrial genome. This year, we investigated how the mitochondria of cancer cells can maintain their nucleotide levels even under conditions of rapid growth, nutrient deprivation and exposure to nucleoside analogue chemotherapies.

Can we block nucleotide supply to suppress mitochondrial activity in cancer cells?

Mitochondria contain their own genome, packaged into mitochondrial DNA (mtDNA) but lack the ability to synthesise their nucleotides *de novo*. Nucleotides must therefore be imported into mitochondria for the replication and subsequent expression of mtDNA. In addition to providing the building blocks of DNA and RNA, regulated nucleotide transport is required for the exchange of mitochondrial ADP/ATP and GTP for metabolic enzymes. Disturbed mitochondrial nucleotide homeostasis can result in cellular nucleotide imbalance and lead to DNA damage and aberrant innate immune responses.

We tested what happens to proliferating cells when their mitochondrial pyrimidine import routes are blocked. We were surprised to find that loss of the two mitochondrial pyrimidine transporters, SLC25A33 and SLC25A36, had little effect on cell division or levels of mtDNA. We discovered that a poorly characterised nucleoside diphosphate kinase, NME6, could preserve mtDNA in pyrimidine depleted conditions. Further work revealed that NME6 is constitutively required for the supply of pyrimidines for mitochondrial RNA synthesis

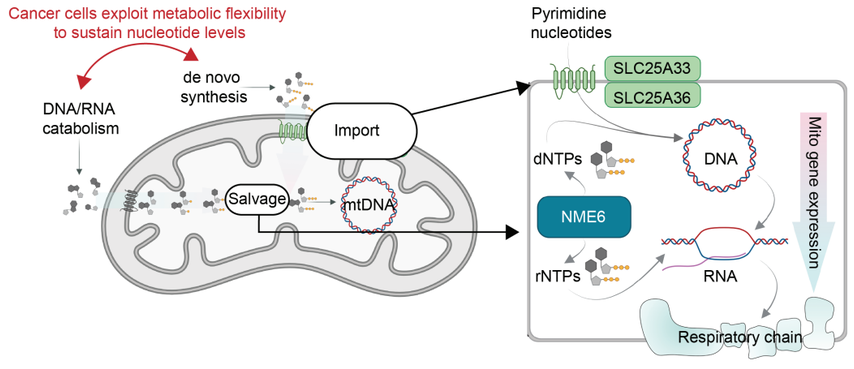

Figure 1. Mitochondrial nucleotide supply and metabolism
Mitochondria obtain nucleotides via different routes. NME6 is a nucleoside diphosphate kinase that supplies pyrimidine ribonucleotides (rNTPs) for transcription and pyrimidine deoxyribonucleotides (dNTPs) for mtDNA synthesis. Cancer cell dependency on NME6 depends on the availability of nucleotides (adapted from Grotehans et al., 2023 *EMBOJ*).

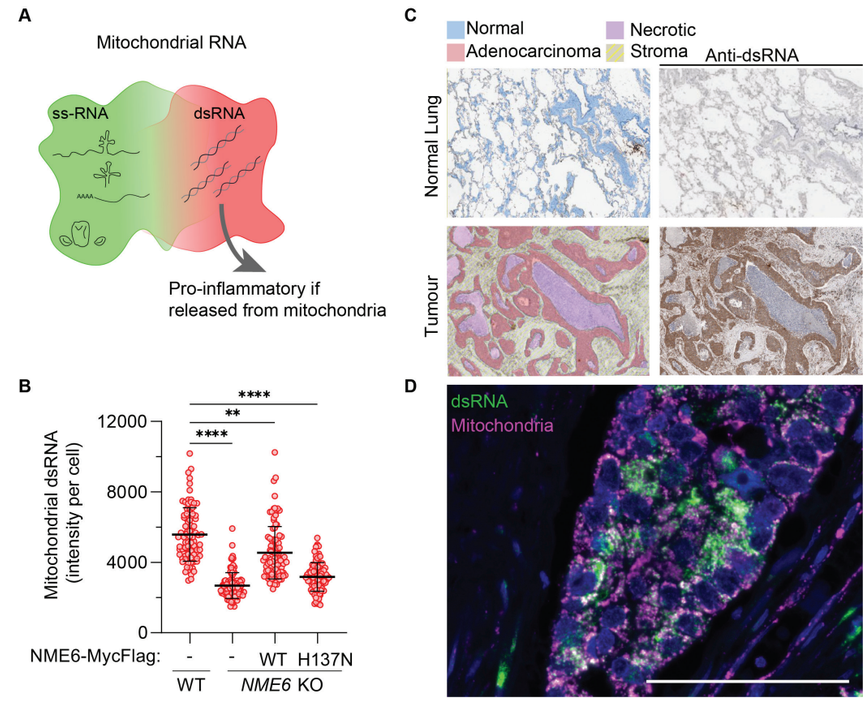

Figure 2. Cancer cells accumulate mitochondrial double-stranded RNA
A Schematic depicting the compartmentalised nature of RNA homeostasis within mitochondria. **B** Accumulation of double-stranded RNA (dsRNA) in proliferating HeLa cells depends on nucleotide supply by NME6 as determined by immunofluorescence using an anti-dsRNA antibody. **C** DsRNA is abundant within patient biopsy lung adenocarcinoma cells and some stromal cell populations. **D** Mitochondrial dsRNA in patient lung adenocarcinoma cells identified by multiplex imaging using anti-dsRNA and anti-ATP5A (Scale bar: 100μm; data obtained by the Deep Phenotyping team at CRUK SI).

(Figure 1). Cells lacking NME6 were deficient in oxidative phosphorylation and could not proliferate in respiration-dependent conditions. NME6 therefore represents a novel node by which we can manipulate mitochondrial gene expression (Grotehans et al., 2023 *EMBOJ*).

Mitochondrial double-stranded RNA in cancer

We aim to target mitochondrial nucleotide supply and mitochondrial gene expression in growing tumours. Interestingly, besides the synthesis of mitochondrial proteins, we found that upregulated mitochondrial nucleotide supply by NME6 also drives the accumulation of mitochondrial double-stranded RNA (dsRNA) in proliferating cancer cells and human tumours (Figure 2). Analogous to viral dsRNA, mitochondrial dsRNA is a potent immunogen when exposed to cytosolic dsRNA receptors. We have characterised the spatial regulation of dsRNA within mitochondria and illuminated a link between mitochondrial dsRNA homeostasis and cellular proliferation. The build-up of mitochondrial dsRNA appears to be a novel marker of cell malignancy and it will be exciting to determine whether mitochondrial dsRNA influences tumour immunogenicity.

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MICROBIAL AND METABOLIC IMMUNE MODULATION

Group Leader
Kendle Maslowski

Senior Scientific Officer
Wilma Hoevenaar

Graduate Student
Marwa Osman[1]

[1]Self-funded

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.

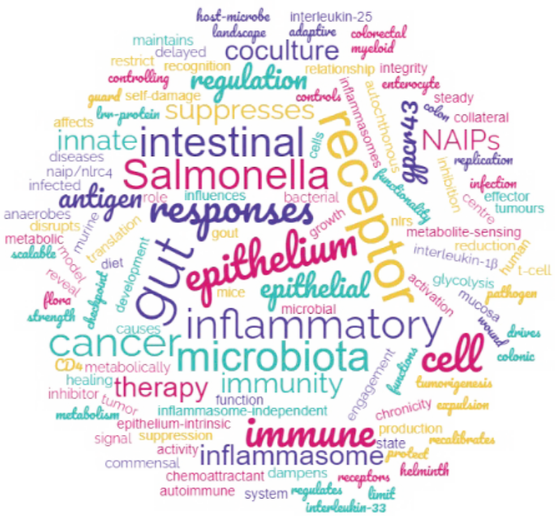
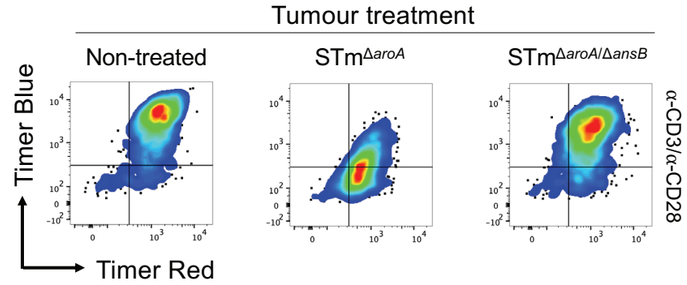


Figure 1. Word cloud generated from publication titles highlighting the laboratory's interests.

This year saw the lab relocate from the University of Birmingham to the CRUK Scotland Institute where we will rebuild. Wilma Hoevenaar joined the group as Senior Scientific Officer. PhD students Gillian Mackie and Lisa Scarfe successfully defended their theses in late 2023.

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 deposited on *BioRxiv* (Copland *et al.*, 2023) and publication pending. 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.



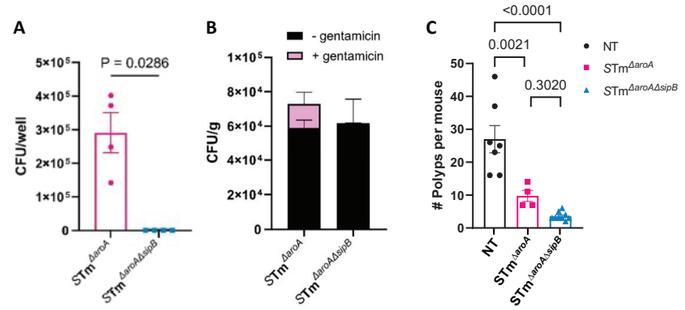


Figure 2. CD4+ T cells cultured in tumour conditioned medium from tumour organoids given the indicated Salmonella strains. aroA=aromatase deficient; ansB=asparaginase deficient. Timer blue and red expression indicates persistent T cell receptor activation. Timer^{red+}Timer^{blue-} indicates terminated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

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.

In a collaboration with the Frickel lab, University of Birmingham, we investigated another pathogen sensor, GBP1, in human colorectal cancer organoids. The Frickel lab had identified a guard mechanism whereby the kinase PIM1 phosphorylates GBP1 to protect an infected cell from GBP1-induced Golgi fragmentation and cell death. If signalling is disrupted, PIM1 is rapidly degraded releasing GBP1, and cell death ensues. We tested whether this mechanism is also present in human tumour organoids using a small molecule inhibitor of PIM1 kinase. PIM1 inhibition indeed led to increased cell death in these patient-derived tumour organoids, and thus this represents an interesting innate guard mechanism that may be targetable in cancers expressing these proteins. (Fisch *et al.*, 2023, *Science*).

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COMPUTATIONAL BIOLOGY

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Alexandrina Pancheva
Andrew Papanastasiou

Graduate Students
Britt van Abeelen
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 and to use data to position our pre-clinical models of disease against these more tightly defined patient subsets to support forward translation from discovery science into the clinic, and back translation into our experimental models. We are together working on 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.

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.

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. Holly Hall, a postdoc in the lab, is 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. Over the last year we have been working with Naveed Khan to commission a High-Performance Computing system that is starting to underpin our data science efforts across the Institute.

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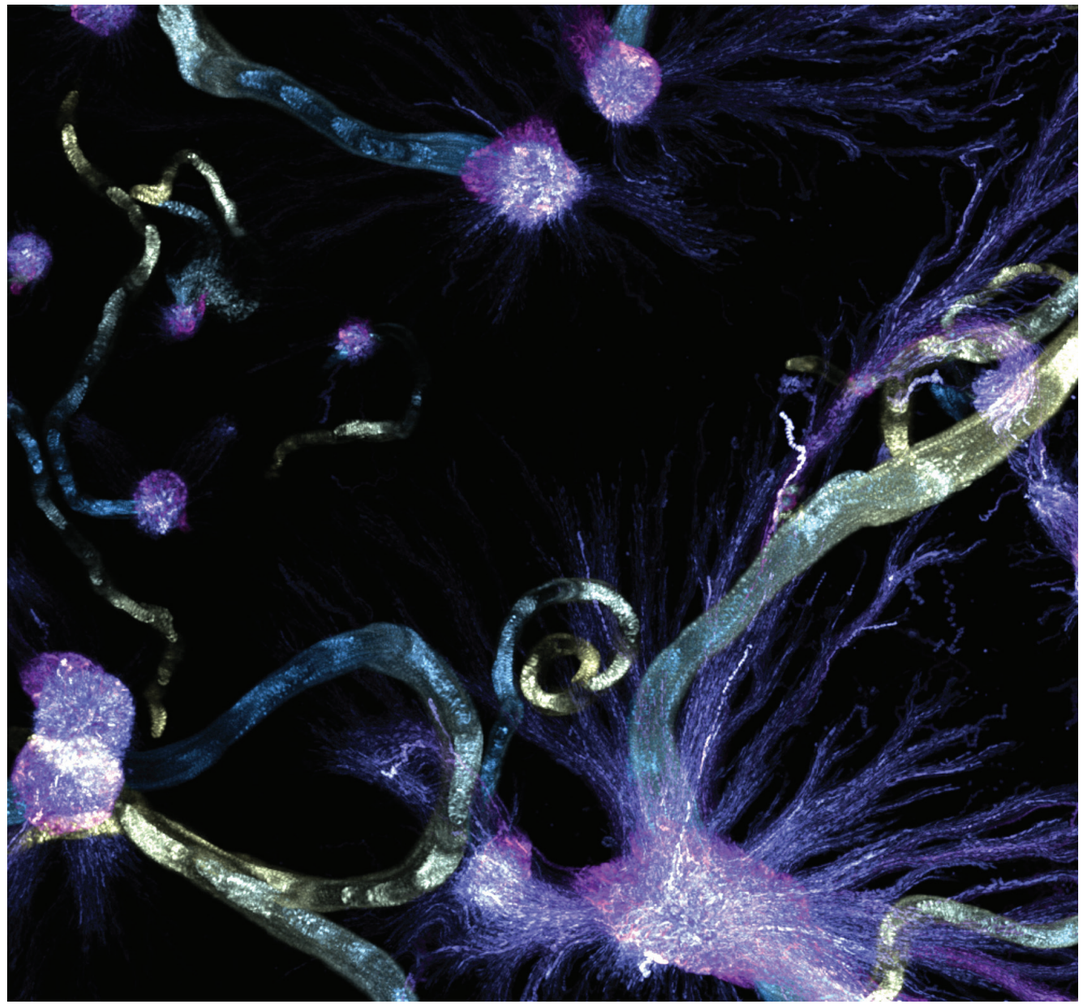

Dictyostelium cells were starved for 4 hours on non-nutrient agar to induce development prior imaging. The cells are expressing Flamindo2, a cAMP sensor that changes intensity of fluorescence dependent on the presence of cAMP in those cells. During development *Dictyostelium* cells utilize cAMP as messenger molecule to orchestrate the formation of fruiting bodies. The image shows an overlay of a timelapse taken between 8 and 14 h of development, when cells are forming streams and slugs as pre-stages before forming a mature fruiting body. Zeiss Airyscan 880, 10x air objective. LUT mpl viridis. Image by Peggy Ingrid Paschke.

PRECLINICAL PANCREATIC CANCER

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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 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, transcriptional subtypes, 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 PDAC 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 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.

PDAC Microenvironment

Tumour-associated macrophages (TAMs) and cancer-associated fibroblasts (CAFs), play a critical role in PDAC progression, but it has only recently been appreciated that significant heterogeneity exists in these cell populations (Yang *et al.*, 2020, *Front Cell Dev Biol*; Helms *et al.*, 2020, *Cancer Discov*). Moreover, the complex interplay between these populations in the tumour microenvironment (TME) is poorly understood. Rare populations of immune cells can also play a role in modulating the phenotype of these different populations, but these too have been under-studied. The stroma can have profound effects on therapeutic response (Beatty *et al.*, 2021, *Genes Dev*); however, therapeutic interventions may also have significant effects on the stroma. For example, radiotherapy causes CAFs and TAMs to alter their secretory output, remodelling the TME to favour tumour growth and treatment resistance (Krisnawan *et al.*, 2020, *Cancers*). By developing a clearer understanding of the complex signalling between different stromal cell subtypes, and the effects on individual signalling pathways on tumour progression and chemoresistance, we should be able to develop rational stromal targeting strategies for this disease. Thus, we are investigating how signalling between different cellular subsets and phenotypes can support fibrosis and

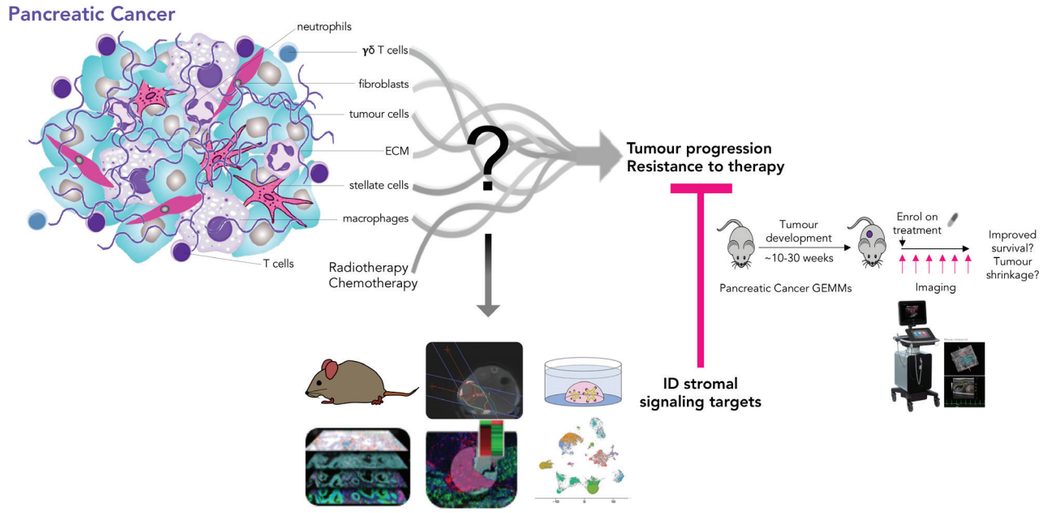

Figure 1. Schematic of workplan to investigate tumour-microenvironment signalling networks driving PDAC progression and therapeutic response and test newly identified therapeutic strategies.

tumour progression, how these phenotypes are controlled, and how therapy, particularly radiotherapy, can drive microenvironmental changes. Ultimately, we hope to identify signalling networks that could be exploited for therapeutic benefit, and test these concepts in tumour-bearing GEMMs (Figure 1).

Therapeutic Resistance

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, our recent data suggest that resistance can develop quickly. Indeed, most tumours relapse quickly, and display elevated fibrosis, enhanced extracellular matrix deposition, and a re-wiring of signalling, including metabolic pathways, in the microenvironment. We are now investigating how these factors can help tumour cells to adapt to therapeutic intervention and influence the response to treatment. Cancer-associated fibroblasts exhibit distinct expression profiles that can either support or restrict tumour growth (Hutton *et al.*, 2021, *Cancer Cell*). Therefore, to fully understand how best to target different cell types for therapeutic effect, we are investigating signalling within individual cell types. New technologies, such as spatial transcriptomic analysis and CODEX, are allowing us to spatially resolve mRNAs and proteins in individual tissue sections to visualize cells and signalling networks in their native tissue context (Figure 2A), but also spatially link molecular changes to therapeutic responses. Mass spectrometry imaging has also revealed significant metabolic plasticity in response to therapy (Figure 2B). Visualising this in spatial context and longitudinally across treatment experiments is crucial to observe metabolic rewiring/resistance mechanisms in situ. Building a comprehensive understanding of the signalling pathways and metabolic features in tumour cells and the tumour microenvironment following therapeutic intervention will allow us to identify the best strategies to overcome resistance.

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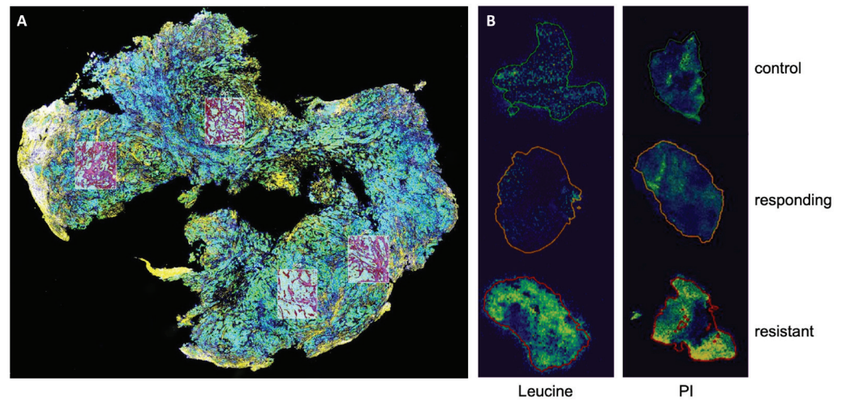

Figure 2. A Region selection and cell type masking for spatial transcriptomic analysis. PDAC tissue immuno-stained for CK19 (cyan, tumour cells), PDPN (yellow, CAFs) and DAPI (blue, CK19-PDPN- cells). **B** Mass spec imaging showing metabolic plasticity in PDAC response to therapy.

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MYC-INDUCED VULNERABILITIES/THORACIC CANCER RESEARCH

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[3]Merck Pharmaceuticals
[4]Asthma + Lung UK, DEBiT Meso
[5]CRUK Accelerator Award, PREDICT-Meso
[6]Self-funded
[7]CRUK Scotland Centre Studentship

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 β-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 Rosa$^{26DM\text{-}lsl\text{-}MYC}$ 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 2023

2023 was a year for consolidation of our lab's position as leaders of Mesothelioma research with publication of 2 papers on the subject, one in collaboration with the lab of Olivier Pardo of Imperial College London. In collaboration with Marion MacFarlane, I led a pre-clinical models education session following the bi-annual iMIG (International Mesothelioma Interest Group) worldwide conference, and I also presented invited lectures to the Cambridge Lung Cancer Symposium and the Oxford Early Detection of Cancer Symposium, along with Keynote lectures at the Indian Institute of Technology International Cancer Conference, held in Chennai, India, and the Chilean Society for Biochemistry annual conference, held in La Serena, Chile.

Within the National Mouse Genetics Network (NMGN), work continued on development of the Tandem Arrayed Regulator (TAR1) mouse that will enable controlled sequential genetic investigation of cancer progression – the allele was successfully integrated into ES cells and the first chimeric mice are expected in Spring '24. Our bid for supplementary funding from the NMGN Business Engagement Fund was approved to support collaboration with OMIC Pharmaceuticals, CA, USA, with work to progress in 2024. Our collaboration with Merck pharmaceuticals and Cancer Research Horizons successfully identified a specific small molecule kinase inhibitor suitable for *in vivo* experimentation.

The work of former PhD student Declan Whyte was published in Molecular Oncology and the lab led or contributed to 4 additional pre-print manuscript released on *BioRxiv* in 2023.

Finally, the lab welcomed new PhD student, Dominika Lubawska, funded through the CRUK Scotland Cancer Centre and commenced recruitment for 2 postdoctoral positions funded by CRUK programme REMIT.

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INTEGRIN CELL BIOLOGY

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Research Scientists
Luis Pardo
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Declan Whyte

Clinical Scientist
Iain Macpherson

Scientific Officer
Madeleine Moore

Clinical Fellow
Sonam Ansell

Graduate Students
Jasmine Peters
Sophie Fisher

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).

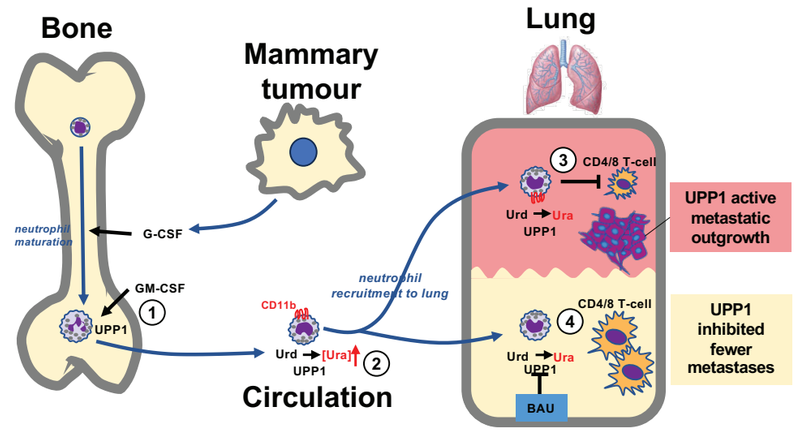

Figure 1A Neutrophil pyrimidine metabolism leads to priming of the lung metastatic niche in breast cancer. **1** Mammary tumours release factors such as G-CSF to drive neutrophil maturation in the bone marrow, and GM-CSF which leads to activation of uridine phosphorylase-1 (UPP1) in neutrophils. **2** 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.

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.

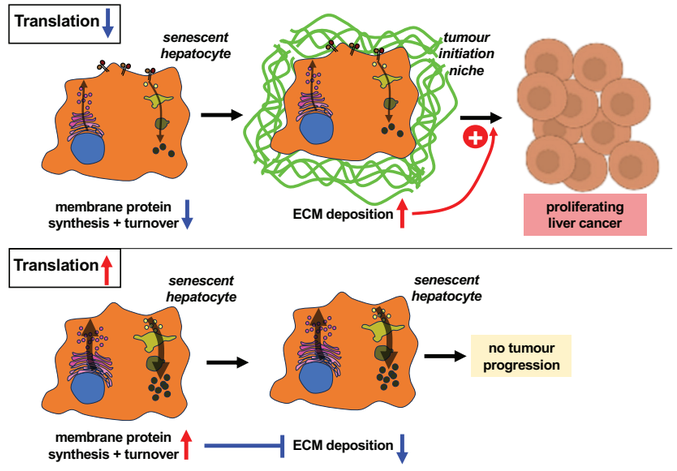

Figure 1B Restriction of mRNA translation is key to efficient initiation of liver cancer. When the translation of secretory mRNAs is restrained (upper panel), protein synthesis and membrane protein turnover is moderate. This maintains membrane trafficking and facilitates ECM protein deposition to promote 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.

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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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. Translating this concept onto cancer cell populations, phenotypic plasticity may lead to the establishment of co-existing phenotypically different metastable states that may grant populational adaptation to fast-paced environments (exposure to drugs, novel niches, etc) even in the absence of genetic divergence. Given the crucial role that 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 of cell plasticity during cancer onset and progression.

Over the past few years and by means of 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 to unravel the molecular mechanisms underlying cellular plasticity, we developed a lineage tracing technology termed Barcode decay Lineage Tracing-Seq (BdLT-Seq) which allows us to build directional cell-lineage trees linking individual branches to metastable transcriptome states, thus uncovering the molecular "rules" of transcriptome plasticity in comparable genetic backgrounds. BdLT-Seq relies on a high-complexity library of non-genetically (episome-)encoded molecular identifiers which, upon transfection, provides each cell within the population with a unique molecular fingerprint (Figures 1A and 1B). Notably, though episomal vectors undergo scheduled replication and therefore are stably maintained within transfected cells, they are also randomly inherited upon cell division. This important feature results in a decay in the number of unique barcodes in daughter cells while maintaining a unique lineage-specific fingerprint which relates each cell to its ancestor (Figures 1A and 1C).

Importantly, applying BdLT-Seq to an $HRAS^{G12V}$ driven clonal *in vitro* tumorigenic model (HA1ER cells) we found that transcriptome states are inherited upon cell division, but are also rewired into distinct states resulting in a progeny displaying different transcriptome profiles, thereby fuelling the generation of non-genetically heterogeneous populations (Figure 1E). Moreover, we reported that the plastic capacity of transcriptome states to generate progeny with a different profile is not stochastic but is encoded in non-genetic networks and restricted in a lineage-linked manner. Notably, restricted transcriptome plasticity and inheritance is not unique to tumorigenic models and has also been observed in immortalized cell systems suggesting its widespread and, perhaps, universal nature. Interestingly, subcloning a parental – clonal – population gives rise to populations of cells that are enriched in subsets of transcriptome states that only partially recapitulate the heterogeneity observed in the parental clone, validating our observations related to lineage-linked transcriptome plasticity. 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.

Following those lines of thought and based on our findings, we used BdLT-Seq to explore if and how lineage-linked transcriptome plasticity plays a role in oncogene-induced cellular transformation. For that, we built a multiplexed cell system in which six RAS mutant variants from different families ($HRAS^{G12C}$, $HRAS^{Q61K}$, $NRAS^{Q61R}$, $NRAS^{Y64D}$, $KRAS^{G12V}$, $KRAS^{E62K}$) are individually expressed in an otherwise isogenic clonal population of immortalised cells (HA1E) and performed a simultaneous analysis of the impact of oncogene expression on lineage-linked transcriptome evolution. Notably, we found that cells expressing different RAS oncogenes are readily enriched in distinct transcriptome clusters, suggesting that the expression of each RAS variant has a distinct impact on transcriptome plasticity, thus promoting the remodelling of the population heterogeneity in an oncogene-specific manner (Figure 1D). Interestingly, despite a similar degree of non-genetic heterogeneity was evidenced for specific RAS variants (HRAS-G12C versus HRAS-Q61K), distinct patterns of transcriptome plasticity were observed for a subset of states (for example, cluster 1 to cluster 4 transitions), thus suggesting that substantial differences in phenotypic output could stem from differential population dynamics (Fig. 1d).

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

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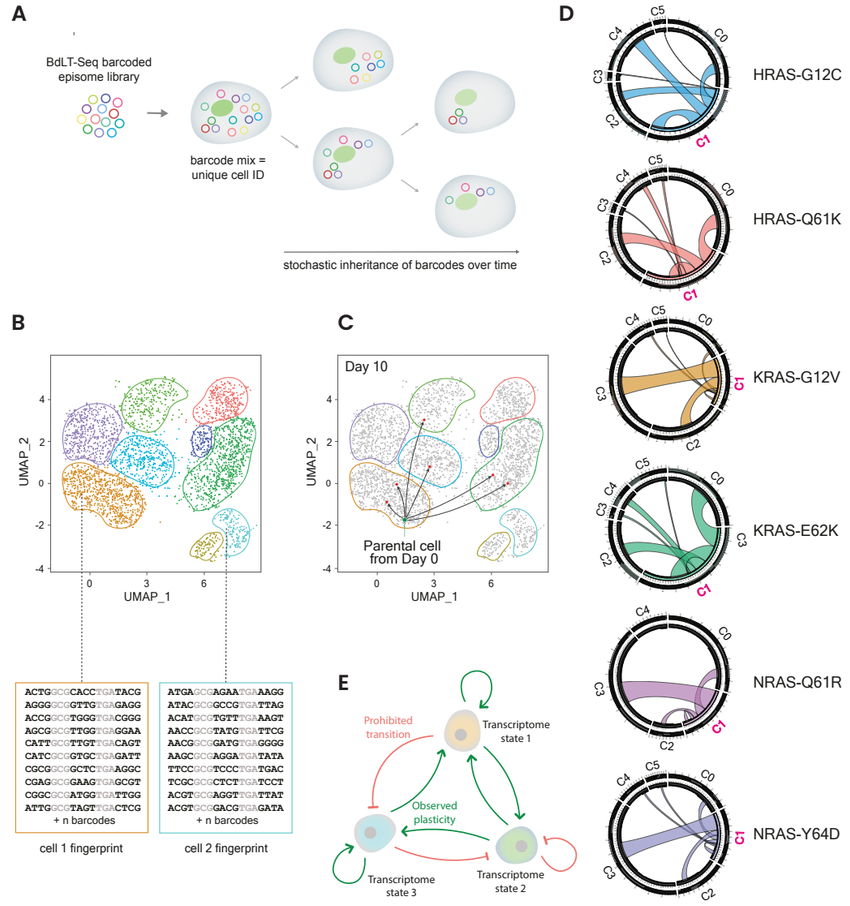

Figure 1. A Scheme representing BdLT-Seq conceptual framework that can be applied to any model system susceptible to be transfected with the episomal library. The combination of barcodes per cell sets its unique fingerprint ID. Barcode decay is exemplified as a function of time. **B** Toy UMAP plot depicting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

Credits for figure
Bianca Blochl and Maxi Portal

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.

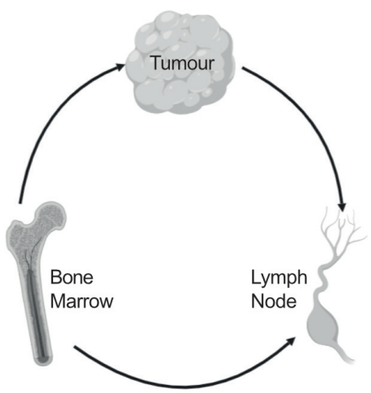

Figure 1. The DC lifecycle
DC precursors develop in the bone marrow and migrate to the tumour and the lymph node. Once within the tumour, they sample proteins from the microenvironment and then mature and migrate to the lymph node. There are the DC which migrated straight to the lymph node and those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

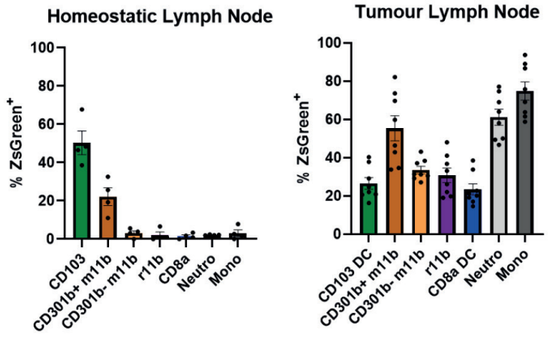

Figure 2. Tumour antigen is handled uniquely
ZsGreen expressed within the lung is carried to the lymph node by migratory DC, but the protein remains restricted to the migratory DC. When the same protein is expressed in a tumour, the protein is carried to the lymph node by migratory DC in a similar fashion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

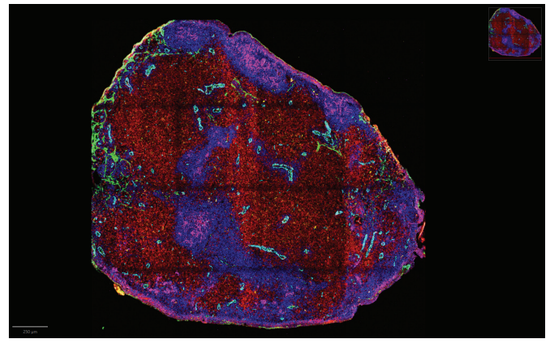

Figure 3. Lymph node organisation
A whole cleared lymph node stained for T cell, B cell and DC markers shows the organisation of a lung tumour-draining lymph node.

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.

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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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 that 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*., *Cell*, 2006), its related family member p73 (*CDD*, 2007), the hypoxia inducible transcription factor HIF-1α (Wilkinson *et al*., *Genes Dev*., 2009) and the chromatin modifier BRD4 (Sakamaki *et al*., *Mol Cell*, 2017) are all regulators of autophagy, indicating that several key cancer-related pathways impact on autophagy. More recently, our attention turned to 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, 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 (Figure 2). 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 (Figure 2).

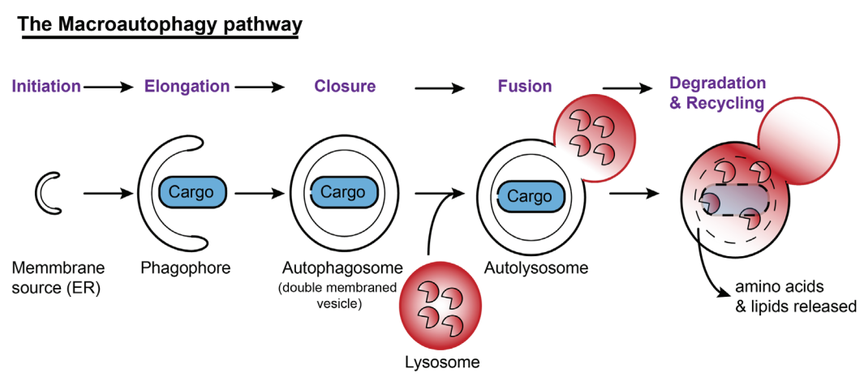

Figure 1: The (Macro) Autophagy pathway.
The process of macroautophagy occurs in the cytoplasm of cells and proceeds through various stages to encapsulate cargoes destined for degradation. Ultimately, fusion occurs with a lysosome that provides hydrolases required for degradation. The breakdown products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

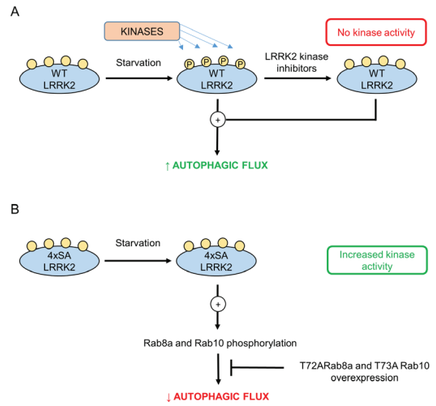

Figure 2: Phosphorylation of LRRK2 regulates autophagy
Upon nutrient deprivation, the Leucine-Rich Repeat Kinase (LRRK2) is phosphorylated at a number of 'constitutive' sites and cells undergo induction of autophagy. Although the treatment of cells with LRRK2 kinase inhibitors ablates the phosphorylation at the 'constitutive' sites, it does not affect autophagy (**A**). In contrast, mutation of the constitutive sites in LRRK2 results in increased LRRK2 kinase activity and an impairment of autophagy flux. This process is dependent on the LRRK2-mediated phosphorylation of Rab8a and Rab10, indicating that the lack of phosphorylation of LRRK2 'constitutive' sites affects autophagy by inducting the kinase activity of LRRK2 (**B**). The figure is reproduced from Kania et al. *Cell Death and Disease* (2023) (https://doi.org/10.1038/s41419-023-05964-0)

These findings therefore provide insight into an additional control point for autophagy that is relevant to human disease.

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 development of the early stages of PDAC development.

Using a mouse model of autophagy that we previously described as having excess PanIN formation (Rosenfeldt *et al*., *Nature*, 2013), we have utilized proteomics, metabolic mass spectrometry and microRNAseq to identify potential serum or urine biomarkers of PanIN formation (Figure 3). 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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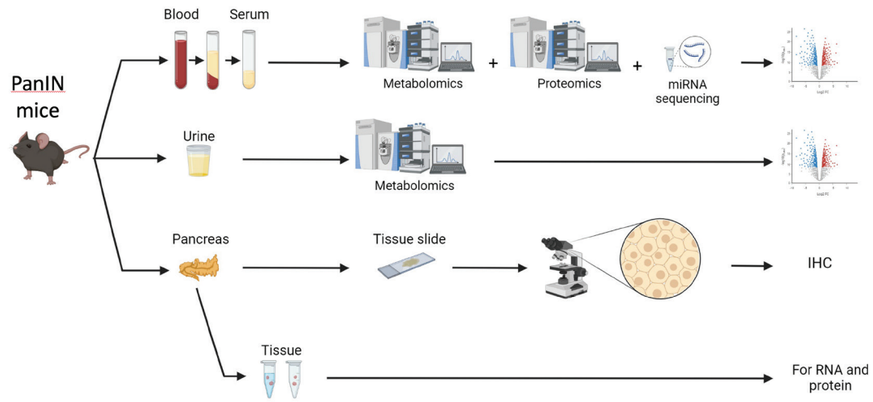

Figure 3: Utilizing autophagy to identify biomarkers of pre-cancerous pancreatic cancer.
Detection of pre-cancerous lesions enables the selection of individuals who are at risk and facilitates tumour formation at an early stage at which it can be treated. Mouse models were used to identify potential 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.

COLORECTAL CANCER AND WNT SIGNALLING

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[6]University of Glasgow/CRUK Accelerator Award
[7]Boehringer Ingelheim
[8]Cancer Research Horizons
[9]CRUK Therapeutic Catalyst Award
[10]AstraZeneca
[11]Novartis
[12]CRUK/University of Oxford
[13]Beatson Cancer Charity
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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" technologies and state-of-the-art preclinical models harbouring key driver mutations, we are interrogating the molecular underpinnings of CRC initiation and progression, with overarching goals to identify early-stage diagnostic biomarkers and stage- and subtype-specific targeted therapies.

Loss of the Wnt-antagonist APC—a key initiating event in most CRCs—drives a hyperproliferative crypt progenitor phenotype, recapitulating the early stages of intestinal transformation and adenoma formation. Progression to adenocarcinoma is associated with compounding genetic alterations in oncogenes and tumour suppressors (e.g., *KRAS*, *PTEN*, *TP53*, and *TGFβ*).

To model the 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 *ex vivo* organoid cultures complementing the study of disease pathogenesis and the preclinical testing of prospective therapeutics. With the ultimate goal of translating our preclinical findings to a tangible clinical benefit, we aligned our GEMMs to human CRC subtypes by integrating human and mouse multi-omics data, and demonstrated that they recapitulate key features of human disease and response to therapy.

Metabolic profiling stratifies CRC and reveals adenosylhomocysteinase as a therapeutic target

Motivated by the need to identify biomarkers and/or druggable targets for early-stage and advanced CRC, we investigated how different genetic drivers of CRC shape the intestinal metabolic landscape both in the early stages of transformation and in established tumours. We used multimodal mass spectrometry-based metabolomics to interrogate the metabolite profiles of premalignant hyperproliferative intestinal tissues from GEMMs harbouring loss of *Apc* (and *Pten*), in the presence or absence of oncogenic *Kras*, and CRC-patient tumours.

We first employed rapid evaporative ionising mass spectrometry (REIMS)—the transformative technology behind the intelligent knife (iKnife) that aids real-time metabolite profiling and detection of malignant tissue intra-operatively. Based on REIMS metabolic profiling, we developed a support vector machine-based algorithm that can stratify hyperproliferative transgenic mouse intestinal tissues (Figure 1A) and CRC-patient tumours (Figure 1B) according to *KRAS* mutation status, a finding of potential clinical utility given that transcriptomic analyses alone fail to distinguish wild-type and *KRAS*-mutant tumours.

As REIMS is best suited to lipidomic analyses, we used desorption electrospray ionisation (DESI) and matrix-assisted laser desorption ionisation (MALDI) to detect small metabolites, peptides, and proteins *in situ* at high spatial resolution. Indeed, these mass spectrometry imaging technologies revealed that tumour, stromal, and normal adjacent tissues exhibit profoundly different metabolic profiles. Such approaches may offer valuable insights into the metabolic heterogeneity of colorectal tumours, before and after therapy, and help identify metabolically distinct cell types or transient cellular states emerging during tumour evolution.

Using conventional liquid chromatography–mass spectrometry, which separates tissue/tumour components based on mass-to-charge ratio, we found that hyperproliferative APC-deficient intestinal tissues harbour dysregulation of the methionine cycle, underpinned by elevated expression of the enzyme adenosylhomocysteinase (AHCY). Indeed, *AHCY* transcripts were enriched in the Wnt-driven consensus molecular subtype 2 (CMS2) of human CRC and correlated with poor survival in stage I–III CRCs, prompting us to evaluate AHCY as a therapeutic target. Pharmacological inhibition of AHCY with DZNeP curtailed the growth of APC-deficient organoids, dampened the intestinal hyperproliferation following acute *Apc* loss both in the presence and absence of oncogenic KRAS^{G12D}, and perturbed tumour growth in *Apc*$^{Min/+}$ mice (Figure 1C).

This work (Vande Voorde *et al*., 2023, *Nat Metab*)—undertaken in collaboration with the rest of the Cancer Grand Challenges "Rosetta" team—illustrates the power of combining multimodal metabolomics approaches to visualize regional metabolite variability across different tumour compartments (which is obscured in bulk tissue analyses), stratify CRC-patient tumours based on underlying genetic drivers, and identify targetable metabolic vulnerabilities of CRCs.

Kras allelic imbalance drives tumour initiation but suppresses metastasis in CRC models

Based on recent studies showing loss of the wild-type allele in *KRAS*-mutant CRCs, which suggested that wild-type KRAS may influence the function of its oncogenic counterpart, we sought to understand how wild-type KRAS impacts tumour initiation, progression, and drug responsiveness in *KRAS*-mutant CRCs. Towards this aim, we developed GEMMs enabling deletion of wild-type *Kras* in intestinal tissues expressing oncogenic *Kras*^{G12D} (Najumudeen *et al*., 2024, *Nat Commun*).

Loss of wild-type *Kras* potentiated oncogenic KRAS^{G12D} activity and downstream MAPK signalling, and increased the tumorigenic capacity of KRAS^{G12D}-mutant APC-deficient (*AKras*$^{fl/G12D}$) cells, suggesting that wild-type KRAS functions as a tumour suppressor in this setting. On the other hand, loss of wild-type *Kras* rendered tumours addicted to oncogenic KRAS signalling and sensitized them to MEK inhibition, unveiling an exploitable therapeutic vulnerability (Figure 2A). Unfortunately, retention of wild-type KRAS dampened the dependence of *AKras*$^{+/G12D}$ tumours on MAPK signalling and conferred resistance to MEK inhibition (Figure 2A), limiting treatment options for this group.

Deletion of wild-type *Kras* in oncogenic KRAS^{G12D}-driven, aggressive tumour models accelerated tumorigenesis and shortened survival (Figure 2B) but, remarkably, abrogated metastasis (Figure 2C) by tempering aggressive and invasive features. Molecularly, KPN KF (VilCre^{ER}*Kras*$^{fl/G12D}$*Trp53*$^{fl/fl}$*Rosa26*$^{Nlicd/+}$) tumours, lacking wild-type KRAS, exhibited significantly elevated Wnt-pathway activity and reduced expression of neutrophil chemoattractants (*Tgfβ2* and the chemokines *Cxcl1*, *Cxcl3*, and *Cxcl5*; Figure 2D) in their premetastatic niche, which blunted metastasis formation, compared with KPN (VilCre^{ER}*Kras*$^{+/G12D}$*Trp53*$^{fl/fl}$*Rosa26*$^{Nlicd/+}$) lesions retaining the wild-type *Kras* allele (Figures 2C and 2E). These studies illuminate how wild-type KRAS influences the disease and treatment trajectory of mutant KRAS-driven CRCs and may lend new insight into earlier findings that Wnt-high tumours confer a better prognosis than metastasis-prone, Wnt-low CRCs. They further advocate that, in addition to screening CRC patients for *KRAS* mutation status, stratification for *KRAS* allelic status might discern those patients likely to derive benefit from inhibition of downstream effector signalling.

Disease positioning of preclinical models and patient tumours

In collaboration with Dr Philip Dunne (Queen's University Belfast and CRUK Scotland Institute), we developed the MmCMS transcriptional classifier, which aligns GEMMs to the CMS classes of human CRC, overcoming species differences and achieving a more robust classification based on biological pathways rather than individual gene expression patterns (Amirkhah *et al*., 2023, *Br J Cancer*). MmCMS (publicly available from https://github.com/MolecularPathologyLab/MmCMS) will enable us and other researchers to select the mouse models that best recapitulate the human CRC subtype of interest for studies into subtype pathogenesis, response to therapy, and the emergence of resistance.

We are also leveraging single-cell and spatial multi-omics technologies to disentangle intra- and inter-tumour heterogeneity during CRC progression and response to therapy. In collaboration with Prof Nigel Jamieson (University of Glasgow), we applied spatial transcriptomics to samples from patients undergoing synchronous resection of primary

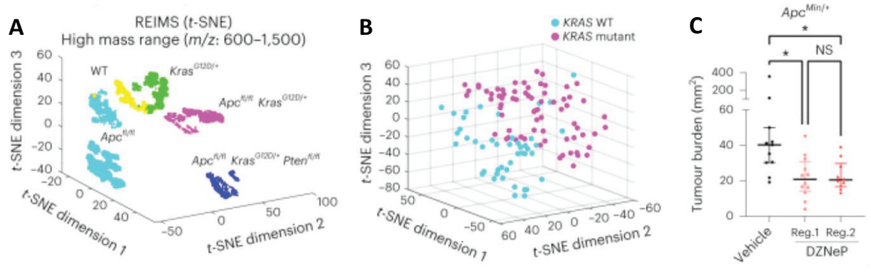

Figure 1: Metabolic profiling stratifies CRCs according to *KRAS* mutation status and reveals AHCY as a therapeutic target. A) t-distributed stochastic neighbour embedding (t-SNE) plot of REIMS mass spectra acquired from the small-intestinal epithelium of mice of the indicated genotypes focused on ions within a mass range of m/z 600–1,500. WT, n = 3; *Kras*$^{G12D/+}$, n = 4; *Apc*$^{fl/fl}$, n = 11; *Apc*$^{fl/fl}$*Kras*$^{G12D/+}$, n = 4; *Apc*$^{fl/fl}$*Kras*$^{G12D/+}$*Pten*$^{fl/fl}$, n = 5. **B)** Three-dimensional t-SNE visualization of REIMS data collected from CRC-patient tissues, using only 50 significant classification features, within a mass range of m/z 50–1,200. KRAS-WT, n = 8; *KRAS*-mutant, n = 16. In (A) and (B), each dot corresponds to a single mass spectrum acquired using the REIMS forceps. **C)** Small-intestinal tumour burden in *Apc*$^{Min/+}$ mice (aged day 50 to day 85) treated with vehicle or DZNeP. Vehicle (i.p. PBS), n = 11; Regime 1 (2 mg DZNeP per kg body weight i.p.; 4 days on and 3 days off), n = 12; Regime 2 (5 mg DZNeP per kg body weight i.p.; twice per week), n = 10. Median and interquartile range are plotted. *P < 0.05; Vehicle versus Reg.1, P = 0.0114; Vehicle versus Reg.2, P = 0.0243; Reg.1 versus Reg.2, P > 0.999; Kruskal–Wallis test followed by Dunn's correction. m/z, mass-to-charge ratio.

colorectal tumours and matched liver metastatic lesions. This approach correlated the presence of adaptive immune cell populations at the invasive edge with improved cancer-specific survival. In contrast, poor-prognosis patients were distinguished by high stromal content and an abundance of immunosuppressive Treg cells and neutrophils, underscoring the importance of the tumour microenvironment and the host immune response as determinants of outcome (Wood *et al.*, 2023, *Cancer Res*).

In collaboration with Prof Sabine Tejpar (KU Leuven, Belgium) and Prof Simon Leedham (University of Oxford), we are investigating whether the intrinsic CMS (iCMS) classification, which recognises two major epithelial cell-intrinsic transcriptional programs (iCMS2, arising from *LGR5*$^{+}$ stem cell populations, and iCMS3, manifesting a metaplastic, foetal-like/regenerative cell phenotype), can stratify our GEMMs. Using single-cell RNA-sequencing of human clinical samples, we have identified a hitherto unrecognized subtype of iCMS3 CRCs, comprising up to 50% of human colorectal lesions, which is characterized by metaplastic gene signatures, foetal-like reprogramming, and damage response/chronic inflammation. Molecularly, iCMS3 tumours are driven by YAP-associated signalling rather than the canonical Wnt-pathway activation, which underpins the iCMS2 group. We are investigating how the metaplasia-related iCMS3 phenotype impacts tumour initiation, evolution, metastasis, and therapeutic response, and whether our GEMMs recapitulate the iCMS2 and iCMS3 epithelial cell states (Figure 3; Gil Vazquez *et al.*, 2022, *Cell Stem Cell*). Integrating insights from preclinical models and clinical studies will help identify molecular drivers of the metaplastic iCMS3 subtype and may inform iCMS3-targeted therapeutic strategies. Together, these studies have the translational potential to drive clinical decision-making and CRC-patient stratification.

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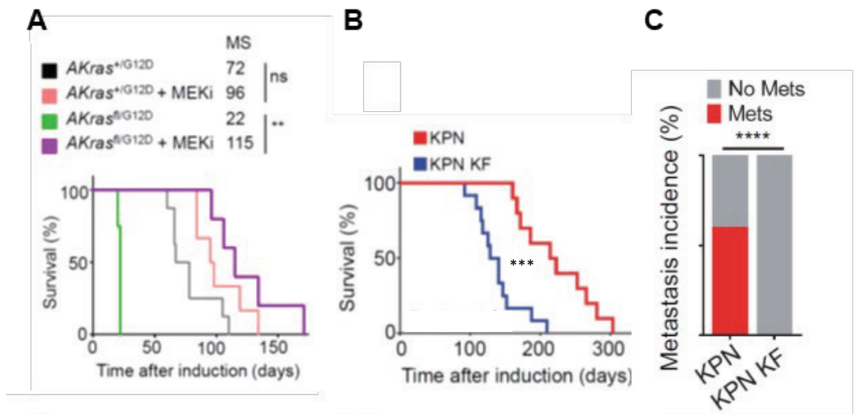

Figure 2: Loss of wild-type *Kras* sensitises to MEK inhibition and suppresses metastasis of KrasG12D-mutant colorectal tumours. **A)** Kaplan–Meier survival curves for VilCre^{ER}*Apc*$^{fl/+}$*Kras*$^{+/G12D}$ (*AKras*$^{+/G12D}$) and VilCre^{ER}*Apc*$^{fl/+}$*Kras*$^{fl/G12D}$ (*AKras*$^{fl/G12D}$) 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). $^{***}P$=2×10^{-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). $^{****}P$=1×10^{-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.

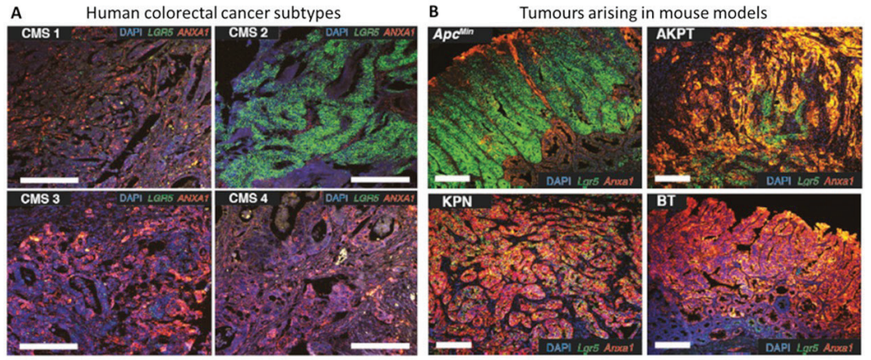

Figure 3: iCMS3-like populations in human and murine CRCs. Representative *in situ* hybridization for *LGR5* (marker of canonical intestinal stem cells; green) and *ANXA1* (marker of regenerative/iCMS3-like stem cells; red) in **A)** human CRC and **B)** GEMM tumours. *ANXA1*$^{+}$ regenerative/iCMS3-like stem populations are enriched in human CMS1, CMS3, and CMS4 CRC and *Kras*^{G12D}- and *Braf*^{V600E}-mutant GEMMs, whereas canonical/iCMS2 *LGR5*$^{+}$ populations are enriched in human CMS2 tumours and *Apc*$^{Min/+}$ adenomas. AKPT, VilCre^{ER}*Apc*$^{fl/fl}$*Kras*$^{G12D/+}$*Trp53*$^{fl/fl}$*Alk5*$^{fl/fl}$; KPN, VilCre^{ER}*Kras*$^{G12D/+}$*Trp53*$^{fl/fl}$*Rosa26*$^{Nlicd/+}$; BT, VilCre^{ER}*Braf*$^{V600E/+}$*Alk5*$^{fl/fl}$. DAPI, blue. Scale bars, 100 µm.

ADVANCED COLORECTAL CANCER

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Patients die from colorectal cancer due to spread/metastasis to other organs, in particular the liver. My 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. My 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 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).

Modelling immunosuppressed metastatic CRC and understanding microenvironmental influences for therapeutic gain

We have worked closely with Professor Owen Sansom's 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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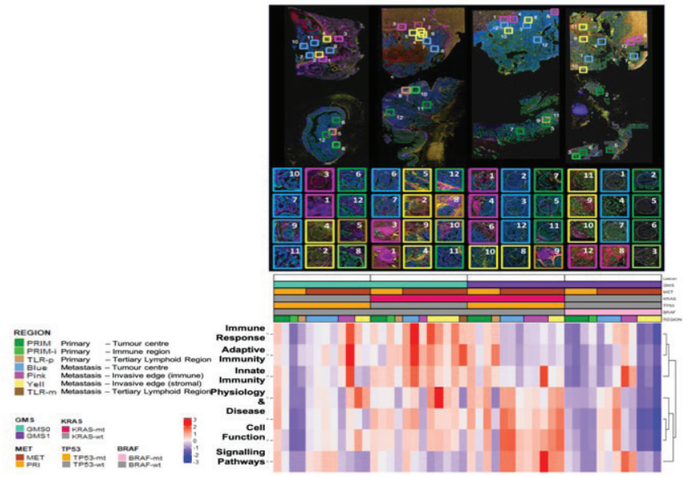
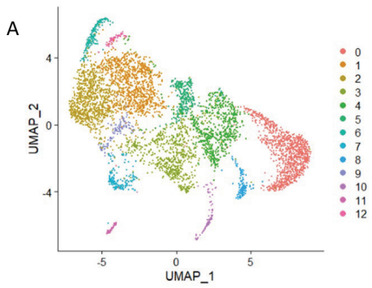
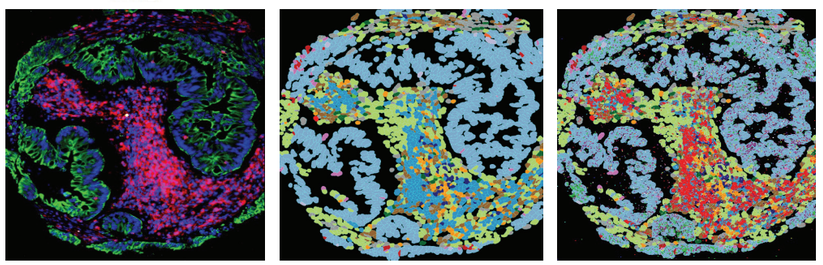


Figure 1. Transcriptomic assessment of heterogeneity, primary and metastatic sites in 4 patients

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. 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.

Apoptotic stress promotes the inflammatory phenotype of senescent cells

The textbook view of senescence and apoptosis is that they are wholly distinct processes – senescence associates with irreversible cell cycle arrest, whereas apoptosis is cell death. We have recently discovered that while distinct processes, apoptosis and senescence are linked via similar mitochondrial dependent mechanisms. Senescent cells are pro-inflammatory, releasing a variety of cytokines collectively termed the senescent associated secretory phenotype (SASP). Importantly, the SASP is associated with various detrimental effects including promotion of metastasis, chemoresistance and age-related tissue dysfunction. Together with Joao Passos (Mayo Clinic), our previous work found a key role for mitochondria in the promotion of SASP, the key question is how do mitochondria promote this?

Investigating this question, we found that senescence cells release mitochondrial DNA (mtDNA) that can promote the SASP through activation of cGAS-STING DNA-sensing pathway (Figure 1). This led us to address how mtDNA can egress mitochondria. Here we found that apoptotic stress induced minority MOMP plays a key role. Genetic or pharmacological inhibition of MOMP (through inhibition of BAX and BAK) supressed the SASP both *in vitro* and *in vivo*. Importantly, blocking MOMP, thereby targeting the SASP, improved the health of aged mice. Collectively, our data demonstrate that apoptosis and senescence are connected by fundamentally similar processes – namely MOMP. Future research will investigate the importance of this connection in cancer development and treatment response, potentially opening new therapeutic avenues.

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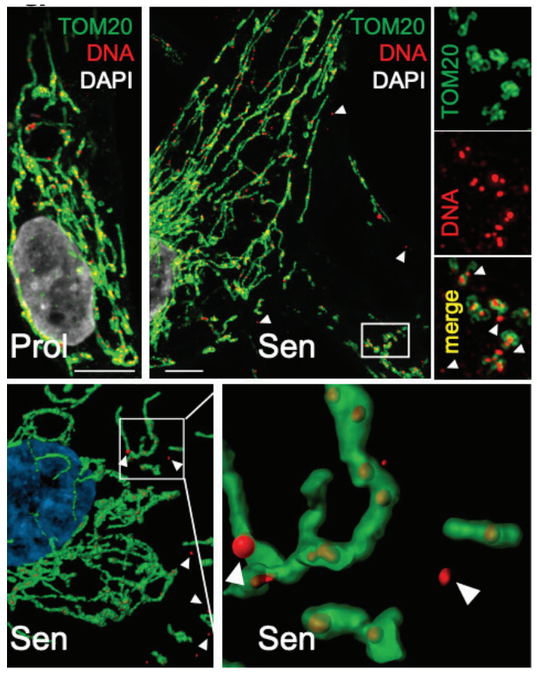

Figure . Cytosolic release of mitochondrial DNA in senescent cells
Proliferating (Prol) or senescent (Sen) MRC5 fibroblasts were stained with anti-DNA (red) and TOM20 (green) antibodies to detect DNA and mitochondria. In senescent cells, mtDNA (red) is often detected in the cytosol.

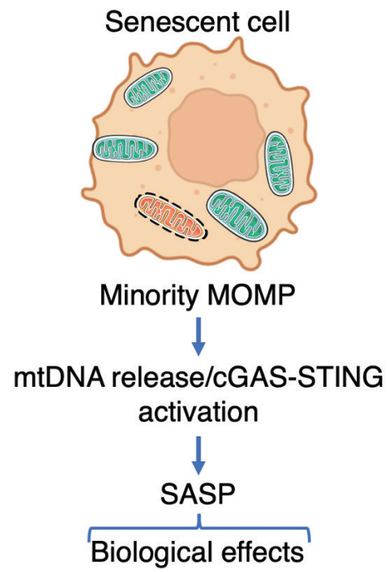

Figure 2. Mitochondrial permeabilization connects apoptotic signalling and senescence
Non-lethal apoptotic stress in senescent cells triggers minority MOMP. This enables mtDNA release activating cGAS-STING to promote the pro-inflammatory SASP with subsequent, pleiotropic biological effects.

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ONCOMETABOLISM

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The transfer of chemical energy from nutrients into macromolecules is the foundation of cellular and tissue growth. Tumours are no exception to this principle, and their metabolic state ultimately supports anabolism and growth. Our vision is that the tissue of origin influences the biochemical pathways utilised by tumours to grow in two ways. On the one hand by imposing environmental constraints, the tissue of origin exposes metabolic vulnerabilities of the tumour. On the other hand, enzymes normally restricted to a defined population of differentiated cells and required for tissue physiological functions, can be hijacked by cancer cells to enhance their metabolic fitness.

Glutamine metabolism in liver homeostasis and cancer

The canonical activity of glutamine synthetase catalyses the production of the amino acid glutamine from glutamate and ammonia. This reaction regulates glutamine metabolism from prokaryotes to mammals and is fundamental for processes such as ammonia detoxification and neurotransmission in humans. In the context of cancer, this enzyme is highly upregulated in a subset of liver cancer, affecting ~1 in 3 patients, which is driven by oncogenic mutations in β-catenin. We are currently studying the role of this enzyme in tumour initiation and progression but a pre-requisite to advance our understanding of its role in cancer is the elucidation of its functions in normal liver.

We demonstrated that a small molecule with uncharacterised biological activity, methylamine, was released by the intestinal microbiome, and it was used by the hepatic glutamine synthetase to produce a glutamine analogue which we identified as N^5-methylglutamine (Figure 1).

Technically, the identification of N^5-methylglutamine as a novel product of glutamine synthetase demonstrated the discovery potential of state-of-the-art metabolomics when applied to *in vivo* models.

Finally, we showed the translational relevance of our findings in a novel genetically modified mouse model of liver cancer that recapitulates the disease of those patients with β-catenin mutant tumours. We demonstrated that the urine levels of N^5-methylglutamine significantly correlated with liver tumour burden, substantiating the value of this metabolite as a biomarker for patients with this genetically-defined subset of tumours that express high levels of glutamine synthetase. These findings were published in *Nature Chemical Biology* volume 19, 292–300 (2023), highlighted in "*A collaborative synthetase*" *Nature Chemical Biology* volume 19, 255–256 (2023), and featured on the journal cover (Figure 2).

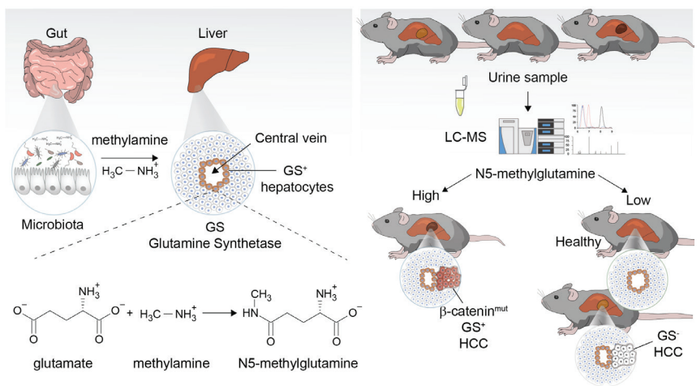

Figure 1 N^5-methylglutamine is a previously unreported product of glutamine synthetase and can predict liver tumour burden. Hepatic glutamine synthetase accepted methylated ammonia to synthesise N^5-methylglutamine. In a mouse model of β-catenin mutant hepatocellular carcinoma, the urine levels of this glutamine analogue were predictive of tumour burden.

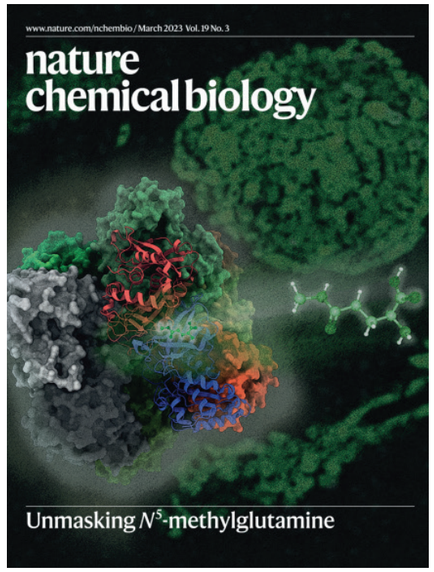

Figure 2. Journal cover featuring N^5-methylglutamine, the metabolite newly identified by the Oncometabolism group.

Figure 3. Plasmax™ is a physiological medium based on the levels of nutrients and metabolites found in human plasma that has been developed at the CRUK Scotland Institute. Plasmax™ is available in the original formulation and 'glucose-free' at https://cancertools.org/cell-culture-media/plasmaxtm-156371/.

A more physiological cell culture medium improves the relevance to *in vivo* biology

Despite it seeming obvious that the nutrient composition of culture medium affects the phenotypic behaviour of the cells, very little attention has been devoted to perfecting the formulation of historic media.

Indeed, the vast majority of biomedical research employs historic growth media, based on the pioneering work done 60 years ago by Harry Eagle. However, these formulations were not designed to reproduce the physiological cellular environment, but rather to enable the continued culture of cells with minimal amount of serum (i.e. Minimal Essential Medium, MEM). Consequently, the standard culture medium known as DMEM is distant from the nutrient levels found in normal human blood. For example, glucose in DMEM is at five-fold the normal glycaemia, and a similar ratio applies to glutamine. Conversely, non-essential amino acids normally circulating in blood are completely missing from DMEM formulation (Ackermann *et al.*, 2019, *Trends in Cancer*). On this basis, we developed Plasmax™ (Figure 3) a cell culture medium with nutrients and metabolites at the concentration normally found in human blood (Vande Voorde *et al.*, 2019, *Science Advances*).

In 2020, Plasmax™ became the first physiological medium to be commercially available, and in 2023 a new 'glucose-free' formulation suitable for glucose-restriction and metabolic tracing experiments was released by Cancertools.org. Implementing physiologically relevant cell culture conditions is a key step to achieve clinical success in targeting cancer metabolism, as emphasized in our review article 'Rethinking our approach to cancer metabolism to deliver patient benefit' (Tardito S, MacKay C., 2023, *Br J Cancer*). See Figure 4.

In 2023, we also published a study in collaboration with Dr Aye's group from the Wellcome-MRC Cambridge Stem Cell Institute, on the impact of Plasmax on human trophoblast stem cells (hTSCs), crucial players for placental development (Avellino et al., 2023, *Am J Physiol Cell Physiol*). The results indicate that hTSCs cultured in Plasmax™ exhibit increased proliferation and differentiation into syncytiotrophoblasts and extravillous trophoblasts compared to those cultured in the commonly used DMEM-F12. Despite the lower glucose concentration, hTSCs in Plasmax™ exhibit increased glycolytic activity and mitochondrial respiration. The metabolic profile of hTSCs emerged in Plasmax™ highlights changes in cellular redox state and methylation capacity that modulate hTSCs differentiation and functions. Indeed Plasmax™ enhances the secretion of human chorionic gonadotrophin and improves syncytial fusion during syncytiotrophoblasts formation.

In summary, the study suggests that the physiological medium Plasmax™ improves the proliferation, differentiation, and metabolic activity of hTSCs compared to the standard DMEM-F12 medium. These findings highlight the importance of using physiological media to better understand developmental mechanisms.

Publications listed on page 122

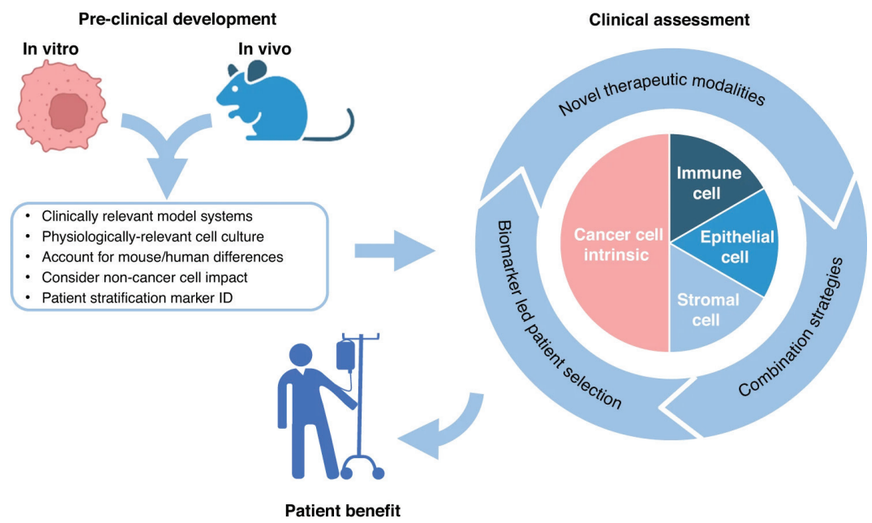

Figure 4. A schematic that illustrates how the pre-clinical developments can impact the clinical success of novel agents targeting cancer metabolism. From Tardito S, MacKay C. Br J Cancer. 2023 Aug;129(3):406-415.

TUMOUR MICROENVIRONMENT AND PROTEOMICS

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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.

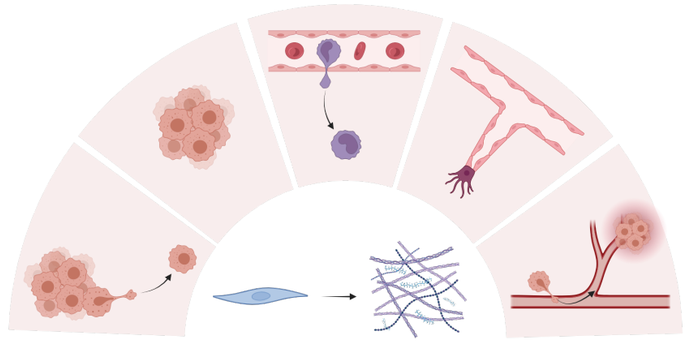

Figure 1. CAFs influence the behaviour of cancer and endothelial cells. Cartoon showing that cancer associated fibroblasts (CAFs), particularly those that secrete abundant ECM, influence various aspect of tumour development. Our works have shown that CAFs regulate mechanisms such as (from left to right): 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.

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 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, Kay *et al.*, 2024, *Sci Signal*).

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.

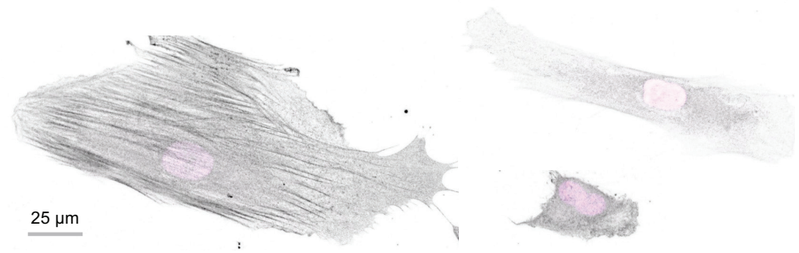

Figure 2. CAFs have different phenotypes. αSMA staining (marker of myCAF) of patient-derived HGSOC CAFs in culture suggesting that the bigger cell is a CAF with a myCAF phenotype while the elongated one is a CAF with an iCAF phenotype. Scale bar = 50 µm.

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, Kunal and Ashton have joined our team to push forward our research on cancer metabolism. Moreover, Emily Kay has presented her work on the immune-regulatory roles of CAFs in breast cancer at the International Beatson Cancer Conference in Glasgow and ISCaM2023 - Systemic Metabolism and Cancer Meeting in London.

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ADVANCED TECHNOLOGIES

BEATSON ADVANCED IMAGING RESOURCE

Head
Leo Carlin
Fellow of the Royal Microscopical Society (FRMS)

Scientific Officers
Ryan Corbyn
Tom Gilbey
Lynn McGarry
Ewan McGhee (until Sept 2023)
Claire Mitchell
Nikki Paul
Peter Thomason

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

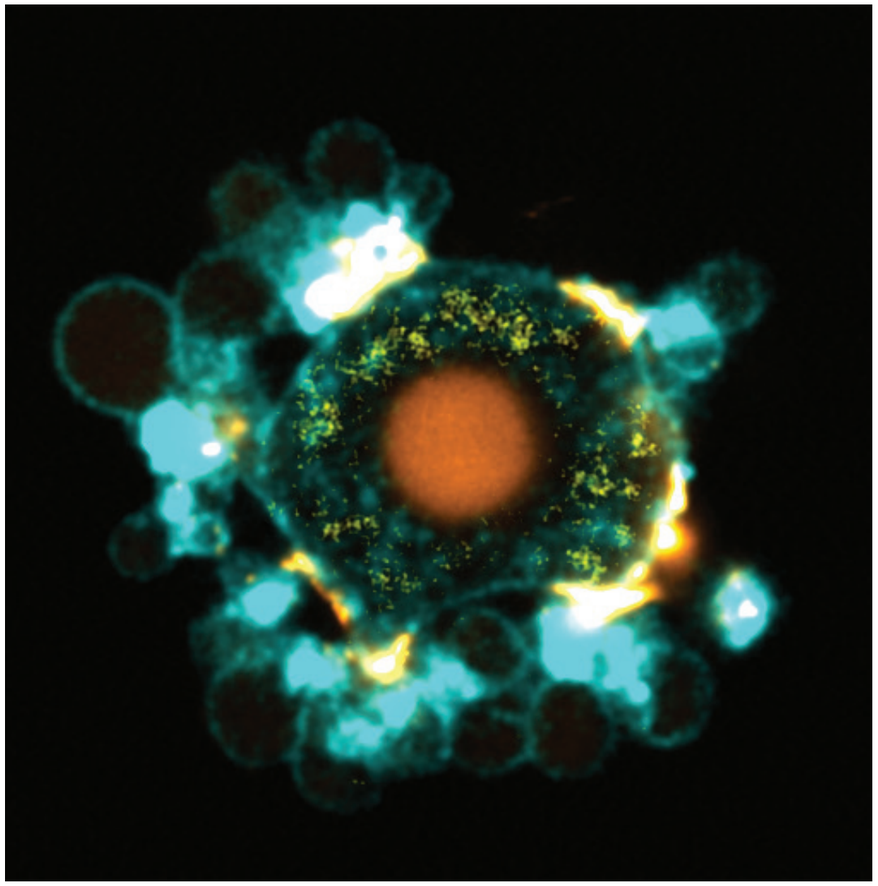
We have the expertise and instruments to:

- Address multiplexed panels of up to 15 markers in liquid phase and dissociated tissue samples by flow cytometry and sort cell populations for downstream analysis (e.g. proteomics or transcriptomics using other advanced technologies at the Institute)
- 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 (3D) 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

In this way, we underpin cancer research at the Institute 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, coordinated by Claire Mitchell, the team delivered a theory and practical course in microscopy covering fundamentals of light microscopy and image analysis in Image J / FIJI and using Python. This was so well received that the team will try to run it twice next year! Demand for flow cytometry from both the Institute and the School of Cancer Sciences (SCS), University of Glasgow has constantly grown in previous years. Therefore, with our successful bid for a University funded spectral flow cytometer fulfilled, we decided to bring this together formally with Tom Gilbey joining SCS colleagues Yi-Hsia Liu and Jennifer Cassels in a cross-faculty team led by Alison Michie from the SCS. We are excited to continue to work closely with the new facility which will consolidate equipment and expertise next door in the Wolfson Wohl Cancer Research Centre.

In the Summer we were delighted to learn that our application to the Medical Research Council for £575k to purchase an Airybeam, multiplexing light sheet microscope to image large volumes of tissues and tumours had been recommended for funding! This instrument will support beyond state-of-the-art research in the Institute, University and MRC national mouse genetics network and will be available from February 2024.



Apoptotic Flower. Live cell imaging of mouse endothelial cells (SVEC4-10) undergoing apoptotic cell death. The cells were imaged 90 min. after BH-3 mimetic addition which induces mitochondrial outer membrane permeabilization. Blebbing cell membranes are visualized by Annexin V (magenta) and the cell nucleus using CellToxGreen (yellow). Images were taken using the Zeiss Airyscan 880, 63x oil objective.

Image by: Rosalie Heilig

BIOINFORMATICS AND COMPUTATIONAL BIOLOGY

Head
Crispin Miller

Scientific Computing Specialist
Naveed Khan

Bioinformaticians
Ryan Kwan
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. A major aspect of our work continues to be the analysis of single cell sequencing data and we have been developing workflows that use a mixture of specific packages, such as Seurat, along with other software tools and packages from the Bioconductor project.

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 Shaw 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 data preprocessing. 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.

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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.

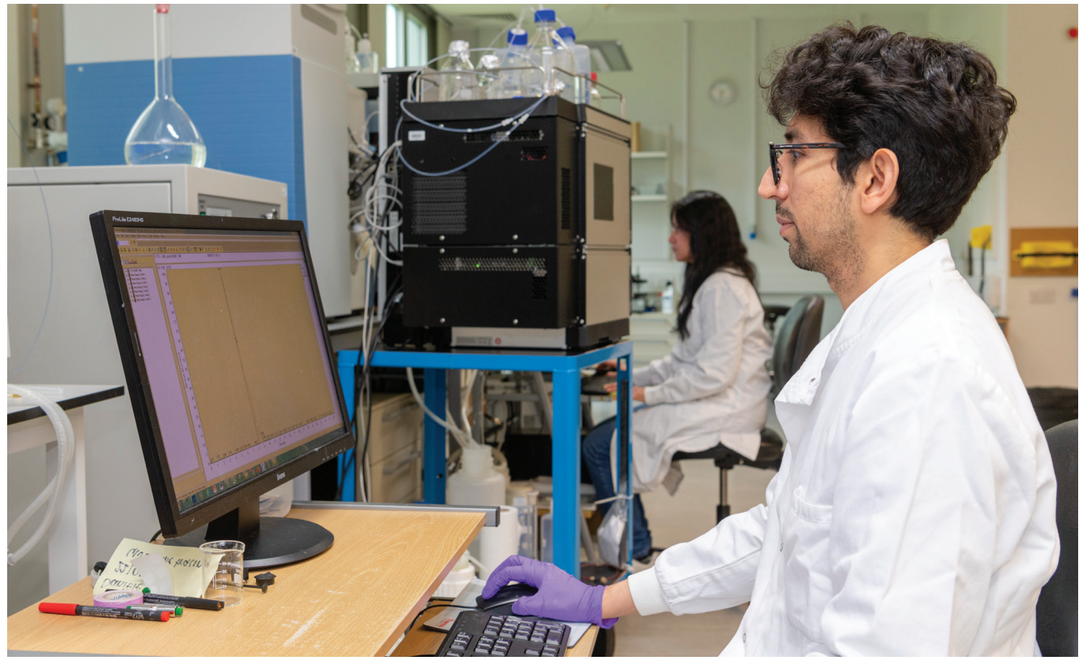
The facility's core aim is to provide access to state-of-the-art MS technology that is optimised for the detection of metabolites and lipids. We maintain and operate the instrumentation, providing both metabolite profiling and custom analysis when needed. This year, for example we have implemented methods for the measurement of deoxynucleotides alongside their much more abundant nucleotides/sides counterparts and the chiral separation of 2-Hydroxyglutarate enantiomers by derivatisation. We have also carried on developing targeted lipidomics methods to complement our existing untargeted approaches, recent efforts focusing on ceramides, sphingomyelins and cardiolipins. As well as measuring sterols with a particular focus on cholesterol in both its free and esterified forms.

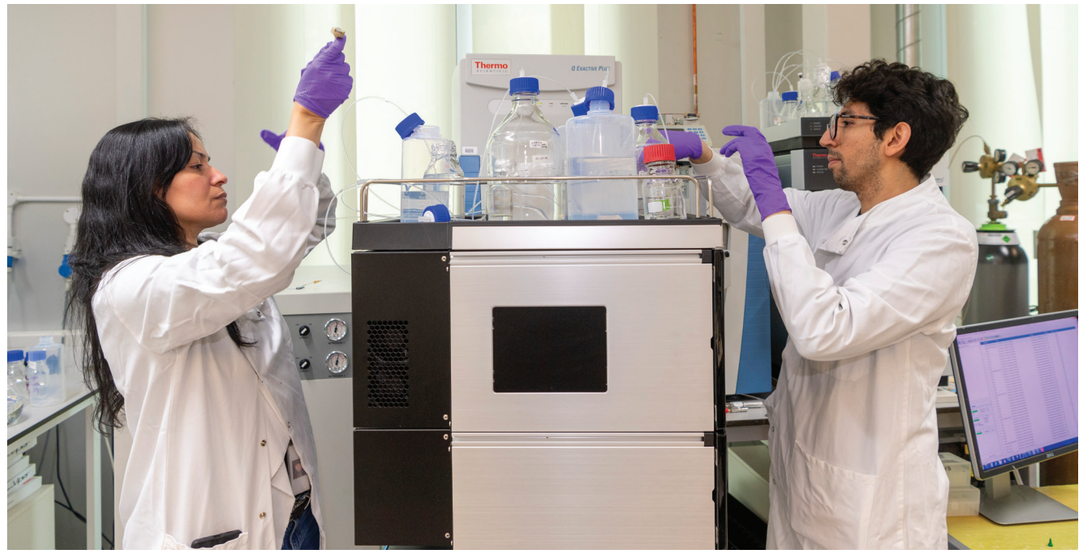
We offer expertise and assistance in data analysis, data interpretation and experimental design. This year, we have rolled out an update to our data analysis pipeline for targeted experiments delivering five in-house workshops over the course of the year. The workshops trained users to carry out their 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 particular highlight this year was working with Saverio Tardito's group on the identification of N5-methylglutamine, an unreported and novel metabolite. Remarkably and showing translational potential, N5-methylglutamine levels can act as a reporter of tumour burden in β-catenin-driven model of liver cancer.

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 2023 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 125





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.

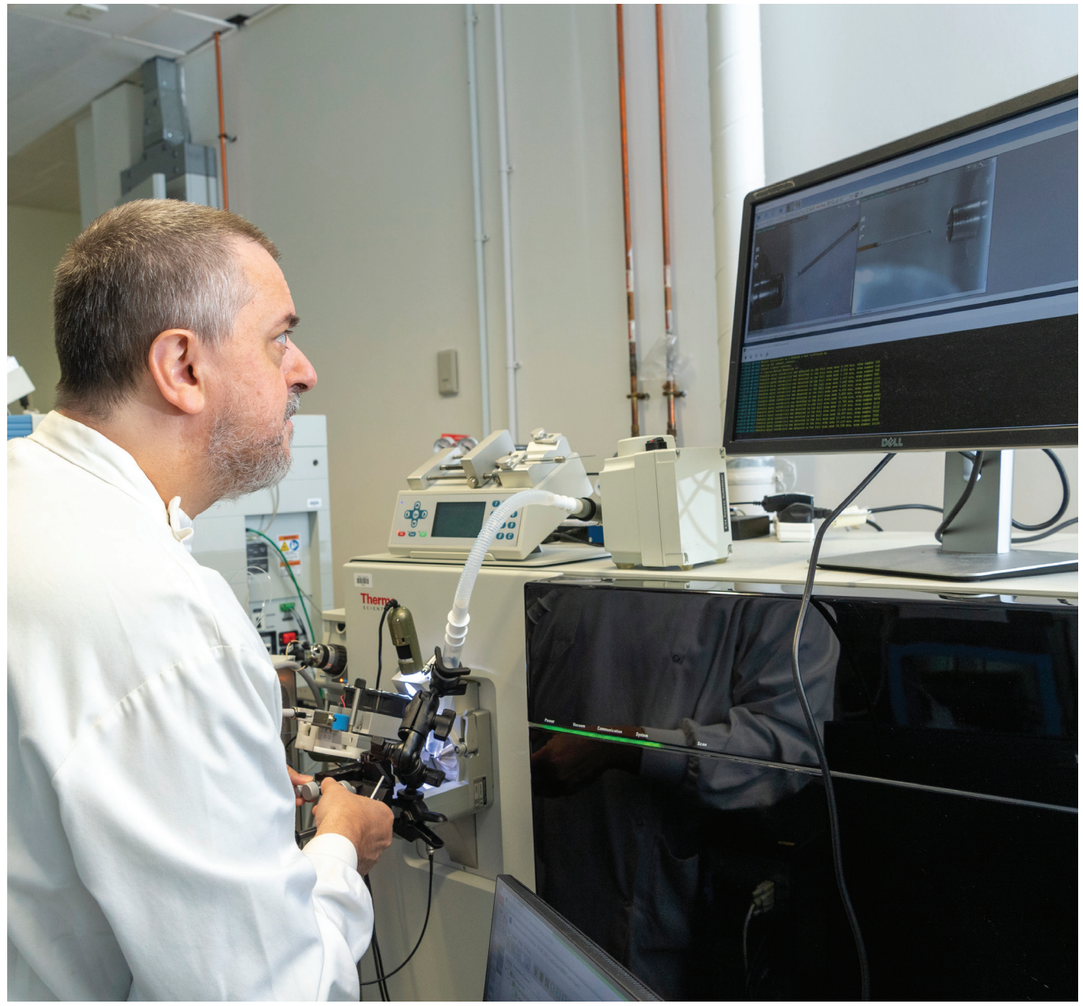
To achieve this, we are equipped with three nano liquid chromatography (nLC)-MS systems, including an Orbitrap Fusion-Lumos. All our instruments are coupled online to Easy-nLC systems, and high-resolution chromatography is achieved by packing our nano-columns in house.

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 2023, 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.

Publications listed on page 125



TRANSGENIC MODELS OF CANCER

Head
Karen Blyth

Lead *In Vivo* Scientist
Louise Mitchell

Scientific Officers
Jayanthi Anand
Dimitris Athineos
Laura Galbraith
Dale Watt[1]

[1]MRC National Mouse Genetic Network

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*

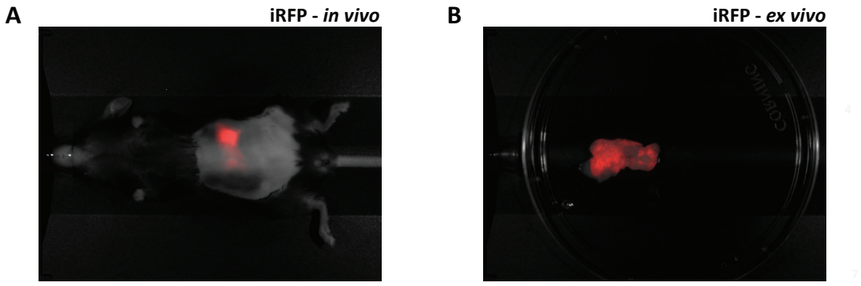
The Institute is internationally renowned for its scientific excellence using mouse models of cancer in a physiologically relevant way to gain insights on complex human diseases. This is important when we consider that tumour cells exist in a highly dynamic microenvironment which involves an intricate 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 plastic 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 have to 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) that carry 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 in 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 locally, nationally and internationally to translate i*n vitro* discoveries.

Research Collaborations

The lab is involved in many diverse projects across all the 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 presents an important therapeutic opportunity. Our colleague Oliver Maddocks recently identified specific transporters that are crucial for uptake of serine in colorectal cancer cells. We collaborated with Oliver to show that blockade of these specific transporters reduced serine uptake and inhibited colorectal cancer cell growth, offering potential therapeutic strategies (Papalazarou *et al*, *Nature Metab*., 2023). We have also collaborated with Saverio Tardito and his lab, in highlighting a new role for glutamine synthetase in producing a previously uncharacterised metabolite, N5-methylglutamine which correlates with liver tumour burden, indicating its potential as a biomarker for tracking liver cancer progression (Villar *et al*, *Nat Chem Biol*, 2023). In a separate study with Saverio we also showed that selenocysteine production facilitates lung seeding in a model of breast cancer metastasis (Ackermann *et al*, *bioRxiv*, 2023).

It was exciting to see our fruitful collaborations with David Bryant and his group being published this year. Building on their exciting observations in 2D/3D assays that the ARF3 GTPase interacts with N-cadherin to control invasion of prostate cancer cells, we showed that ARF3 was also instrumental in driving prostate cancer metastasis *in vivo* (Sandilands et al., *J Cell Biol*. 2023). Similarly, Podocalyxin (PODXL), found to be upregulated in patients with metastatic prostate cancer, was shown to be a key mediator of cancer invasion in both *in vitro* and *in vivo* prostate cancer models (Roman-Fernandez *et al*, *Sci Adv*. 2023).

Figure 1. Using iRFP expression to visualise and monitor pancreatic tumour progression *in vivo*.
A. Fluorescent imaging captured from a Pdx1-Cre;Kras$^{LSL-G12D}$;Trp53$^{LSL-R172H}$;HPRT$^{LSL-IRFP}$ mouse model. The iRFP reporter expression in the Pdx1-expressing cells reveals the distinct visualisation of the pancreatic tumour (depicted in red).
B. Pancreatic tumour shown in Figure 1A was resected and imaged *ex vivo*. Images were acquired on a PEARL Odyssey Imager and data was analysed using Image Studio Software.



In projects studying the tumour microenvironment we worked with Seth Coffelt to show how two subsets of lung γδ T-cells respond differently to cancer signals, influencing their regulation and potential for immunotherapy (Edwards *et al*, *J Exp Med*, 2023). And with Sara Zanivan's team, we have been investigating the importance of cancer-associated fibroblasts (CAFs) in driving cancer metastasis (Santi *et al*, *bioRxiv*, 2023). The lab also collaborated with Martin Bushell on a project to refine sample preparation techniques from primary mouse tissue to safeguard RNA integrity for accurate assessment of translational control mechanisms (Munro, *et al, Cancers*, 2023).

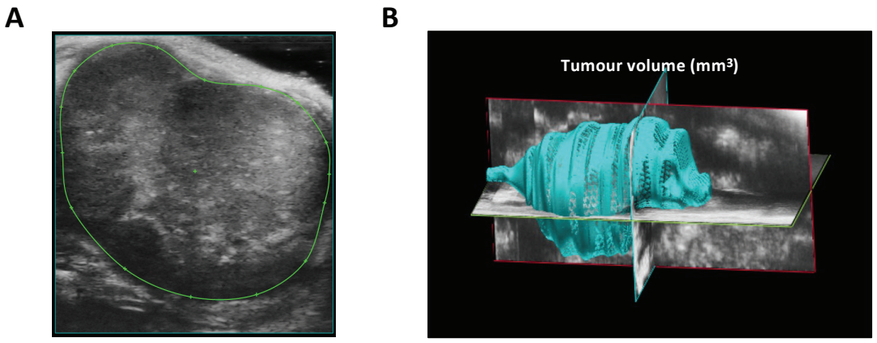
Finally, in partnership with colleagues at Glasgow, Oxford, London and Belfast, the lab also co-leads the Cancer Cluster within the MRC's newly established National Mouse Genetic Network (NMGN), (https://nmgn.mrc.ukri.org/clusters/cancer/). To this end we have been working closely with the MRC Mary Lyon Centre at Harwell and the rest of the network to develop and improve mouse models of human disease.

Resources & News

At the end of 2022, we welcomed Dr Louise Mitchell as Lead *In Vivo* Scientist to the group. Louise has been instrumental in motivating the team, steering our collaborative projects and leading researcher engagement and compliance. Louise also hosted and mentored Black in Cancer Intern Saharla Warsame for an 8-week internship, allowing Saharla to enhance her analytical and technical skills.

The Institute underwent a Strategic Review this year with a high commendation from the international panel of experts for our world-class animal modelling, and cutting edge-technologies. These achievements underscore our commitment to innovation and excellence in research. As a lab, we continue to focus on innovation to refine and improve cancer models for the benefit of the Institute. In pursuit of advancing our surgical expertise and repertoire, our team visited collaborating institutes to learn new methods. Dale Watt visited IRB in Barcelona to train on a resection model for colorectal cancer which mirrors the progression of metastatic colon cancer in patients. Meanwhile, Laura Galbraith was welcomed at the Netherlands Cancer Institute to train on specialised mammary intraductal injection techniques. In all our approaches we continually promote the 3Rs, including using longitudinal imaging protocols such as the PEARL near-infrared fluorescence detector (Figure 1), IVIS fluorescence/bioluminescence, and ultrasound imaging (Figure 2).

Figure 2. High-resolution ultrasound imaging to visualise and monitor prostate tumour progression *in vivo*.
A. Representation of a high-resolution ultrasound image of a prostate tumour in an orthotopically transplanted mouse model. Images were acquired using the Vevo 3100 instrument.
B. Tumour volume of a prostate tumour (measured in mm^3) can be calculated using the Vevo Lab 5.7.1 software from 3D scans.



TRANSGENIC TECHNOLOGY

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Scientific Officers
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Farah Naz Ghaffar
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The Transgenic Technology Laboratory uses molecular genetic techniques to help understand the function of genes in the onset and progression of cancers. By using techniques like genome editing and gene targeting, we are able to introduce precise genetic changes into endogenous genes in stem cells, allowing us to accurately model the specific changes in genes detected in human cancers. By using specific combinations of these genetic changes, we can generate more sophisticated models of human disease and understand how the genetic changes work together to contribute to the development of cancer.

Making better models of clinically relevant cancers

Stem cells are a valuable tool to help with the generation of new models of cancer. For example, embryonic stem (ES) cells have a number of properties which help in analysis of gene function. Firstly, ES cells exhibit high levels of homologous recombination (HR), a property we can exploit to make exact genetic alterations in cellular genes. So, by taking advantage of HR, we can replicate the identical mutations to those found in human cancers, directly in ES cells' genes. We can then use the cells carrying these mutations to study the impact of the mutations on the affected proteins, and the role that these altered proteins play in the progression of cancers.

A second useful characteristic of ES cells is that they will differentiate into a wide variety of cell types from different tissues. So, having generated stem cells carrying the gene mutations we want to analyse, we can subsequently differentiate them into cells from the tissue of interest. So, for example, if we need to investigate the effects of a mutation originally detected in pancreatic cancer, we can generate pancreatic cells from the altered stem cells carrying the mutation.

Rapid generation of an allelic series of conditional point mutations

KRAS is the most commonly mutated oncogene in human cancers. The vast majority of KRAS mutations are single-base mutations, found most frequently at codons 12, 13, or 61 in exons 2 and 3. Different KRAS mutations have been shown to activate distinct signalling pathways. For example, G13D mutations display a high affinity for RAF and preferentially activate the MAPK pathway. G12C and G12V activate RAL signalling whereas G12D mutations have been shown to activate AKT. As different mutations in KRAS can result in different outcomes it is important to be able to analyse each of the different mutations independently.

The first step in creating an allelic series was to generate a conditional point mutation in ES cells by gene targeting. Although a bit more labour intensive than editing, it allows the complex genetic alterations required for a conditional point mutation. Creating a conditional point mutant for Kras in ES cells requires taking into account various considerations around targeting of the allele, stability of the targeting vector and ensuring expression of the gene in both mutated and non-mutated forms (Figure 1A). We and others have found that inverted repeats within targeting vectors can have the effect of undermining plasmid stability. To avoid this, we chose to replace the mouse exon 2 sequences with those for human exon 2 (including 25bp of intronic sequence 5' and 3' of the exon) and to have an inverted mouse G12C exon 2 (flanked by 25bp of mouse intron sequence) inserted downstream of this. The human exon 2 encodes the same amino acid sequence as the wild-type mouse exon but varies in DNA sequence. The flanking sequences were retained with a view to maintaining splicing and reducing plasmid instability. To allow replacement of the hKRAS exon 2 with mKras G12C exon 2, we adopted the strategic positioning of variant loxP sites which allow inversion of the mutated exon and deletion of the human exon on the controlled expression of Cre recombinase. The selection cassette used for targeting of mouse ES cells can be removed by expression of Flp recombinase.

Once we had established the G12C allele in ES cells, the next step is to use this as a basis for generation of an allelic series. Generation of a separate mutant allele by gene targeting would require building a separate targeting vector, then repeating the targeting and subsequent Flp deletion. A more direct method is to make use of genome editing to directly modify the site of the G12C mutation and convert this to a different mutation. To directly edit the G12C sequence we made use of Cas 9. Cas9 is a CRISPR-associated (Cas) endonuclease, or enzyme, that functions as "programmable molecular scissors" to cut DNA at a site which is determined by a guide RNA (Figure 1B). Including a small DNA donor template along with Cas9 allows the cut in the DNA to be resolved by the homology directed repair pathway using the donor DNA. This allows the replacement of the G12C mutation with the G12R sequence (Figure 1C). Using the model discussed above we were able to successfully modify the Kras G12C mutation to G12D, G12R, G13C, G13D, G12V and back to wild-type glycine using genome editing. The success of these experiments rested on the ability to use a gRNA (Figure 1B) which overlapped the original G12C point mutation. This guide only targeted the inverted modified mouse exon 2 sequences while leaving both the endogenous mouse exon 2 and the human exon 2 intact, thus preventing the adverse effects of disrupting the Kras gene. A different donor template containing the desired point mutation was designed for each point mutant and 'knock-in' success ranged from 21%-33%. Additionally, by generating the original line by ES cell targeting and then using genome editing technology to modify the original allele, versus generating all seven lines using ES cells we were able to reduce the timeline required to generate these alleles significantly.

Publications listed on page 126

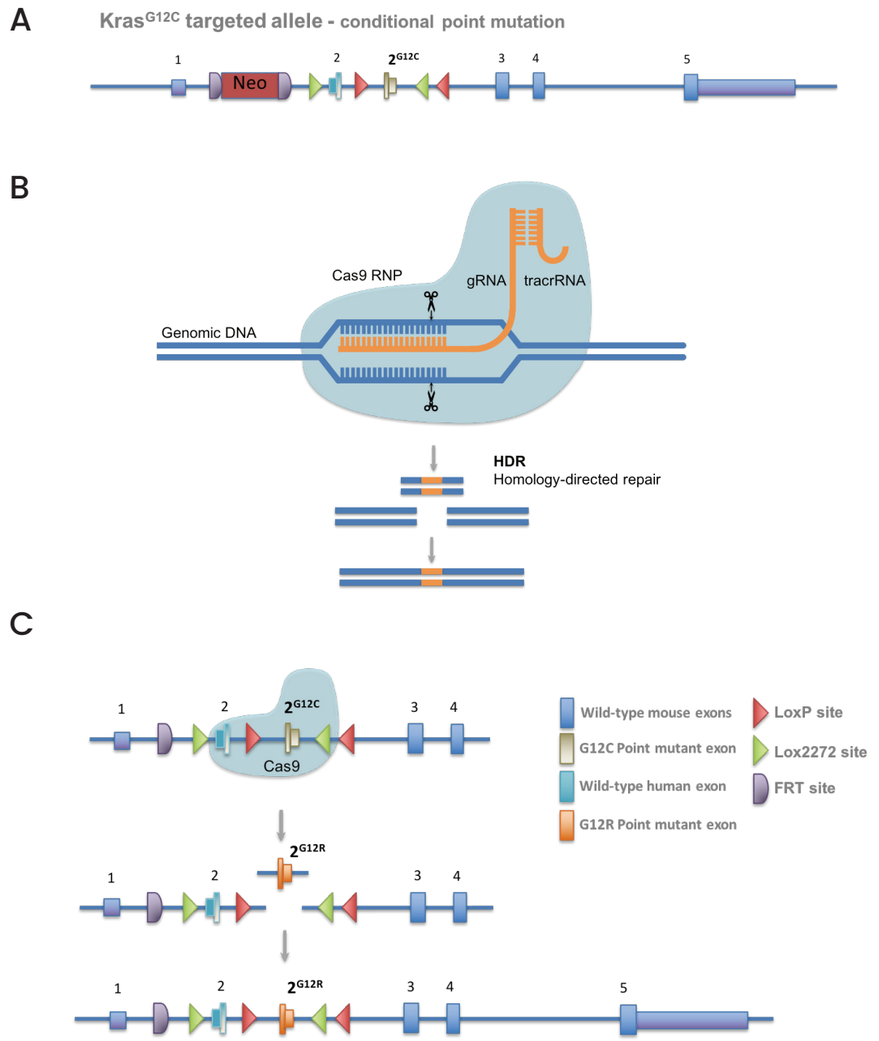

Figure 1.
(A) Diagram of the Kras conditional G12C point mutant allele generated by gene targeting. (B) Using CRISPR-Cas9 for Genome Editing. The RNP complex (Cas9 with gRNA) on its target sequence. Cas9 generates a DNA double strand break 3 - 4 bp upstream of the gRNA target sequence, triggering endogenous DNA repair pathways in the process. This can result in the formation of insertion or deletions if repair occurs via the non-homologous end joining pathway or a knock-in of precise sequence changes via the homology directed repair pathway, if template DNA is included in experimental design, as illustrated. (C) The G12C mutation in the Kras allele is replaced by G12R. Cas9 is used to introduce a cut in the DNA close to the G12C mutation, A small repair template of 120bp is introduced along with Cas9 allowing the exchange of the G12R sequence in place of the original G12C.

TRANSLATIONAL MOLECULAR IMAGING

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

The R&D radiochemistry platform is fully equipped for developing novel carbon-11 and fluorine-18 labelled PET probes from a range of radiolabelled precursors. This platform has allowed us to develop a panel of fluorine-18 and carbon-11 labelled radiotracers for *in vivo* metabolic studies. 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) and [^{11}C] leucine. In 2023, we published two papers improving the radiochemical synthesis of [^{18}F] FSPG and [^{18}F]TFB for *in vivo* imaging of tumour redox and *in vivo* tracking of tumour cells.

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 2023, we initiated a collaboration with the University of Edinburgh to establish the Scotland Total-body PET Facility. This endeavour secured £12M in funding from the Medical Research Council, enabling the facility to acquire one of only two total-body PET scanners in the UK, thereby establishing itself as a national PET imaging facility. This successful award also granted us inaugural membership in the national PET imaging platform (NPIP). As a result, the Translational Molecular Imaging Facility will engage in collaborative efforts on new national projects focusing on total-body PET development. Additionally, this grant will support the creation of three new positions in chemistry and image analysis.

Preclinical and translational imaging

Members of the TMI are also contributing to a number of UK-wide projects through the CRUK Radiation Centre of Excellence (RadNet) Molecular Imaging and Radiotherapy Working Group. We obtained funding for the MIGRATES project (Multi-centre deployment of preclinical multi-modal imaging-guided radiotherapy), a partnership between four RadNet sites, which is creating a system agnostic animal cradle for multimodal imaging and is facilitating image-guided radiotherapy programmes in Glasgow.

We further supported Tom Bird's group to identify and target mouse models of hepatocellular carcinoma (HCC) with radiotherapy, including validation of CT contrast agents. Also in HCC, with Saverio Tardito's group we validated liver volumetric MR imaging and correlated tumour burden with urinary excretion of N^5-Methylglutamine (Figure 1). In collaboration with Saverio Tardito and Tom Bird, this work has now been converted into a successful Sir Jules Thorn Trust application to develop [^{11}C]methylamine as a PET imaging biomarker of elevated glutamine synthase, as a consequence of Wnt-driven β-catenin in HCC. Finally in 2023, we made and imaged radiolabelled vitamin B3, [^{11}C]nicotinamide as a biomarker of glioblastoma with Saverio Tardito.

Publications listed on page 126

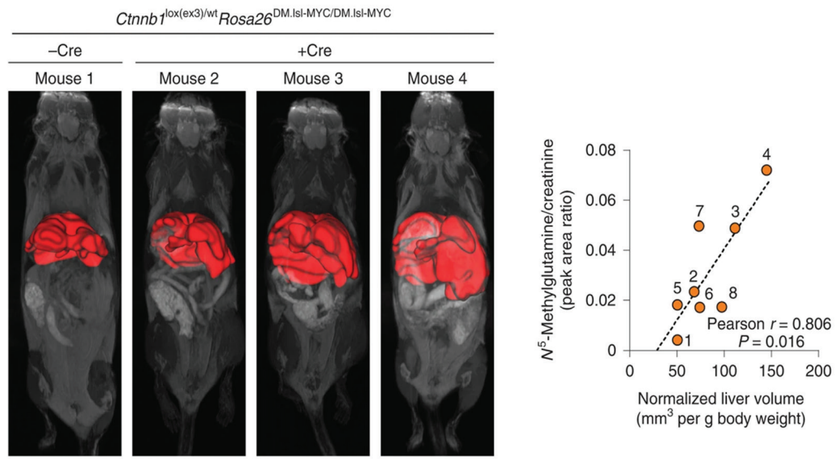

Figure 1.
MRI images of *Cnntb*$^{fl(ex3)/wt}$*Rosa26*$^{DM.Isl-MYC/DM.}$Isl-MYC liver tumour bearing mice. Three-dimensional reconstructions of the livers are highlighted in red. Liver volumes correlated to urinary levels of the methylated glutamine analog N^5-methylglutamine, a product of liver glutamine synthetase activity (Villar *et al.*, 2023, Nat Chem Biol).

HISTOLOGY

Head
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Saira Ghafoor
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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.

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. 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, 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, 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.

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

A recent advancement in *in situ* hybridisation technique (BaseScope) now means where a probe is available or one can be specifically designed to the meet the researcher's needs, we can label and visualise much smaller targets, around 50 base pairs in size.

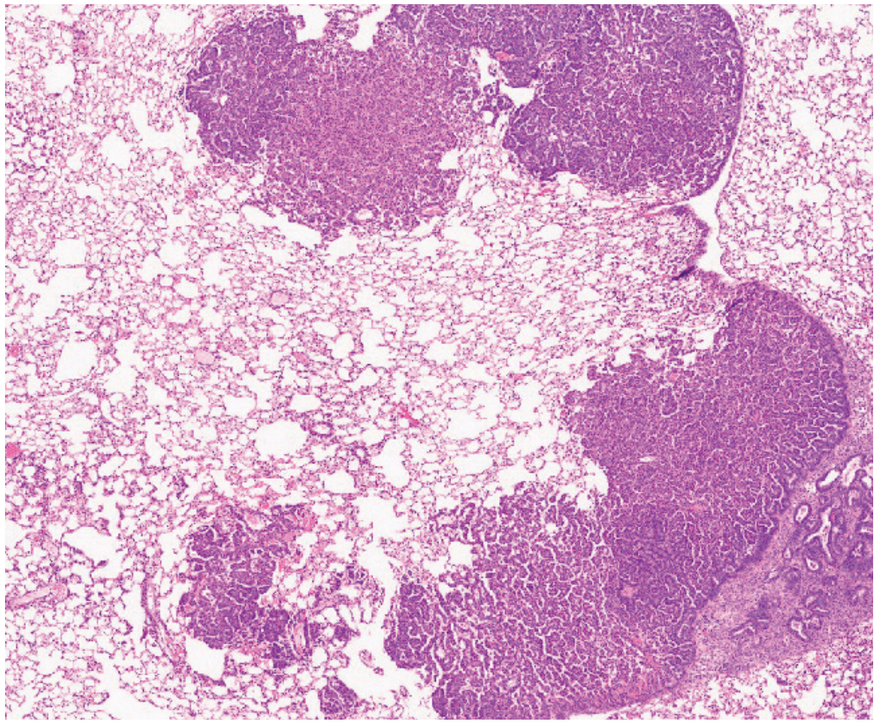
The service offers a wide range of specialised histological stains such as Alcian Blue (+/-PAS), Elastin Van Gieson, Gram, Grimelius, Martius Scarlet Blue, Picro-Sirius Red, Retic, Toluidine Blue and TUNEL staining.

Material for DNA/RNA investigation, immunofluorescence staining, 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. We can cut sections 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.



H&E - Lung tumour

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.

This year, 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 utility cost increases, as well as inflationary pressures on routine consumables and equipment. This has led to increased improvement activities around utility management and investing our capital wisely into equipment to help reduce ongoing costs, as well as improving reliability and services. Investment into projects to replace our lighting to LED, replacing two of our older Liquid nitrogen storage tanks and improving metering on our utility usage will all help reduce the running costs in these areas giving year on year benefits.

Alongside these investments, our operational teams continue to look for opportunities to adapt our ways of working to provide improved services to our researchers, as well as efficiencies in how we operate. The introduction of the supply centres in our stores area has been an excellent example of this type of improvement, introducing a service that is more aligned with researchers' needs, whilst also providing time savings on ordering and environmental improvements through reducing packaging and deliveries.

The coming year will continue to pose challenges, particularly around utility costs, but 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

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 another busy year with several projects being taken through to completion. We have continued our upgrading of lighting across the Institute to LED which provides significant energy savings alongside a reduction in maintenance requirements. In addition, we have also completed the installation of two new autoclave systems in our Biological Services Unit which was a substantial project and now provides increased resilience and reliability in this area.

Alongside these larger projects, there have also been several smaller laboratory improvement projects to help accommodate the changing requirements of our researchers, as well as fabric and signage upgrades related to our successful Quinquennial Quality Review (QQR) and recent name change to the CRUK Scotland Institute. We have also just recently completed the retender process for the Institute's catering provision which will see a new provider commence from March 2024 with an exciting new menu and service.

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

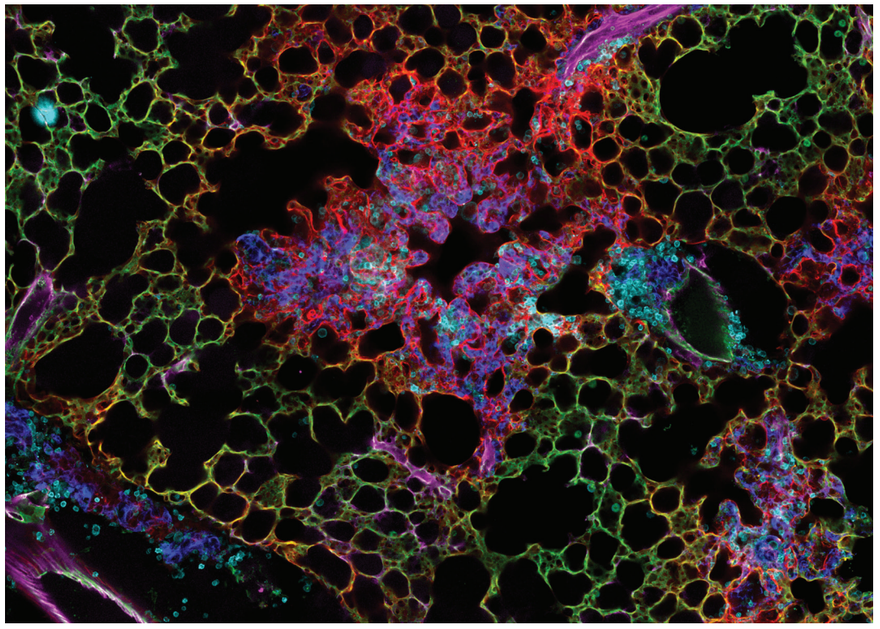
Euan Cameron, **John Kinsella**, Karen Thomas, James Dyball

The Laboratory Management team (Euan Cameron, Karen Thomas, and James Dyball) 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 ensure 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.



A pancreatic cancer micrometastasis to the lung, where green highlights the vasculature (PECAM-1), red and magenta show immune adhesion molecules (ICAM-1 and -2), and in cyan are immune cells stained with the pan-marker CD45. Image by Marco De Donatis

Laboratory Support Services

Angela Miller, **Tracy Shields**, Abbie McFarlane, Anna Shearer, Dilhani Kahawela, Isobel Dawes, Jonny Sawers, Kirstie McPherson, Lauren McGowan, Linda Scott, and Nicola O'Hagan.

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 new sub team within Lab Support Services, called Specialised Lab Support has been created to focus on the preparation of a repertoire of thirteen widely used buffers, *Drosophila* fly food and antibiotic containing agar plates for bacterial selection. This area has been transferred from the Molecular Technologies team, and by doing so we can offer alternative buffers to users if their research projects require this, allowing for potential growth of this department within the overall Laboratory Support team.

Stores

Angela Miller, Alistair Horton, Lauren McGowan, 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 66 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.

At the end of 2023, Stores opened a Merck supply centre, and we were also the first organisation to hold Peprotech items as consignment stock. This was launched on-site in November with an in-house exhibition establishing a new relationship and opening talks about future items to be in the supply centre repertoire.

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 best use of the space available within the department, particularly as new items are introduced to support researchers and their consumable needs. We continue to focus on cost saving methods and ensure the items held are essential to the world-renowned research that is ongoing at the Institute. Moving forward, we will be looking at more sustainable products and in collaboration with suppliers about how to offer these whilst also ensuring best value for money when purchasing.

Molecular Technology Services

Graeme Clark, Andrew Keith, Jillian Murray

The Molecular Technology Service provides a number of services to researchers and collaborators across the Institute.

We continue to provide high-throughput plasmid (miniprep) DNA purifications, and have transitioned processing onto our Biomek FXp liquid handler, utilising a Promega paramagnetic bead-based kit. Processing has remained consistent over this transition, whereby researchers submit pelleted overnight bacterial cultures for plasmid isolations. Similarly, we continue to offer and maintain large scale plasma purifications (maxiprep), and process these at limited numbers manually utilising the PureLink HiPure Plasmid Maxiprep kits (Thermo Fisher).

Previously, we conducted all Sanger sequencing (and associated tests) on an in-house Applied Biosystems® 3130xl (16 capillary) Sequencer. Unfortunately, during the past year, this instrument reached end-of-life, and was no longer actively supported, which put this service at risk. Thus, to mitigate this risk, we have retired the instrument, and have introduced a send-out model for processing all Sanger sequencing reactions, and Cell line authentications (via Eurofins Genomics). This service is currently conducted on a tri-weekly basis and requires researchers to submit samples at set volumes and concentrations ready for sequencing. This adaptation to the service has meant a new submission process has been implemented, however the service is still wholly administered by the Molecular Technology Service staff.

We also continue to offer Mycoplasma screening on a weekly basis. Researchers are encouraged to screen imported cell lines as soon as possible after arrival, to detect any infected cell lines early. Supernatant from cell lines are tested using the Venor GeM qONEStep Mycoplasma detection kit for qPCR (Cambio). We also routinely select a subset of samples to be screened via external providers to verify sensitivity of the current testing regime and will be further investigating the potential to outsource this service in its entirety throughout the early period of next year.

The Molecular Technology Service provides Next Generation Sequencing (NGS) services to all Scotland Institute research groups, along with close collaborators (primarily housed in the School of Cancer Sciences, University of Glasgow). The service assists researchers from the early stage of study design, through initial sample QC, library preparation, sequencing and finally data return. We currently perform our in-house sequencing on an Illumina NextSeq 500 benchtop sequencer; however, we also regularly access high throughput sequencing on an Illumina NovaSeq 6000 platform. This additional sequencing capacity has allowed us to perform larger sequencing projects (e.g. whole exome, genome & 10X single cell RNA) which would have been otherwise cumbersome and uneconomical to perform in-house. We are continuing to perform relatively large numbers of bulk RNAseq preparations but are also now performing a range of other assays, including small RNA, total RNA, ChIPseq, RRBS, amplicon-based & whole exome preparations. In terms of in-house sequencing, we have performed ~110 runs over the past 12 months, which in turn has generated over 6Tbp of data. These runs have been performed primarily on libraries prepared by the service; however, we do also continue to provide sequencing only services to several research groups. Similarly, over this period we have performed 15 NovaSeq sequencing runs, which has returned ~17Tbp of sequencing. Our NGS based services have been used by ~20 research groups both within the Institute and UoG.

Finally, having been involved heavily in the library preparation and sequencing of 10X single cell libraries, we have recently taken on the service entirely, and now offer this as a complete end-to-end service to researchers both within the Institute and close collaborators. Indeed, over the past 12 months we have prepared and sequenced ~185 samples as part of this service, a trend which we expect to continue into next year.

As a service, we are now working closely with colleagues in the core bioinformatics team to provide a streamlined service from sample submission through to final data return, with a focus on tracking sequencing quality and improving overall sequencing data management.

PUBLICATIONS

Imran Ahmad (page 14)
Models of Advanced Prostate Cancer

Primary Research Papers
Bennett SS, Leung HY, Ahmad I.
A cohort analysis of patients receiving neoadjuvant androgen deprivation therapy prior to robot-assisted laparoscopic prostatectomy during the Covid-19 pandemic. *J Clin Urol*. 2023;16:131-139.

Taggart R, Dutto L, Leung HY, Salji M, Ahmad I.
A contemporary analysis of disease upstaging of Gleason 3 + 3 prostate cancer patients after robot-assisted laparoscopic prostatectomy. *Cancer Med*. 2023.

Tibbo AJ, Hartley A, Vasan R, Shaw R, Galbraith L, Mui E, Leung HY, Ahmad I.
MBTPS2 acts as a regulator of lipogenesis and cholesterol synthesis through SREBP signalling in prostate cancer. *Br J Cancer*. 2023;128: 1991-1999

Other Publications
Hartley A, Ahmad I.
The role of PPARγ in prostate cancer development and progression. *Br J Cancer*. 2023;128:940-945.

Tom Bird (page 16)
Liver Cancer, Disease and Regeneration

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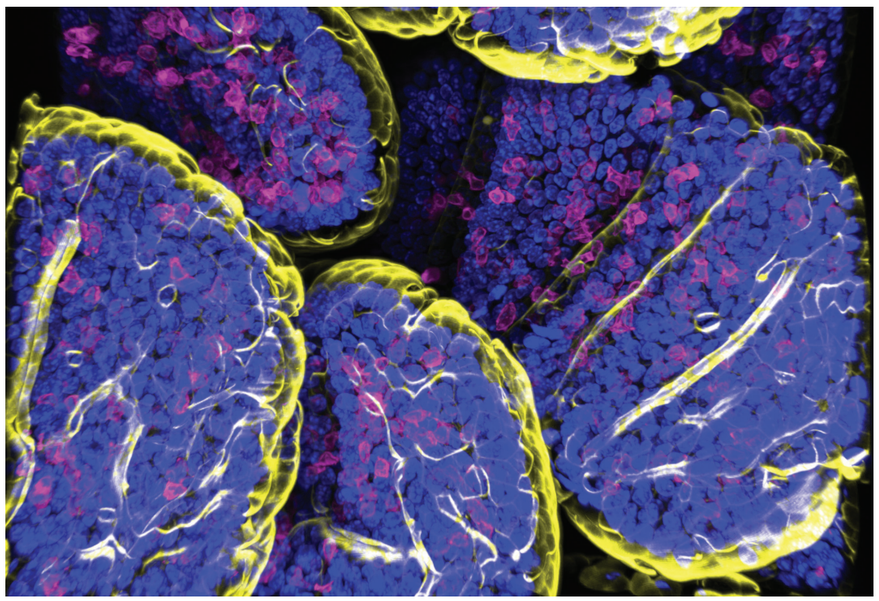

Confocal microscopy of mouse small intestine, stained for actin (yellow), nuclei (blue) and CD3 positive immune cells (magenta). Image by: Nikki R. Paul and Federico Lupo

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Leo Carlin (page 26)
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Seth Coffelt (page 28)
Immune Cells and Metastasis

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Fieke Froeling (page 36)

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Payam Gammage (page 40)

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Danny Huang (page 42)

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Gareth Inman (page 44)

Growth Factor Signalling and Squamous Cancers

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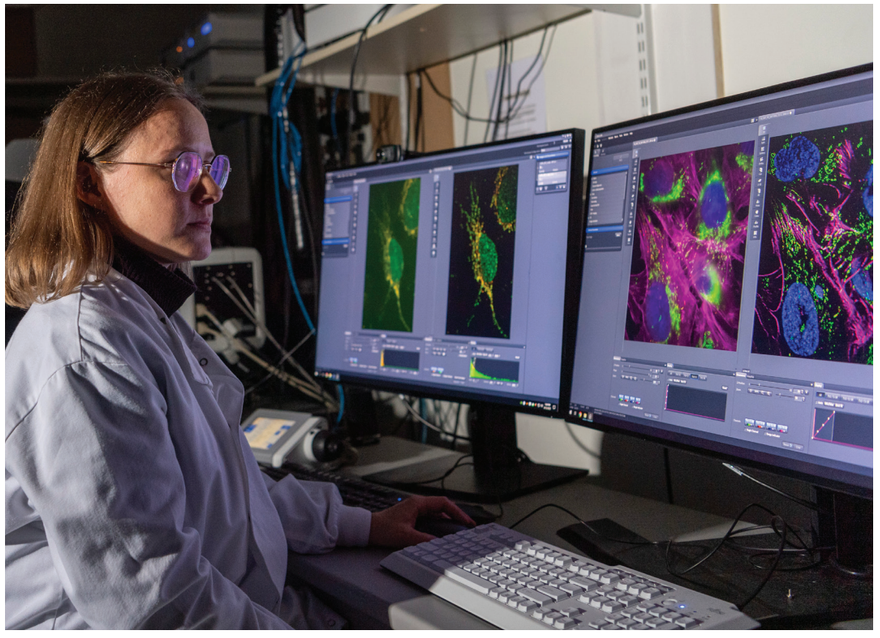

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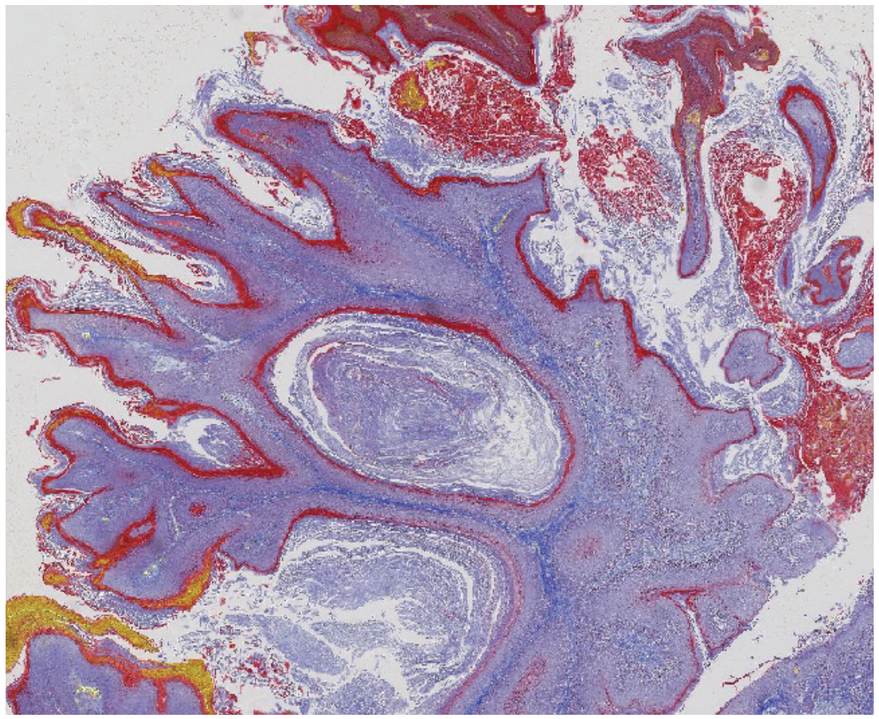

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Tumour Cell Death

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Owen Sansom (page 72)
Colorectal Cancer and Wnt Signalling

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Tumour Microenvironment and Proteomics

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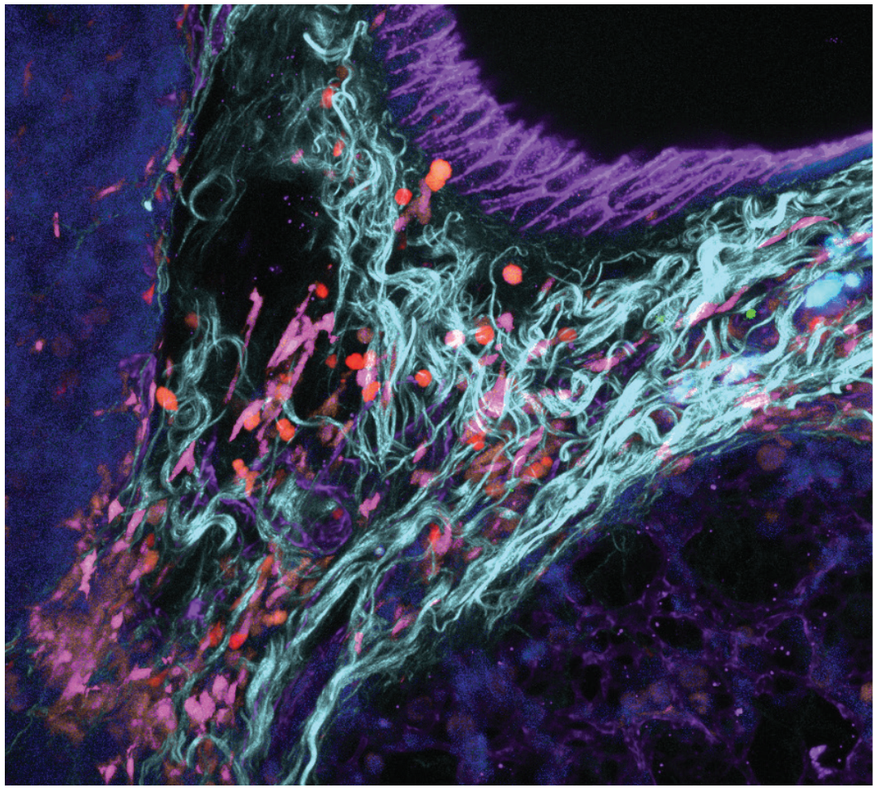

Interactions among macrophages (orange, pink, green) within the fibrillar collagen-rich (cyan) tumor adventitial cuff of a KM mouse model of lung adenocarcinoma crossed with a multi-chemokine receptor reporter mouse." The image was taken using the Zeiss 880 multiphoton confocal microscope and the fibrillar collagen was imaged using the non-descanned detector. Image by Lindsey Arnott

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Proteomics

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Douglas Strathdee (page 96)

Transgenic Technology

Primary Research Papers

Hennequart M, Pilley SE, Labuschagne CF, Coomes J, Mervant L, Driscoll PC, Legrave NM, Lee Y, Kreuzaler P, Macintyre B, Panina Y, Blagih J, Stevenson D, Strathdee D, Schneider-Luftman D, Gronroos E, Cheung EC, Yuneva M, Swanton C, Vousden KH.
ALDH1L2 regulation of formate, formyl-methionine, and ROS controls cancer cell migration and metastasis. *Cell Rep.* 2023;42(6):112562.

Perera G, Power L, Larson A, Codden CJ, Awata J, Batorsky R, Strathdee D, Chin MT.
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David Lewis (page 98)

Translational Molecular Imaging

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Brown G, Soloviev D, Lewis DY.
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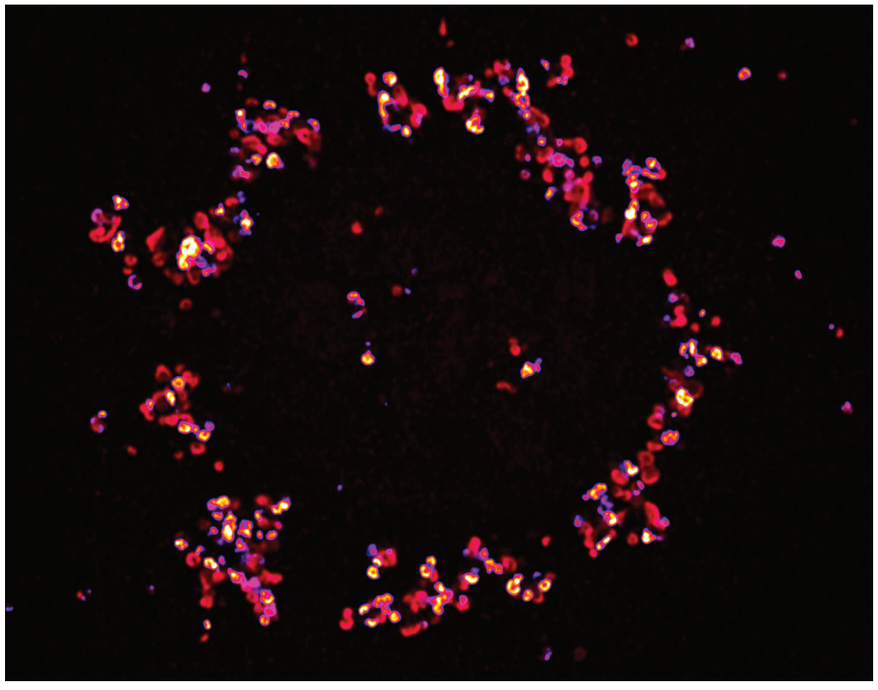

Mitochondrial circle. Imaging of mouse endothelial cells (SVEC4-10) with a permeabilized outer mitochondrial membrane. GFP-NEMO (Red) localizes to the outer mitochondrial membrane stained for TOM20 (Fire) a mitochondrial outer membrane protein. A z-stack image visualized as a maximal intensity projection was taken by the Zeiss Airyscan 880 microscope using a 63x oil objective. Image by Rosalie Heilig

Hollis RL, Thomson JP, van Baal J, Ilenkovan N, Churchman M, van de Vijver K, Dijk F, Meynert AM, Bartos C, Rye T, Croy I, Diana P, van Gent M, Creedon H, Nirsimloo R, Lok C, Gourley C, Herrington CS.
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Karantanou C, Minciacchi VR, Kumar R, Zanetti C, Bravo J, Pereira RS, Tascher G, Tertel T, Covarrubias-Pinto A, Bankov K, Pfeffermann LM, Bonig H, Divieti-Pajevic P, McEwan DG, Giebel B, Munch C, Dikic I, Krause DS.
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O'Rourke CJ, Salati M, Rae C, Carpino G, Leslie H, Pea A, Prete MG, Bonetti LR, Amato F, Montal R, Upstill-Goddard R, Nixon C, Sanchon-Sanchez P, Kunderfranco P, Sia D, Gaudio E, Overi D, Cascinu S, Hogdall D, Pugh S, Domingo E, Primrose JN, Bridgewater J, Spallanzani A, Gelsomino F, Llovet JM, Calvisi DF, Boulter L, Caputo F, Lleo A, Jamieson NB, Luppi G, Dominici M, Andersen JB, Braconi C.
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Other Publications

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Traject3d for studying 3D cellular heterogeneity. Nat Rev Cancer. 2023.
Santibanez JF, Villar VH, Echeverria C. Current and Future Cancer Chemoprevention Strategies. *Pharmaceutics.* 2023(5).

Villar VH, Subotički T, Đikić D, Mitrović-Ajtić O, Simon F, Santibanez JF.
Transforming Growth Factor-β1 in Cancer Immunology: Opportunities for Immunotherapy. *Adv Exp Med Biol.* 2023:309-328.

THESES

Albilasi, Hakem (2023) Contribution of chronic myeloid leukemia niche to metastasis and treatment resistance [PhD thesis, University of Glasgow, CRUK Scotland Institute]

Caldera Quevedo, Irene (2023) Investigating the impact of lung cancer cell-of-origin on tumour metabolic phenotype and heterogeneity [PhD thesis, University of Glasgow, CRUK Scotland Institute]

Cumming, Erin (2023) The role of Podocalyxin in colorectal cancer [PhD thesis, University of Glasgow, CRUK Scotland Institute]

De Donatis, Marco (2023) Addressing vascular and immunological features in a model of pancreatic ductal adenocarcinoma lung metastasis [PhD thesis, University of Glasgow, CRUK Scotland Institute]

Drake, Tom (2023) Mechanisms of immunotherapy resistance in hepatocellular carcinoma [PhD thesis, University of Glasgow, CRUK Scotland Institute]

Georgakopoulou, Anastasia (2023) Targeting epigenetics in preclinical models of HCC for translational therapy [PhD thesis, University of Glasgow, CRUK Scotland Institute]

Gilchrist, Ella (2023) Investigating the effect of KDM6a in intestinal homeostasis and tumorigenesis [PhD thesis, University of Glasgow, CRUK Scotland Institute]

Köessinger, Anna (2023) Targeting therapeutic cell death in glioblastoma [PhD thesis, University of Glasgow, CRUK Scotland Institute]

Mahmood, Mahnoor (2023) Dissecting the function of recurrent complex I truncations in melanoma [PhD thesis, University of Glasgow, CRUK Scotland Institute]

Shergold, Amy (2023) A novel screen to understand preDC migration and improve recruitment to the tumour microenvironment. [PhD thesis, University of Glasgow, CRUK Scotland Institute]

Walsh, Christopher (2023) Methods for tumour aggression prediction in colorectal cancer through virtual immunohistochemistry, supervised, and self-supervised deep learning [PhD thesis, University of Glasgow, CRUK Scotland Institute]

Walsh, Peter (2023) Deciphering the role of liquid-liquid phase separation in oncogenic gene expression [PhD thesis, University of Glasgow, CRUK Scotland Institute]

Wiesheu, Robert (2023) Unravelling the diversity of anti-tumour gdT cells with implications for cancer immunotherapy [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 winner of this year's award was **Amy Shergold** from Ed Roberts' group. Her talk was entitled "cDC recruitment to the tumour microenvironment - A novel screen determining the heterogeneous chemokine signals driving preDC migration".

CONFERENCES AND WORKSHOPS

High School Open Evening

19 April 2023

In April 2023, we ran 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 Seth Coffelt, Tom Drake, and Holly Hall.

Beatson International Cancer Conference 2023

16-19 July 2023

CRUK Scotland Institute, Glasgow
Scientific Committee: Kevin Ryan (Chair), Julia Cordero (Co-Chair), David Lewis, Jim Norman, Ed Roberts, Saverio Tardito

The Beatson International Cancer Conference 2023 on 'Interorgan Communication in Cancer' took place at the Cancer Research UK Scotland Institute (formerly the Beatson Institute) and hosted approximately 200 attendees. These included scientists from 4 continents and 10 countries, ranging from early and mid-career researchers to senior investigators. There were talks by 17 invited world leading figures and 9 early career applicants, and over 40 posters were presented across the event.

Some of the main themes that were presented on were cancer paraneoplastic syndromes, immune, endocrine, and metabolic signalling, microenvironmental interactions and behavioural and neuronal interactions.
The poster prize was awarded to PhD student Jade Phillips (CRUK Scotland Institute) and the best short talk prize went to PhD student Felipe Rodrigues (The Francis Crick Institute).

We look forward to welcoming everyone back in 2024 for our 25th Anniversary Beatson International Cancer Conference – Cancer Models: Cages to Clinic (8-11 July 2024, Glasgow, UK).

Scottish Biomedical Postdoctoral Researcher Conference 2023

8 September 2023
Sir Charles Wilson Building, University of Glasgow

Organising Committee: Nuray Gunduz, Denise Giovana Carrasco Gonzalez, Adelaide Young, Silviya Dimova, Lawrence Bates, Emily Miedzybrodzka, Catarina Mendes Correia, Marisa Di Monaco.

This year, the Scottish Biomedical Postdoctoral Researcher Conference (SBPRC) was held at the University of Glasgow in the Sir Charles Wilson Building and had around 90 attendees. The morning session focused on Genetics and Multiomics, this was followed by a poster session and then a final afternoon session on Cancer Immunology and Microbiology.

The conference also included a career discussion with Dr Emma Hall and Dr Rachel Smith – scientific writers from Envision Pharma and Dr Catherine Winchester – Head of the Research Integrity Service from the CRUK Scotland Institute.

Finally, the conference concluded with a prize giving for Presentations: 1st and 2nd Oral (Alicia Ware and Georgios Kanellos), People's Choice (Jana Travnickova), Flash Talk (Lucas Zeiger), People's Choice for Flash Talk (Ikhlas Ahmed), Poster Prizes (Lucas Zeiger and Fiona Haward) and Most Engaged Audience Member (Emma Hall).

Betty MacGregor Memorial Award Showcase

2 November 2023

CRUK Scotland Institute, Glasgow
Organising Committee: Ed Roberts, Vicky Cowling, Jen Morton, Kate Schraut, Cassie Clarke, Asma Elsheikh, Hannah Donnelly, George Skalka, Amy Shergold, Marco De Donatis, Mahnoor Mahmood, Jasmine Peters, James Ettles.

In November, the CRUK Scotland Institute hosted the first ever Betty MacGregor Memorial Showcase to highlight the contributions of women in cancer research. As part of the event, an award named after the pioneering cancer researcher Elizabeth "Betty" MacGregor OBE FRCOG FRCPath (1920-2005) will be awarded each year to a woman scientist who has made a huge impact in the field of cancer research.

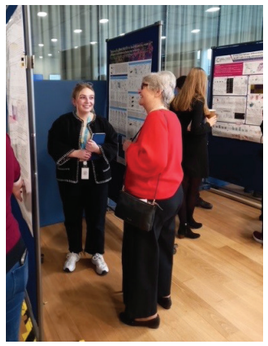
Betty MacGregor grew up in Glasgow, graduated in medicine from the University of Glasgow and pioneered the first screening in cervical cancer in Scotland which was later emulated across the world. Her work led to a significant decrease in the number of women dying from cervical cancer.

This year the Betty MacGregor Memorial Award was awarded to **Professor Margaret Stanley OBE** for her pioneering work on the pathogenesis of Human Papilloma Virus and investigating how this leads to cervical cancer. Her work highlighted how integration precedes chromosomal instability in cells and has investigated the immune response to HPV and highlighted particularly at-risk populations. Her work was also key to the generation of HPV viral like particles which subsequently led to the development of the HPV vaccine.

Margaret has also been active in policy having been on numerous boards of research councils as well as government panels including currently being on the Joint Committee on Vaccines and Immunization as the UK's HPV subcommittee's invited HPV expert. She is a vocal proponent of pre-pubertal HPV vaccination and of both boys and girls. She received an OBE in 2004 for services to Virology, a Lifetime Achievement Award by the American Society for Colposcopy and Cervical Pathology (ASCCP) in 2010, a lifetime award for achievement from the International Papillomavirus Society and in 2023 the Maurice Hilleman Award from the International Papillomavirus Society. She is a fellow of the Academy of Medical Sciences and an honorary fellow of Christ's College, Cambridge.

As part of the showcase, a second early career researcher is invited to present their work. In 2023, **Dr Hélène Salmon**, a group leader at l'Institut Curie in Paris, was invited to speak. Hélène investigates the organisation of lung cancer, and she has shown that the presence of different fibroblast subsets controls T cell infiltration and response to immunotherapy.

Between the talks, a poster session was held, and PhD student Ella Boswell from the University of Glasgow was awarded the poster prize for her work developing a lateral flow test for HPV in low resource settings.



SEMINARS

The following seminars were held at the Cancer Research UK Scotland Institute during 2023. We would like to thank all of our 2023 speakers and look forward to many more insightful seminars in 2024.

January

KJ Patel, Chief Scientist, Cancer Research UK

David Kent, Department of Biology, University of York, UK

Johannes Meiser, Luxembourg Institute of Health, Luxembourg

February

William Grey, Department of Biology, University of York, UK

Ram DasGupta, Genome Institute of Singapore, Singapore

Andrea Oeckinghaus, Institute of Moleculer Biology, University of Muenster, Germany

David Crosby & Anbu Paramasivam, Prevention and Early Detection Research, Cancer Research UK, UK

Samantha Stehbens, Institute of Molecular Bioscience, University of Queensland, Australia

March

Adele Fielding, UCL Cancer Research Institute, London, UK

Sara Sigismund, European Institute of Oncology, Milan, Italy

Didier Devaurs, Institute of Genetics and Cancer, University of Edinburgh, UK

Julie Aspden, School of Molecular and Cellular Biology, University of Leeds, UK

Sarah Aitken, MRC Toxicology Unit, University of Cambridge, UK

April

Oliver Rocks, Charite – University Medicine, Institute of Biochemistry, Berlin

Eugene Wong, Department of Medical Biophysics, University of Western Ontario, Canada

May

John Wood, Wolfson Institute for Biomedical Research, UCL, London, UK

Theodore Alexandrov, European Molecular Biology Laboratory, Heidelberg, Germany

Richard Burkhart, John Hopkins Hospital, USA

Srikala Raghavan, A*STAR Skin Research Labs, Singapore

June

Eoghan Mulholland & Joshua Bull, Leedham Lab, Oxford, UK

Vivian Li, The Francis Crick Institute, London, UK

July

Christopher Halbrook, University of California, USA

August

Alan Ramsay, Kings College London, UK

Mariia Yuneva, The Francis Crick Institute, London, UK

September

Carol Leung, University of Oxford, UK

Prabhu Arumugam, Genomics England, UK

Stephen Carter, MRC-University of Glasgow Centre for Virus Research, UK

October

Karuna Ganesh, Memorial Sloan Kettering Cancer Centre, New York, USA

Hemant Kocher, Barts Cancer Institute, London, UK

Simon Cook, Babraham Institute, Cambridge, UK

November

David Kuninger, R&D Director, Thermo Fisher Scientific,

Johanna Ivaska, University of Turku, Finland

Kalle Sipilä, Kings College London, UK

Martin Turner, Babraham Institute, Cambridge, UK

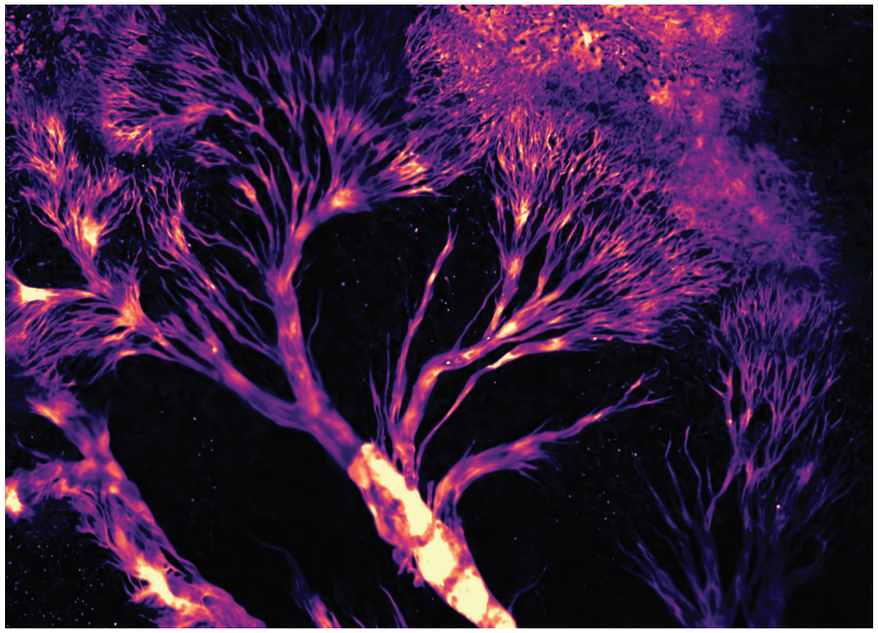
Chris Tape, UCL, London, UK

Pedro Pinheiro, MRC Mitochondrial Biology Unit, University of Cambridge, UK

Amaya Viros, CRUK Manchester Institute, UK

December

Tatjana Stankovic, University of Birmingham, UK



Dictyostelium cells were starved for 4 hours on non-nutrient agar to induce development prior imaging. The cells are expressing Flamindo2, a cAMP sensor that changes intensity of fluorescence dependent on the presence of cAMP in those cells. During development *Dictyostelium* cells utilize cAMP as messenger molecule to orchestrate the formation of fruiting bodies. To encourage the formation of large streams the cells were plated under a thin layer of agarose. Shown are large streams of *Dictystelium* cells captured in a single snap after about 7 hours of development. Nikon AX NSPARK. 4x air objective. LUT Fire. Image by Peggy Ingrid Paschke

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

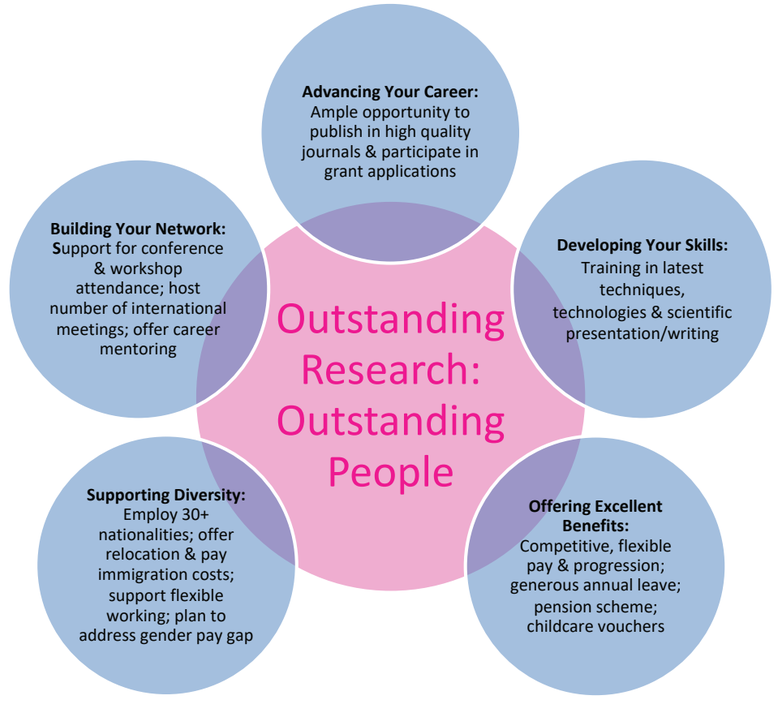
We see postdocs as pillars of the research and intellectual activities of their own groups and of the Institute as a whole. Our postdoctoral training, which is overseen by a senior member of the Institute (Professor Jen Morton), is designed to promote the development of outstanding and dedicated early 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 two 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.seeglasgow.com give general information about Glasgow and other useful links.

Postdoc opportunities at CRUK Scotland Institute



OPERATIONAL SERVICES

Finance

Gary Niven CA, Richard Spankie CA, Nicki Kolliatsas, Jo Russell, Jacqui Clare, Karen Connor, Patricia Wylie, Sandra Watt

The Finance team is responsible for the provision of all financial management information to Institute senior managers, budget holders and the Board of Directors (Trustees). They work with all managers, providing them with relevant information, to help manage and control their budgets and, thus, ensure that decisions concerning the allocation of the Institute's research resources provide the best use of stakeholders' funding.

2023 has seen a return to pre-Covid levels of activity (and spend) in the labs against a backdrop of reduced funding. The Finance function has therefore been heavily focussed on re-forecasting our spend profile on a regular basis and careful management of costs. As a result, the Institute continues to be in a healthy financial position.

In addition, the Finance team is also an important link in our association with the University of Glasgow through the coordination and administration of grants, payment of suppliers and staffing.

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 professionally qualified team provides support and advice across, Human Resources (including recruitment, performance management, pay and grading, absence management, employee relations and employee engagement), Learning and Organisational Development (including culture and change), Equity Diversity & Inclusion and Health & Wellbeing.

The team plays a vital role in shaping organisational culture to create a positive working environment in which our staff are engaged. We strive to achieve this through aligning our people plan to the Institute's strategic plan and creating the desired culture through defining and embedding our values and supporting our leaders to embody these values.

In 2023, much of the team's focus was on transitioning from a transactional HR centric service to the broader portfolio of People and Culture. We delivered leadership development to support an efficient and equitable working environment and implemented a new appraisal process emphasising a quality conversation, setting of objectives and personal development. In response to staff feedback we introduced a new approach for pay uplift, which we agreed in consultation with the union, and we adapted our recruitment practices to ensure more gender balanced interview panels. We are committed to our Equality, Diversity and Inclusion agenda supported by our EDI Advocates and collaborations with University of Glasgow, VOICE Committee.

Administration

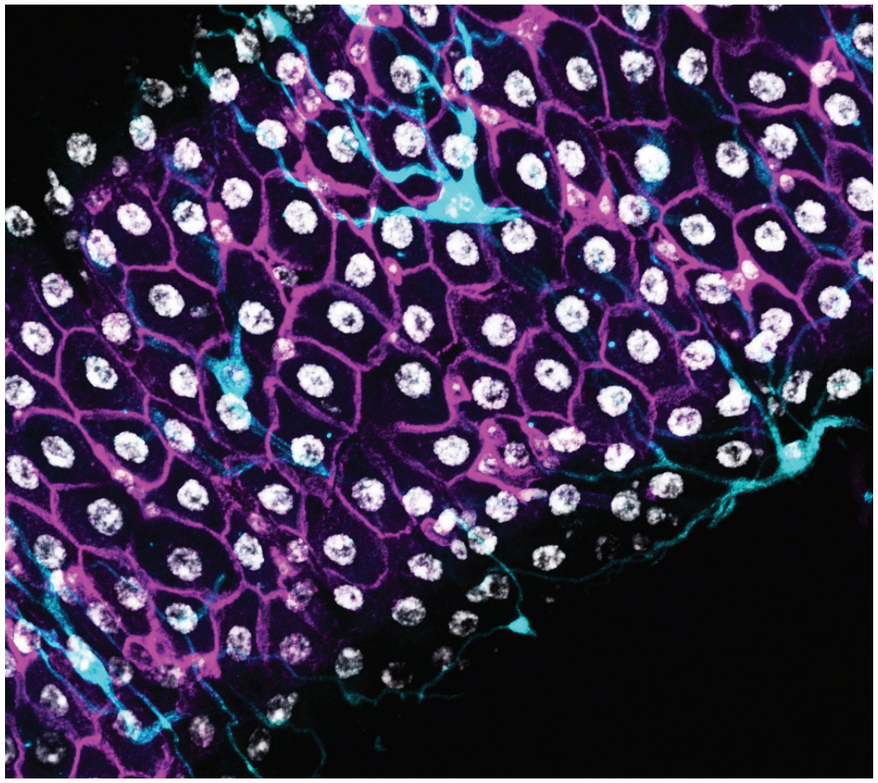
Catriona Entwistle (Receptionist/Administrator), Emma McLean (Events and Administration Officer) Rebecca Gebbie (PA to Director), Shona McCall (PA to Director), Laura Hughes (PA to Senior Management)

The Administration team provides an extensive range of secretarial, admin and office services. These include organising travel and accommodation; internal and external seminar arrangements; organisation of Institute events including reviews, the annual conference, workshops and open evenings; the operation of Reception for the Institute; and onboarding new starts. 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 with which our scientists have contact.

Research Management

Jackie Beesley PhD, Catherine Winchester PhD, Fiona Paulin-Ali PhD, Katharina Schraut PhD, Rebecca Sharland MSc

Members of the Research Management team are all scientifically trained and between them have considerable research experience. They support researchers at the Institute in a variety of ways, including assisting them in applying for external grant funding; overseeing all aspects of the graduate student training programme; providing training and advice on good practice in research; checking manuscripts for research integrity prior to submission; providing external communications for the Institute via its website, social media channels and annual reports; and setting agendas and taking minutes at scientific meetings and reviews. Team members also provide project management support for the CRUK Scotland Centre and larger network awards such as the Colorectal Cancer Accelerator.



Adult *Drosophila* intestinal epithelium (magenta) and guts associated vascular like tracheal cells (cyan). Cell nuclei (white). Image credit: Dr. Jessica Perochon

EQUALITY, DIVERSITY AND INCLUSION

The CRUK Scotland Institute is committed to promoting Equality, Diversity and Inclusion (EDI) within our community.

To support our EDI strategy, we have,

- adapted our recruitment practices to ensure more gender balanced interview panels and applicant shortlists
- offered flexible work patterns
- provided maternity cover of up to 18 months to enable continuation of research careers. For example, cover was provided for 8 positions under this policy over the last 3 years
- reviewed grades to identify and address any gender pay gap issues
- promoted development opportunities for women, including coaching, mentoring and leadership training
- maintained strong links with the School of Cancer Sciences, University of Glasgow VOICE (Athena Swan) Committee
- ensured equal representation in our seminar series and at our scientific conferences

Jointly with the University of Glasgow VOICE Committee, we have delivered several campaigns and events including Inclusion week, Ramadan Awareness, International Women's Day and a High School Open Evening. We are currently supporting event planning for Pride Week.

To support retention of women in science we have engaged with our early career researcher's community to understand career development challenges. We have a career development event being planned for September 2024. We also gather data on our alumni to obtain a measure of retention of women in science.

We are actively working with a committee to recognise cancer researchers of colour and their significant contributions to cancer research. We gather racial diversity data to identify and address any ethnicity pay gap issues.

We published our Gender Pay Gap Report for 2023 (see following page), which is under continuous review by our Senior Management Team and Board of Directors and are currently re-visiting our EDI vision and aims for 2024/25.

Vision

To create a diverse and inclusive culture that attracts and retains research and support staff with a shared vision of collaborative world class cancer research.

Action Plan

Transparency, Evidence and Improvement	EDI Awareness and Training	Career Support and Development	Equitable Recruitment Practices and Opportunities	Scientific Engagement
To monitor, analyse and publish diversity data to develop an evidence base to learn and drive change/ improvements	To ensure that all staff, students and associates understand their responsibilities with respect to EDI, and are appropriately trained and engaged	To enable all researchers and support staff to reach their potential regardless of their gender, age, disability, ethnicity, sexual orientation or other protected characteristics	To ensure that all recruitment practices promote Equality, Diversity and Inclusion not just in words but in actions	To engage with external organisations and networks to promote and encourage equity of opportunity

GENDER PAY GAP 2023

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

Creating a diverse work culture where everyone can be themselves and reach their full potential as individuals is hugely 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 2022, the mean hourly pay gap between females and males increased by 0.16% but the median hourly pay gap decreased by 4.96% in 2023.

Table 1: Pay Gender from April 2021 to April 2023

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	2023	2022	2021
Mean	18.03	20.39	11.60%	11.44%	10.24%
Median	18.55	20.01	7.28%	12.24%	10.08%

Gender pay gap vs equal pay

Equal pay has been a legal requirement in the UK for nearly 50 years; the gender pay gap is not the same as this. 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. There was a slight increase in the mean difference between female and male salaries, by 0.16%, however the median decreased by 4.96%. This reflects our efforts in both recruitment and internal processes of salary review.
In year 2022/23, 69% of new starts were female. In research roles 75% of new starts were female. There is still, however, a disproportionate number of women in the lower paid roles.

In 2023, our workforce was 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. Whilst it is encouraging to see more women in the upper middle quartile, the majority of our newly appointed female staff have been recruited to the lower and lower middle quartiles (66% of all staff recruited within these quartiles). However there has been a high percentage of females recruited into the upper middle quartile (76% of those recruited at this level were female) resulting in a 6% increase in females in this quartile.

We continue to review our grades to identify variance in pay between males and females and make adjustments to salaries accordingly. In 2023, 50% of all promotions were women and 75% of advancements in grades (salary increases above our cost-of-living increase) were also women.

It is important to note that our senior research faculty, e.g. Group Leaders, are not reflected in our gender pay gap analysis. This is because they are employed on hybrid contracts and are technically employed by the University of Glasgow.

GENDER PAY GAP (CONTINUED)

Table 2: Comparison of Quartiles 2021 to 2023

	M-2021	F-2021	M-2022	F-2022	M-2023	F-2023
Lower Quartile	34%	66%	30%	70%	32%	68%
Lower Middle Quartile	37%	63%	33%	67%	29%	71%
Upper Middle Quartile	48%	52%	45%	55%	39%	61%
Upper Quartile	59%	41%	54%	46%	53%	47%

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, which is regularly reviewed by our Board of Directors.

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.

We have previously noted that of those women who start out in a scientific research career as a Postdoc, many subsequently fail to transition into an independent Principal Investigator (PI) position. Almost, two thirds (65.45%) 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.

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.
- Captured EDI data during recruitment.
- Offered flexible work patterns.
- Provided maternity cover of up to 18 months to enable continuation of research careers. For example, cover was provided for 8 positions under this policy over the last 3 years.
- Reviewed grades to identify and address any gender pay gap issues.
- Promoted development opportunities for women, including coaching, mentoring and leadership training. For example, this year our first cohort of women technicians join the Hershel Programme for Women in Technical Leadership.
- Maintained strong links with the School of Cancer Sciences, University of Glasgow VOICE (Athena Swan) Committee.
- 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.

Improving equity is the right thing to do. 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
Aligos Therapeutics, University of Edinburgh, American Friends of Cancer Research

Karen Blyth
MRC, Omideon

Martin Bushell
BBSRC

Kirsteen Campbell
Prostate Cancer Research, PCUK

Leo Carlin
Breast Cancer Now

Vicky Cowling
ERC, MRC, Wellcome Trust

Payam Gammage
EPSRC, NIH

Danny Huang
AstraZeneca, BBSRC, FogPharma

Gareth Inman
British Skin Foundation, DEBRA, SANOFI

David Lewis
Beatson Cancer Charity/Beatson Endowment, NIH

Jennifer Morton
Pancreatic Cancer UK, UCB Biopharma, MRC

Jim Norman
Chief Scientist Office, MRC
Ed Roberts
Beatson Cancer Charity

Kevin Ryan
The Kay Kendall Leukaemia Fund

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

Douglas Strathdee
Scenic Biotech

Saverio Tardito
University of Bergen

Sara Zanivan
Breast Cancer Now, Breast Cancer Charity

THANKS FOR SUPPORTING US (CONTINUED)

School of Cancer Sciences, University of Glasgow

Imran Ahmad
Beatson Cancer Charity, Wellcome

David Bryant
UKRI

Seth Coffelt
Breast Cancer Now, McNab, MRC, Pancreatic Cancer UK, Worldwide Cancer Research

Julia Cordero
China Scholarship Council, Wellcome Trust

Kristina Kirschner
Blood Cancer UK, European Hematology Association, CRUK Scotland Centre studentship, MRC, Saudi Government Scholarship, Turkish Government Scholarship

John Le Quesne
Celgene, Jean Shanks Foundation

Daniel Murphy
Asthma + Lung UK, Merck, MRC

Colin Steele
Chief Scientist Office

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.

American Friends of Cancer Research
Andrew Anderson & Sons Funeral Directors – *Donation in memory of the Late Mrs Margaret McQue*
Beckman & Coulter
Cardiff University
Charities Aid Foundation
Charities Trust
Senga Dempster
Mr Alistair Duguid – *On behalf of Mrs Dorothy Duguid*
Dunblane Bowling Club
First Mortgage
Mrs Grace Flynn – *In memory of her mother, Betty Meiklem*
Genefirst
Genescript
Avril Haddow
Ideal Catering Consultancy Ltd
Victoria Lambert – *on behalf of her mum, Elizabeth Bolton*
Mrs Janette Law – *on behalf of Mr David Law*
Mrs Fiona McNeill – *Donation from family in lieu of sending Christmas cards*
Mrs R Montgomery
The Partick Burns Club
Police Scotland
Mrs Jennifer L Rentoul
Sengenics
Mrs Patricia Simpson
St Machan's Primary School
Strathmore Funeral Directors – *Donation received in memory of the Late Mr Michael Millar*
Ann Soutar
Mr John Teevan – *In memory of their Late mother*
Carolynn Thompson
The Tron Church – *Donation raise in memory of Aidin Pourmoghadan*
Mr & Mrs J Ward
West of Scotland Women's Bowling Association Annual Donation
Mrs Olive Wilson
Mrs Irene Young
Zenith Bridge Club

Just Giving
Paypal – Giving Fund

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 Beatson 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, Strategy and Research Funding, Cancer Research UK

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

Company Secretary
Mr Gary Niven
The Beatson Institute for Cancer Research

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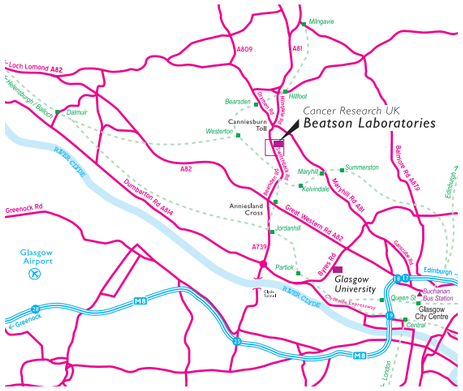
The Beatson Institute for Cancer Research [trading as the Cancer Research UK Scotland Institute] is a registered charity in Scotland (SC006106) and registered as a company limited by guarantee in Scotland (84170). Registered address: Cancer Research UK Beatson Institute, Garscube Estate, Switchback Road,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

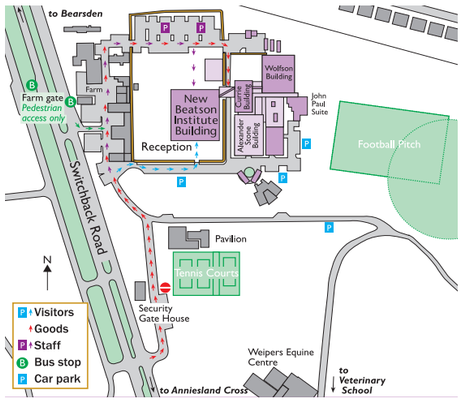
Tel +44(0) 141 330 3953

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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