SCIENTIFIC REPORT 2022

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COVER IMAGE

A Vortex of pink: Dictyostelium cells streaming

Vegetative amoebas expressing the cAMP sensor Flamindo2 under a thin layer of agar were starved for 8 hours to induce the formation of streams. Mediated by cAMP signalling of the starving amoeba, cells are attracted by each other and form streams that later result in the formation of tipped mounds, slugs and finally a mature fruiting body. Zeiss 880 Airyscan -fast mode – tile scan/ timelapse; 10x objective; 488 nm; snap of a single tile. LUT: pink or LUT: Fire.

Image by Peggy Paschke

SCIENTIFIC REPORT 2022

BEATSON INSTITUTE

University of Glasgow

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Cancer Research UK Beatson Institute building.

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DIRECTOR'S INTRODUCTION

Director of the Cancer Research UK Beatson Institute

Professor Owen Sansom FRSE, FMedSci, FRCPS(Glasg)

Our future plans aim to solve the most important emerging biological questions and clinical problems facing oncologists worldwide. This is aligned with CRUK's new research strategy, published this year, where discovery science is central and importantly, where barriers between discovery and clinical research are removed, allowing the effective and rapid translation of findings.

Our intertwined discovery research themes continue to be energetic stress and metabolic vulnerabilities in cancer; understanding the tumour microenvironment to target metastasis; and the biology of early disease. Importantly, our progress with these research themes and our future plans will be assessed during the Institute Quinquennial Review (QQR) in March 2023 and the latter part of this year has been spent preparing for this.

In October, we held a Scientific Advisory Board (SAB) focused on matters related to the Institute QQR and treated it as an opportunity to rehearse the presentation of our strategy and progress. The SAB noted how critical our unparalleled ability to generate and use new disease-relevant, predictive genetically engineered mouse models (GEMMs) of cancer is to our mission, and fully endorsed our increased emphasis on linking these to relevant human samples. SAB members were also impressed with our new recruits (Professor Vicky Cowling and Dr Tom MacVicar), our collaborative ethos, and the progress of our early career researchers.

We continue to assess our individual group leaders on a regular basis. This year, Professors Laura Machesky and Kevin Ryan both had extremely positive QQRs, and we held an advisory mid-term review for Dr Ed Roberts. The panel felt Ed's work, which is aimed at exploiting T cell responses for cancer therapy, was excellent and well prioritised.

We welcomed the Irving family to the McNab Centre for Cancer Innovation - newly-established through the Annie McNab bequest

We made good progress this year in targeting additional external funding to support our key research areas. Professors Stephen Tait and Daniel Murphy were both awarded CRUK Programmes that will utilise our colorectal cancer and mesothelioma GEMMs, respectively. My joint CRUK Programme application with Simon Leedham (Oxford)—work that came out of the CRUK Colorectal Accelerator—was funded and I received funding from Boehringer Ingelheim for studies focused on targeting β-catenin. In addition, Dr Payam Gammage was awarded an NIH R01 grant with Ed Reznik (Memorial Sloan Kettering Cancer Center) exploring the connections between mitochondrial DNA mutations and cancer.

We were delighted that **Ron and Gail Irving** were able to visit the Institute in September and hear what the McNab legacy has supported so far. To date, we have established a Centre for Cancer Innovation, led by Professors Ross Cagan, Jim Norman and Dr Chiara Braconi, to address the significant challenge of metastasis to effective cancer treatment. We have also funded fellows, Dr Seth Coffelt (now CRUK Career Establishment Award), Dr Fieke Froeling and Dr Colin Steele (now UKRI Future Leaders Fellowship) while they establish their own independent research programmes focused on key aspects of cancer biology and treatment. In addition, we have been able to support several students and postdocs at a critical stage of their research career.

We continue to make strategic appointments where possible. **Professor Vicky Cowling** (from University of Dundee) joined us as a Senior Group Leader in June. Vicky is an international leader in studying the mechanisms of translation initiation and will make a key addition to our energetic stress theme. Vicky has recently secured a Wellcome Trust Senior Fellowship and already holds an ERC award as well as industry funding. We also completed our junior faculty recruitment and next year, will be hosting Xiao Fu (from Francis Crick Institute), Kendle Maslowski (from University of Birmingham) and Zoi Diamantopoulou (from ETH Zürich) at the Institute, maintaining our junior faculty pipeline.

Finally, I would like to congratulate Professor John Le Quesne on becoming a Trustee of the Pathological Society, Professor Laura Machesky on becoming President of the British Society for Cell Biology, Professor Kevin Ryan on being appointed a Member of Academia Europaea/The Academy of Europe and Fellow of the European Academy of Cancer Sciences.

RESEARCH HIGHLIGHTS

This year, we have continued to be highly collaborative and make important advances in our key research themes and some of our major findings from 2022 are described here:

Bader AS et al. (2022).
DDX17 is required for efficient DSB repair at DNA:RNA hybrid deficient loci.
Nucleic Acids Res 50: 10487–10502

This study from Martin Bushell's group shows that the RNA helicase DDX17 is required for efficient DNA double strand break repair. At damage sites, DDX17 is key for RNA:DNA hybrid formation – essential for effective repair – especially at genomic locations where RNA:DNA hybrids have failed to form.

Baudot AD et al. (2022).
Glycan degradation promotes macroautophagy.
Proc Natl Acad Sci USA 119: e2111506119

This work led by Kevin Ryan describes the importance of appropriate glycan degradation for successful autophagy and therefore cellular integrity. When glycans accumulate, autophagy is slowed due to impaired lysosomal enzyme activity, resulting in the accumulation of undigested cargo and failed fusion of the autophagosome-lysosome.

Cao K et al. (2022).
Mitochondrial dynamics regulate genome stability via control of caspase-dependent DNA damage.
Dev Cell 57: 1211-25

This paper from Stephen Tait's lab finds a link between impaired mitochondrial function, apoptotic stress and DNA damage. Dysfunctional, fragmented mitochondria engage in a process called minority MOMP that triggers the activity of caspases, which in turn leads to oncogenic DNA damage. Mechanistically, this tumourigenic process involves anti-apoptotic BCL-2, which can be successfully targeted with BH3 mimetics.

Corry SM et al. (2022).
Activation of innate-adaptive immune machinery by poly(I:C) exposes a therapeutic vulnerability to prevent relapse in stroma-rich colon cancer.
Gut 71: 2502-2517

This study gives new insights into the biology of antigen processing and presentation in discrete immune lineages and its role underlying the relapse of colon cancer patients. Treatment with the TLR3 agonist, poly(I:C) in mice shows promising results for patients with the worst prognosis.

Curio S et al. (2022).
NKG2D signaling regulates IL-17A-producing γδT cells in mice to promote cancer progression.
Discov Immunol 1: kyac002

This work shows the importance of the NKG2D receptor in tissue surveillance, γδ T cell recruitment, the production of pro-inflammatory IL-17A and ultimately, cancer progression in mucosal tumours.

Edwards SC et al. (2022).
PD-1 and TIM-3 differentially regulate subsets of mouse IL-17A-producing γδ T cells.
J Exper Med 220: e20211431

This paper led by Seth Coffelt provides a comprehensive analysis of IL-17A-producing γδ T cells in a healthy lung versus a pre-metastatic setting. In response to a tumour, T cell diversity increases, marked by differentially upregulating co-inhibitory molecules such as PD-1 and TIM-3.

Falcone M et al. (2022).
Sensitisation of cancer cells to radiotherapy by serine and glycine starvation.
Br J Cancer 127: 1773-86

This study shows that serine and glycine restriction sensitises to radiotherapy, impacting not only antioxidant response and nucleotide synthesis but also inhibiting the TCA cycle. This suggests dietary restriction of serine and glycine is a viable radio-sensitisation strategy in cancer.

Fisher NC et al. (2022).
Biological misinterpretation of transcriptional signatures in tumor samples can unknowingly undermine mechanistic understanding and faithful alignment with preclinical data.
Clin Cancer Res 28: 4056-69

The advent of population screening in colorectal cancer (CRC) has provided an opportunity for early detection, improved prognosis and reduced mortality. However, while early detection can improve the chances of survival, a subset of early CRC has been identified that has an inherent metastatic potential and exhibits very early dissemination. This study from Philip Dunne leverages the power of large-scale transcriptomics and advanced *in vivo* modelling to drive a fundamental understanding of these 'born-to-be-bad' lesions, and to identify TGF-β signalling as an actionable, predictive biomarker in this setting.

Freckmann EC et al. (2022).
Traject3d allows label-free identification of distinct co-occurring phenotypes within 3D culture by live imaging.
Nat Commun 13: 5317

This paper from David Bryant's group presents Traject3D – an imaging tool that allows the identification of distinct phenotypic patterns in 3D, live cell culture over time. This can give insights, for example, into how cellular phenotypes change with treatment exposure or how drugs can be combined to target resistant cell populations.

Humpton TJ et al. (2022).
p53-mediated redox control promotes liver regeneration and maintains liver function in response to CCl(4).
Cell Death Differ 29: 514-26

This work describes a role for p53 in supporting repair and recovery from acute liver damage. Via the detoxifying enzyme Cyp2a5/CYP2A6, p53 limits cell stress induced by reactive oxygen species and mediates a regenerative process during chronic liver injury. Cyp2a5/CYP2A6 could also be used as a prognostic marker.

Humpton TJ et al. (2022).
A noninvasive iRFP713 p53 reporter reveals dynamic p53 activity in response to irradiation and liver regeneration *in vivo*.
Sci Signal 15: eabd9099

In collaboration with Karen Vousden (Francis Crick Institute), this study introduces a new near-infrared reporter that enables tracking of p53 activity *in vivo*, in this case during liver regeneration and in response to irradiation.

Kay EJ et al. (2022).
Cancer-associated fibroblasts require proline synthesis by PYCR1 for the deposition of pro-tumorigenic extracellular matrix.
Nat Metab 4: 693-710

In this paper, Sara Zanivan's lab describes how extracellular matrix production by cancer-associated fibroblasts - which is pro-tumorigenic - is under strict metabolic control, in particular as a result of increased proline synthesis.

Koessinger AL et al. (2022).
Increased apoptotic sensitivity of glioblastoma enables therapeutic targeting by BH3-mimetics.
Cell Death Differ 29: 2089-104

This work led by Stephen Tait demonstrates that a robust anti-tumour response can be achieved in glioblastoma *in vivo* by sensitising apoptotic pathways through targeting BCL-2 family members with BH3-mimetics.

Leslie J et al. (2022).
CXCR2 inhibition enables NASH-HCC immunotherapy.
Gut 71: 2093-106

This collaboration with Tom Bird, Leo Carlin, Owen Sansom and Derek Mann (Newcastle) shows that non-alcoholic steatohepatic liver cancer can be sensitised to immune checkpoint therapy by targeting neutrophil infiltration. Blocking the neutrophil receptor CXCR2 increases anti-tumour immunity by re-programming neutrophil activity and increasing the activation of T cells and dendritic cells.

Nakasone MA et al. (2022).
Structure of UBE2K–Ub/E3/polyUb reveals mechanisms of K48-linked Ub chain extension.
Nat Chem Biol 18: 422–31

This paper from Danny Huang's group examines the structure of K48-linked polyUb chain as a post-translational modification that can target proteins for degradation. Using chemical biology approaches, the work reveals the function of individual complex components and their domains, and thus, explains their relevance to the ubiquitin-mediated degradation machinery.

Villar VH et al. (2022).
Hepatic glutamine synthetase controls N5-methylglutamine in homeostasis and cancer.
Nat Chem Biol online ahead of print

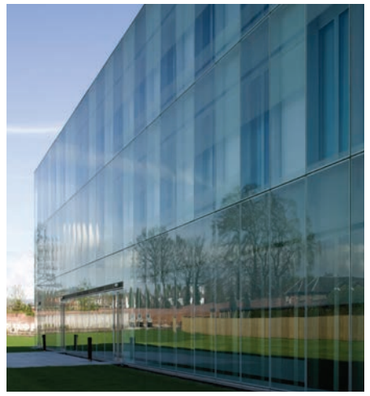
This study led by Saverio Tardito reveals the metabolite N^5-methylglutamine as a previously unreported read out of glutamine synthetase activity. Although also detectable in the circulation, N^5-methylglutamine can act as a urinary marker of tumour burden in a model of liver cancer.

The name Beatson used in our title is in recognition of the early work of Sir George Beatson, who in 1912 established a research department at the cancer hospital in Glasgow. 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 at Garscube Estate in Glasgow.

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.

Sir George Beatson
1848 - 1933

Cancer Research UK
Beatson Institute



CANCER RESEARCH UK BEATSON INSTITUTE

RESEARCH GROUPS

Gut-Vasculature interactions in intestinal homeostasis. Regenerating adult Drosophila melanogaster intestinal epithelium (Edad::tdTomato and Dlg; magenta) and associated vascular like tracheal cell (Cyan).

Image taken by Jessica Perochon & Jade Phillips

www.beatson.gla.ac.uk/imran_ahmad

MODELS OF ADVANCED PROSTATE CANCER

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[1]CRUK Glasgow Centre

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 Hing Leung's 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 suggested that prostate cancer patients could be stratified in terms of PPARγ/FASN and PTEN levels to identify patients with aggressive prostate cancer who might 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 might be driven through alterations in mitochondrial function and AKT3 activations.

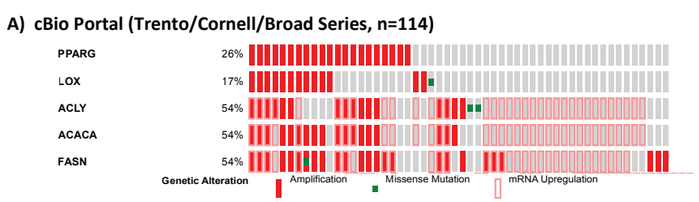
In addition, to our knowledge, we were the first to demonstrate the strength of the Sleeping Beauty transposon model system in successfully determining low-frequency somatic mutations that might drive prostate tumorigenesis. We are further investigating and validating other novel and clinically relevant 'hits' from this screen. (Ahmad *et al., PNAS 2016*; 113 (29) 8290-829; Galbraith *et al., Oncogene*. 2021;40:2355-66; Hartley *et al., BJC*. 2023; 128:940-945))

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).



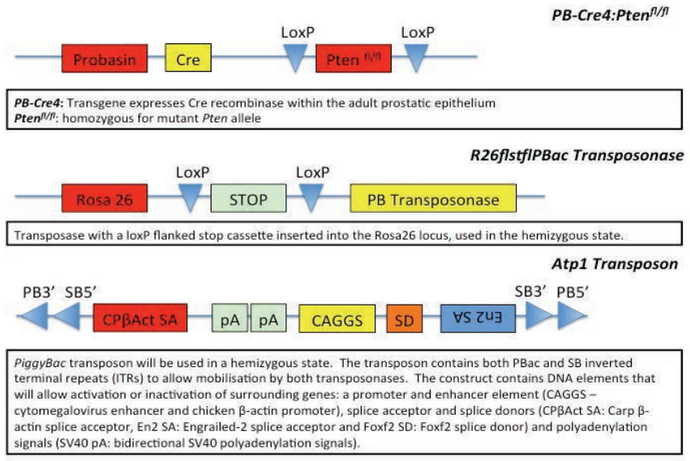


Figure 2
Genetic modifications of the PiggyBac mice.

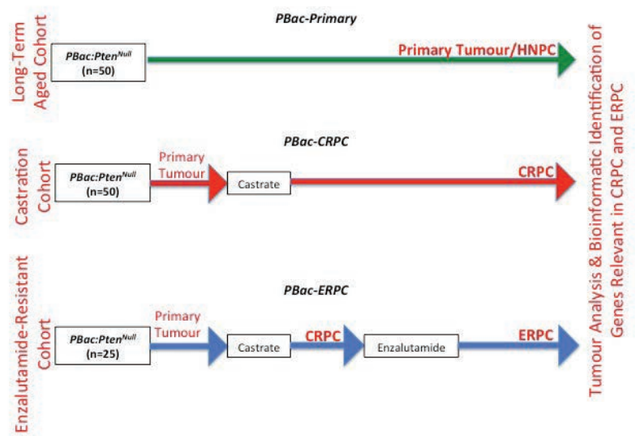


Figure 3
Experimental design for the ageing, castration and enzalutamide-treatment of the PiggyBac (PBac) mice.

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 was reviewed in our recent publication. (Hartley *et al., Expert Opin Drug Discov.* 2021; 16:173-181)

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 was reviewed in our recent publication. (Hartley *et al., Expert Opin Drug Discov.* 2021; 16:173-181)

Role of MBPTS2 in prostate cancer

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

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

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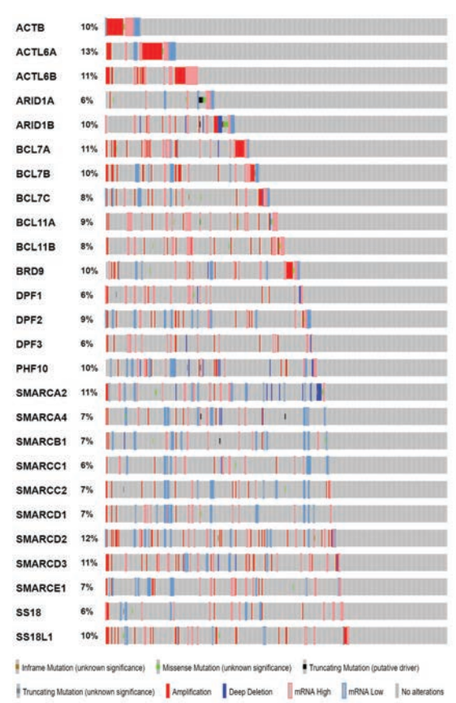
Figure 4
Mutations in the BAF complex in metastatic prostate cancer



LIVER CANCER, DISEASE AND REGENERATION

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Graduate Student
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[1]Edinburgh University
[2]HUNTER CRUK Accelerator
[3]AstraZeneca
[4]Paterson Endowment

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 are able to 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.

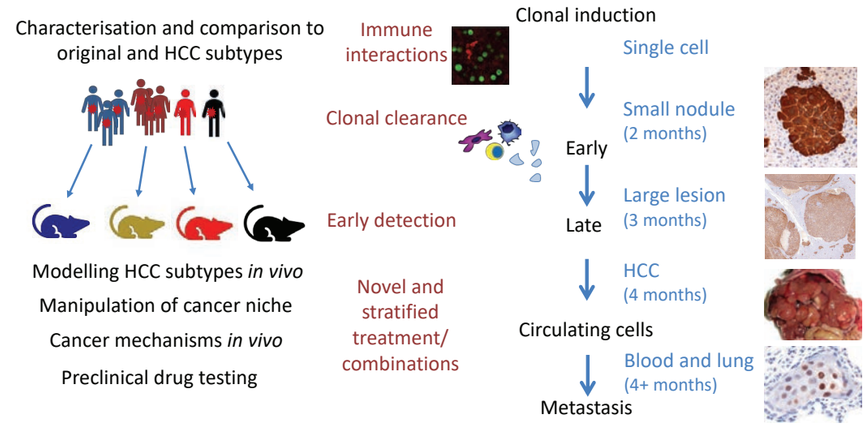

Figure 1
Human HCCs can be grouped into different functional and genetic subclasses.
We are mimicking the genetic alterations in human HCC subclasses using *in vivo* models in the mouse. Our strategy is to induce clonal hepatocytes and then follow the clones as they develop into metastatic HCC. We aim 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.

Early detection of hepatocellular carcinoma

Deaths from liver cancer are likely to continue to increase until we are able to 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. We are very excited about the prospect of working with an industrial partner to explore whether changing the way we do liver tumour surveillance in patients, replacing ultrasound with a state-of-the-art MRI scan, could improve early disease detection. The aim is that through catching and treating these cancers early, and combining this with new and more targeted, therapy we can provide better opportunities and outcomes for patients with liver cancer.

Publications listed on page 100

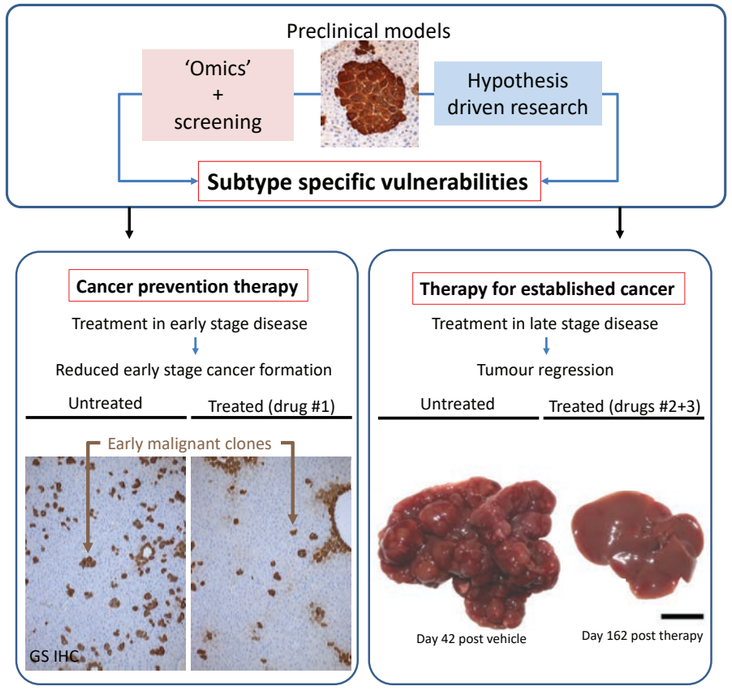

Figure 2
Cancer prevention in preclinical models by targeting early tumour clones.
We are able to explore specific vulnerability of individual liver cancer subtypes. We have identified pathways which are specifically activated in early disease. When we apply therapies to early disease, we are able to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

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IN VIVO CANCER BIOLOGY

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Our lab uses preclinical models to study cancer processes, 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. The group has expertise in modelling different cancer types and co-leads the MRC National Mouse Genetic Network *Cancer Cluster*. 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 and other cancers.

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

Our lab has a long-standing interest in the RUNX/CBFβ transcriptional complex, an essential regulator of mammalian development which is often found dysregulated in cancer. It is not surprising that this family of genes is altered in cancer considering the pathways regulated by this complex (Figure 1), yet there is often a dichotomy on how these proteins manifest their effects in a cancer setting. Genetic aberrations of the *RUNX1* and *CBFB* genes are particularly prevalent in breast cancer, and two PhD students, Kerri Sweeney and Adiba Khan, recently submitted and successfully defended their theses exploring the enigmatic role of these genes.

In collaboration with Prof Ewan Cameron (University of Glasgow) and funded in part by Breast Cancer Now, Kerri's thesis was titled *'Investigating the tumour suppressor function of RUNX1 in breast cancer'*. Deletion of *Runx1* in two independent *in vivo* models of breast cancer accelerated disease onset and led to emergence of multifocal and multicentric tumours. RNAseq analysis revealed an increased stem-like transcriptional signature in *Runx1*-deficient tumours, while loss of *Runx1* predisposed to increased stem/progenitor-like behaviour in functional mammosphere assays. Adiba's studies revealed that while loss of *Cbfb* did not overtly alter normal development of the mammary gland, when combined with oncogenic WNT signalling it dramatically accelerated onset of mammary tumours, providing the first *in vivo* evidence that CBFβ has a tumour suppressor role in a mouse model of breast cancer. Adiba, along with Masters Student Nimrit Kaur, showed however that loss of *Cbfb* did not promote tumour susceptibility within the MMTV-*PyMT* model. Thus, as observed in patients, CBFβ played a context-dependent role in breast cancer. Profiling of RUNX/CBFβ-deleted mammary tumours has revealed that loss of the complex evoked changes to the tumour microenvironment where an important aspect of RUNX/CBFβ activity might be to orchestrate the immune microenvironment, a hypothesis we are pursuing further.

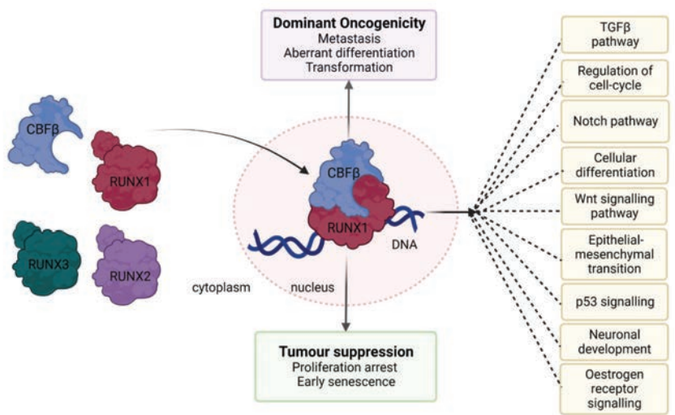
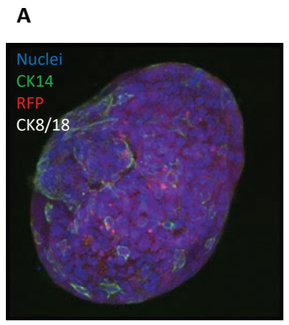


Figure 1
Schematic representation of the RUNX/CBFβ complex and its role in regulation of cell fate processes. RUNX proteins (RUNX1, RUNX2 and RUNX3) interact with their binding partner CBFβ to form a heterodimeric complex which translocates to the nucleus where RUNX can bind to DNA (RUNX1 shown here) to regulate transcription of various target genes involved in a multitude of signalling pathways (yellow boxes). Depending on context, the RUNX/CBFβ complex can support either suppression of cell proliferation (green box) or promotion of growth enhancing signals (purple box).



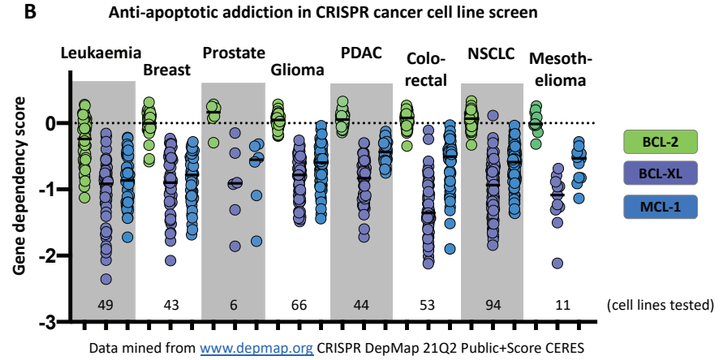


Figure 2
Investigating tumour cell dependency on BCL-2 family proteins.
A Colony forming cell assays conducted on mouse mammary epithelial cells. Red fluorescent protein (RFP)- expressing mammary cells are resuspended in a basement membrane matrix and establish 3D structures to investigate the dependency of tumour-initiating cells on BCL-2 family proteins. 3D structures are stained for basal (anti- CK4/14) **[green]**- and luminal (anti-CK8/18) **[white]**- cell specific cytokeratin antibodies and imaged using confocal microscopy. **B** Frequent genetic requirement for BCL-XL (purple), MCL-1 (blue) but not BCL-2 (green) genes for viability across a panel of cancer cell lines is evident in a CRISPR cancer cell line screen. Gene dependency scores below 0 indicate dependence on that gene for survival, PDAC (pancreatic ductal adenocarcinoma), NSCLC (non-small cell lung cancer). Data mined from www.depmap.org dataset CRISPR DepMAp 21Q2 Public+Score CERES.

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

MCL-1 is a protein best known for its role in cancers of the blood, but we have found a key role for MCL-1 in breast cancer showing that MCL-1 is required for tumour development and maintenance of established tumours (Campbell et al., *Cell Death Dis* 2018 9:19). In collaboration with Prof Stephen Tait, our experiments revealed that the anti-apoptotic function of MCL-1 was required in breast cancer cells (Campbell *et al., Cell Death Diff* 2021 28:2585-600). Interestingly, while thought to be responsible for tumour initiation, metastasis and treatment resistance, we have found that breast cancer stem cells were particularly dependent on MCL-1 and were effectively killed by MCL-1 inhibiting drugs. A focus of PhD student Matthew Winder's work is to further define the requirement for MCL-1 in breast cancer stem cells and unravel the role of MCL-1 at the time of tumour initiation (Figure 2A). We hope that understanding the requirement for MCL-1 at this early stage of tumour evolution may allow the development of cancer preventative treatment approaches. At the same time, we are interested in the role of MCL-1 in treatment resistance where Masters Student Vibhuti Aggarwal's project combined MCL-1 inhibition with novel drug combinations to enhance tumour cell death in models of triple negative breast cancer.

Our data suggested that MCL-1 also has a role in prostate cancer where it could act as a barrier to tumour cell elimination by prostate cancer therapies. Advanced prostate cancer, where the tumour has spread to distant sites around the body, is a lethal diagnosis. Furthermore, bone metastases is a particularly painful and debilitating condition. MCL-1 seems preferentially increased in advanced prostate cancer and bone metastases. Funded by a Prostate Cancer Research grant, Dr Laura Martinez Escardo is investigating whether targeting MCL-1 can improve response to hormone or chemotherapy in advanced prostate cancer. New treatments for prostate and breast cancer are urgently required as these diseases account for over 23,000 deaths in the UK each year. We hope to prove MCL-1 as a valid target in prostate and breast cancer and expedite the use of MCL-1 inhibitors in these cancer types.

Targeting the BCL-2 family to induce radiosenistisation

Pro-survival members of the BCL-2 family such as MCL-1, BCL-2 and BCL-XL are frequently upregulated in cancer where elevated expression acts as a barrier to efficient cell death induction by cancer therapies. In addition to our studies in breast and prostate cancer, we noted that many tumour types show dependence on MCL-1, or it's close relative, BCL-XL (Figure 2B). More than half of cancer patients receive radiotherapy and so, Masters Student Rosie Willis investigated whether inhibition of pro-survival BCL-2 proteins with a class of drugs called BH3-mimetics, could sensitise to radiotherapy and lead to more efficient cancer cell elimination. This project is co-led by Dr Kirsteen Campbell and Dr Joanna Birch who, in collaboration with Prof Karen Blyth and other Glasgow RadNet colleagues, secured a Cancer Research UK RadNet Pump Priming Grant to characterise radiosensitisation by BH3-mimetics in pancreatic cancer and glioblastoma.

MRC National Mouse Genetic Network (NMGN) Cancer Cluster

The lab are excited to co-lead the *Cancer Cluster* within the highly interactive MRC National Mouse Genetic Network (https://nmgn.mrc.ukri.org/). With colleagues in Glasgow, Belfast, London, Oxford, and the Mary Lyon Centre at Harwell, we will utilise state-of-the-art technologies such as spatial phenotyping to study complex cancer-host interactions and position models that recapitulate the human disease. We will also refine models to mirror human tumour evolution more accurately and through robust patient-relevant mouse models, assess responses to novel therapies with improved predictability. To find out more please visit https://nmgn.mrc.ukri.org/clusters/cancer/.

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EPITHELIAL POLARITY

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A feature of most tumours is that they become less organised as they progress. Tissue organisation is thus the strongest 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 studies the gain and loss of collective cell polarity and invasion in tumours. Our research is focused on two intersecting streams: 1) understanding the molecules that regulate collective cell polarity, and 2) developing the computational image analysis tools that allow us to dissect cell polarity.

Developing tools for collective 3-Dimensional (3D) invasion analysis

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 aims to develop methods to overcome such limitations. We have an Industrial Partnership with Essen Bioscience to develop image analysis tools to automate this process and provide bioinformatics solutions to studying 3D cultures via live imaging.

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.

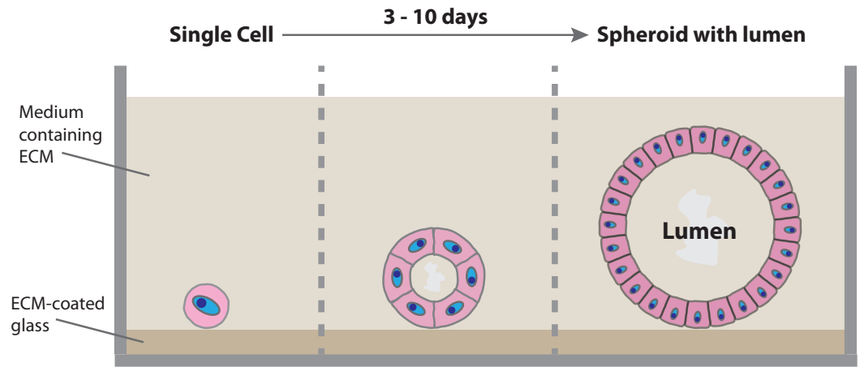
In collaboration with the Blyth, Leung and Zanivan groups, we are interrogating their function in metastasis. We find that many ARFome family members assumed as redundant have highly divergent and sometimes opposing roles in invasion, and show that there is specificity of signalling between family members. 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. 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. 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

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.



prostate cancer metastasis and tumour growth *in vivo*. 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.

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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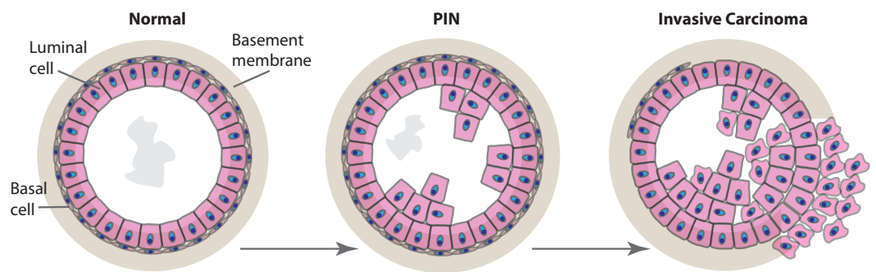
Figure 2
3D cultures of cells to form cysts (also called spheroids or organoids) also allows us to model the loss of normal tissue architecture that occurs in cancer. For example, the progressive disrupted organisation of Normal, to Prostatic Intraepithelial Neoplasia (PIN), to Invasive Carcinoma typifies 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 as well as ensuring their fidelity through the correct folding and cellular localisation.

eIF4A1 and eIF4A2 have opposing roles in tumourigenesis

The translation initiation factor eIF4A1 is a DEAD-box RNA helicase, whose role is to unwind secondary structure in the 5′ untranslated regions (5′UTRs) of mRNAs. While almost all cellular mRNAs require eIF4A1 for their translation, their requirement is not equal, with mRNAs encoding oncogenic proteins depending most upon eIF4A1 activity for their expression, which is thought to explain why eIF4A1 expression is associated with poor survival in human malignancy. Targeting eIF4A1 in cancer is currently an attractive therapeutic and there are several eIF4A inhibitors available, with EFT226 currently in clinical trials. However, 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 acts as a translational repressor in conjunction with the CCR4-NOT complex and the role of eIF4A2 in malignancy and the effect of its targeting in cancer remain unclear.

Data from the Sansom lab showed that knock-out of either eIF4A1 or eIF4A2 in the colon of WT mice was tolerated, yet in colon cancer models, loss of eIF4A1 led to reduced proliferation and increased survival, but the loss of eIF4A2 accelerated tumourigenesis and led to decreased survival. To dissect the mechanisms that explained the divergent roles of eIF4A1 or eIF4A2 in colorectal cancer, we carried out ribosome profiling on epithelial extractions from APC$^{-/-}$/KRAS^{G12D} small intestines, following loss of either eIF4A1 or eIF4A2.

Following loss of eIF4A1, there were 283 genes that were translationally downregulated and 249 that were translationally upregulated. Gene set enrichment analysis (GSEA) showed that those mRNAs that were translationally downregulated encoded proteins that were involved in cell cycle processes (Figure 1B), which correlated with our data that loss of eIF4A1 led to reduced cell proliferation in these cells. Interestingly, those mRNAs that were translationally upregulated encoded proteins involved in the adaptive immune response and this is currently being further investigated. There were far fewer translational changes following the loss of eIF4A2. This was consistent with our data that loss of eIF4A2 did not alter the rate of proliferation in this model. Only 37 mRNAs were translationally upregulated and 1 downregulated with statistical significance. Interestingly, of the 37 mRNAs that were upregulated, 33 were TOP mRNAs, with cytoplasmic translation coming back as the main group of genes enriched in translationally upregulated mRNAs. Although only one mRNA was identified as decreasing translationally with statistical significance, when carrying out GSEA using the log2 fold-changes in translation efficiency for all mRNAs (without considering adjusted p-values), then many processes related to differentiation were identified as being enriched in translationally downregulated mRNAs (Figure 1D), which fits with eIF4A2 expression being limited to differentiated cells.

Investigating the role of stress granules in cancer

Cancer cells use translational control as a means of rapid circumvention of various environmental stresses, in particular hypoxia and oxidative stress, which they must overcome to thrive. Stress granules are liquid-liquid phase separated mRNA- and protein-rich foci that form in the cytoplasm in response to cellular stresses that have been implicated in the pathophysiology of various diseases including cancer. KRAS-mutant cell lines have been shown to have elevated levels of stress granules and overexpression of stress granule markers is associated with a poor prognosis in multiple cancers.

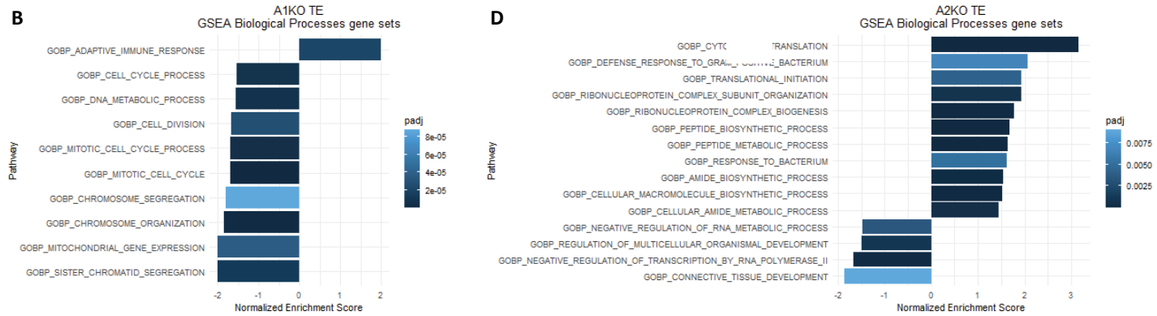

Figure 1
eIF4A1 and eIF4A2 have distinct effects on translation in APC$^{-/-}$/KRAS^{G12D} small intestines.
B GSEA shows the biological processes that are involved in translationally upregulated or downregulated mRNAs following loss of eIF4A1. **D** GSEA shows the biological processes that are involved in translationally upregulated or downregulated mRNAs following loss of eIF4A2.

In order to assess stress granule formation in response to oxidative and hypoxic stress, we expressed a fluorescently tagged stress granule protein GFP-G3BP1 and stressed cells using sodium arsenite or 0.1% oxygen (hypoxia), respectively. Confocal microscopy showed that granule morphology was dependent on the stress applied (Figure 2A), with arsenite-induced stress granules displaying the canonical cytosolic puncta with P-body docking on the outer surface, while hypoxia-induced stress granules were considerably larger, occupying a large proportion of the cytosol and had no P-bodies docking on their outer surface. Distinct upstream signalling events regulating granule formation were also observed between the two conditions (Figure 2B), namely phosphorylation of eIF2α in response to arsenite and hypophosphorylation of 4EBP1 in hypoxia.

We purified stress granules using multiple steps of differential centrifugation followed by an anti-GFP-G3BP1 immunoprecipitation and analysed the proteome by mass spectrometry. This highlighted a number of key differences between arsenite- and hypoxia-induced granules, including the presence of large ribosomal subunits and an enrichment of eIF4A1 in hypoxia. These data, in context with the current literature, suggest that hypoxia-induced stress granules might be even more translationally active than what has already been observed and promote pro-survival gene expression programmes in response to stress. Previous studies have implicated DEAD-box helicases as regulators of liquid-liquid phase separation, and eIF4A1 specifically as playing a pivotal role in regulating stress granule size and limiting P-body docking. Treatment with hippuristanol, an eIF4A inhibitor, reduced stress granule size and restored P-body docking (Figure 2C). G3BP1 and eIF4A1 are coexpressed in lung adenocarcinoma (LUAD) patients in the Cancer Genome Atlas (TCGA) cohort. High expression of both G3BP1 and eIF4A1 in these patients was associated with the worst survival outcomes, whilst low expression of both was associated with the best survival outcomes (Figure 2D). G3BP1 was identified as an eIF4A1-interacting protein by co-immunoprecipitation suggesting that these proteins could directly interact or be in complex together (Figure 2E). We are currently using ribosome profiling and other complementary high-throughput sequencing techniques to better understand how these two RNA-binding proteins regulate liquid-liquid phase separation, and as a result help drive pro-oncogenic gene expression programmes.

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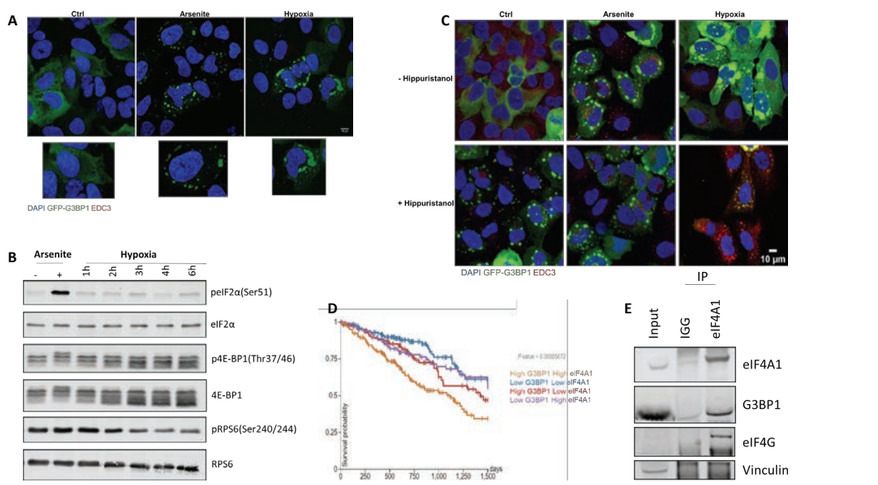

Figure 2
Arsenite- and hypoxia-induced stress granules are distinct.
A Confocal microscopy images of GFP-G3BP1-expressing U20S cells treated with either sodium arsenite or 0.1% oxygen (hypoxia) and stained with DAPI and EDC3 (p-body marker) antibodies. **B** Western blot from arsenite- or hypoxia-treated U20S cells probed with the stated antibodies. **C** Confocal microscopy images of GFP-G3BP1-expressing cells treated with either sodium arsenite or 0.1% oxygen (hypoxia) and stained with DAPI and EDC3 (p-body marker) antibodies, as in (A) plus and minus eIF4A inhibition with hippuristanol. **D** Kaplan-Meier plot of LUAD TCGA cohort stratified by *G3BP1* and *eIF4A1* expression. E Co-IP from A549 cells, demonstrating a direct interaction between eIF4A1 and G3BP1.

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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 to clinical while others fail? We use Drosophila as our lead tool to explore these questions, focusing on developing genetically complex models and using these to develop lead therapeutics including fly-to-bedside clinical trials.

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 have been testing these ideas in experimental fly-to-bedside clinical trials as well as building a new generation of lead therapeutic compounds for cancer and RASopathies.

Colorectal cancer

A key unmet need in the cancer field is effective, durable treatments for solid tumours, the major focus of the laboratory. 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. We are currently using genetic, expression, and metabolite studies to match this and other unique drug combinations to genetic profiles. Our goal is to predict drug response based on a patient's tumour profile.

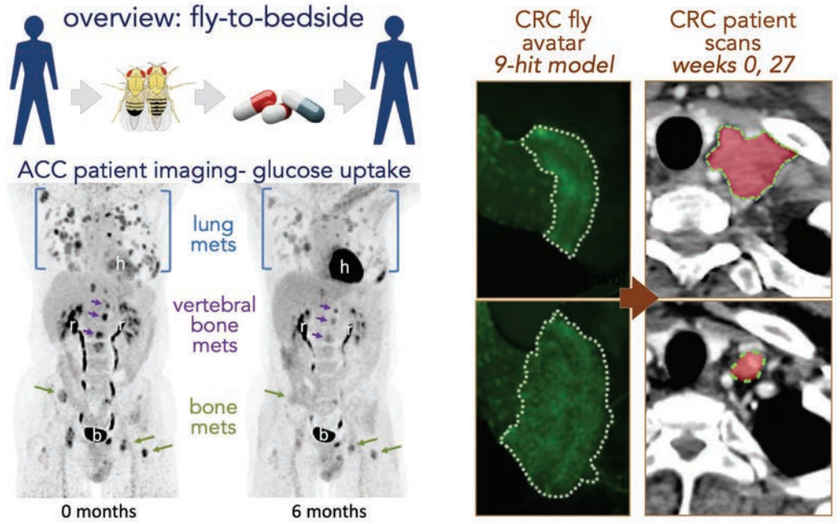
Exploring our set of patient based colon cancer 'fly avatar' lines more deeply, we found evidence that increasing genetic complexity gave rise to multiple avenues of drug resistance. For example, we have identified upregulation of detoxification pathways when specific cancer genes were paired. Blocking 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 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 full omic tumour analysis to develop a 5-hit 'personalised fly avatar'; the resulting fly exhibited multiple aspects of transformation. Our robotics-based approach identified the novel three-drug combination tofacitinib-vorinostat-pindolol, which proved effective: the patient displayed partial response for 12 months on treatment, with tumour burden reduced by 49% across all lung and bone marker lesions (Figure 1). Our recent work has identified key pathways that are required for tumour progression; drugging these pathways with clinically relevant drugs and drug cocktails showed broad efficacy across our set of ACC avatars. We are now exploring whether our most broadly acting drugs are likely to show efficacy in ACC clinical trials.

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



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 indicated 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 have identified promising lead therapeutics that act broadly across our models; 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.

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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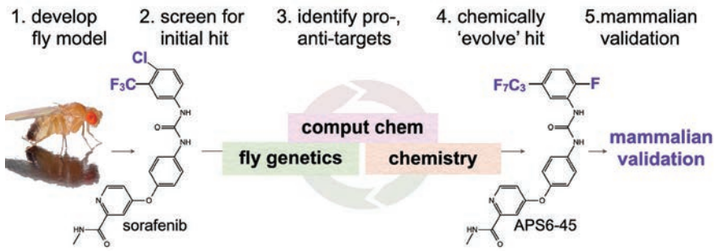
Figure 2
Platform to 'tune' therapeutic leads.



LEUKOCYTE DYNAMICS

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[5]TRACC programme; co-supervised with Sarah Walmsely (Edinburgh) and Jo Birch (SCS)

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 primarily 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, yet.

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 (see Figure 1). 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. Neutrophils 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, a feature previously thought to only apply to other myeloid cells. 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 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. Neutrophils 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.

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. We continue to collaborate with several groups here at the Institute to investigate this in state-of-the-art pre-clinical models. We were excited to contribute to a major collaboration (Leo Carlin as co-senior author with Tom Bird, Owen Sansom, and Derek Mann) that addressed the role of neutrophils in the response of hepatocellular carcinoma on a non-alcoholic steatohepatitis background (NASH-HCC) to immunotherapy. In this context neutrophils were thought to be tumour promoting via their suppression of anti-tumour immunity. We found that by targeting the major neutrophil chemokine receptor CXCR2, we could make our models of NASH-HCC vulnerable to immune checkpoint inhibitors. Intriguingly, there were more, rather than fewer neutrophils in the tumours, but they had changed phenotype, suggesting the possibility of reprogramming neutrophil activity in the tumour microenvironment (Leslie, Mackey, Jamieson *et al.*, Gut 2022). We were excited to see the opening of a clinical trial based on this combination (CUBIC) and also received funding via a CRUK programme grant renewal to continue this highly productive collaboration.

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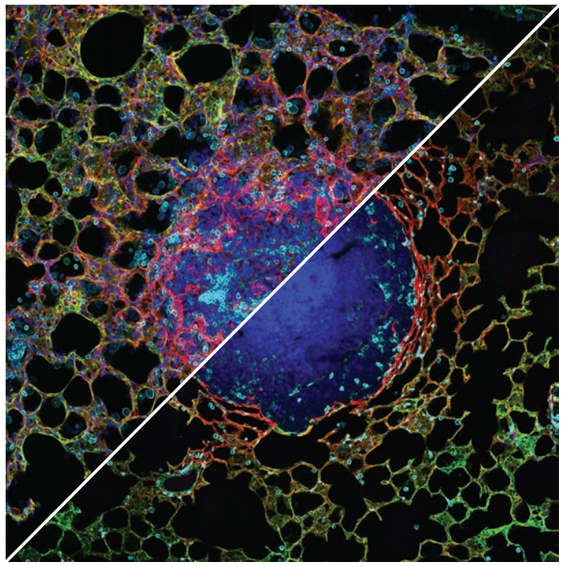

Figure 1
Two different types of vascularisation in mouse lung tumours grown from intravenous tumour cell line injection (experimental metastasis model). Images fused down centre line shown for comparison. **Left**, *Kras*$^{G12D/+}$; *Trp53*$^{R172H/+}$; *Pdx1-Cre* (KPC) cell line with vessel co-optive pattern. **Right**, renal cortical adenocarcinoma (RENCA) cells with a pushing / angiogenic pattern. Both samples were stained to reveal nuclei (left, DAPI; right, Sytox Blue; BLUE), vasculature (CD31; GREEN, ICAM1 (RED) and leukocytes (CD45 ; CYAN). The image also illustrates clear differences in the pattern of leukocyte infiltration to the two similar sized tumours. Images were aquired by Marco De Donatis using Zeiss 880 (BAIR) in spectral detection mode.

IMMUNE CELLS AND METASTASIS

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Our lab focuses on a type of immune cell, called 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 2022, our lab contributed to six scientific papers; two of these publications highlighted our work on IL-17A-producing γδ T cells. We deposited data from other studies as preprints on *BioRxiv*, which centre around tissue-resident subsets of γδ T cells. We attended several conferences to disseminate this work, including the 2nd International workshop on "Receiving and Transmitting Signals via the γδ TCR" in Cefalu, Sicily, and the British Society for Immunology annual congress in Liverpool. Mark and Hannah successfully completed their PhD studies. We welcomed three new members of the group: Kyi Lai, Federico, and Anna.

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 expressed different co-inhibitory molecules on their surface. One subset (Vγ6$^+$ cells) expressed constitute levels of PD-1, while another subset (Vγ4+ cells) upregulated TIM-3 in response to tumour-derived factors. Blocking either PD-1 or TIM-3 signalling in mammary tumour-bearing mice increased proliferation of Vγ6$^+$ or Vγ4$^+$ cells, respectively. This increase in Vγ6$^+$ or Vγ4$^+$ cell number counteracted T cell checkpoint inhibitor immunotherapy, as genetic deletion of γδ T cells sensitized metastatic mammary cancer cells to anti-PD-1 or anti-TIM-3 and prevents lung metastasis (Figure 1). Second, the scRNAseq highlighted different subsets of IFNγ-producing γδ T cells, identifiable by the differential expression of Ly6C. These subsets had cancer-killing functions. We have found that Ly6C+ γδ T cells were maintained by the cytokine, IL-27, which amplified their cancer-killing ability. In adoptive transfer experiments, Ly6C+ γδ T cells delayed mammary tumour growth, while Ly6C$^-$ γδ T cells did 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 counteracted intestinal adenoma formation and killed transformed enterocytes in mice. When tumours developed, however, these cells were 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, was 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 induced the downregulation of *Btnl1* mRNA using organoids derived from our mouse models. This down-regulation of *Btnl1* was accompanied by decreased expression by gut-specific transcription factors, such as HNF4A and HNF4G. Interestingly, inhibition of β-catenin in mouse models reversed the downregulation of *Hnf4a*, *Hnf4g*, and *Btnl1* in tumours, which was associated with higher numbers of γδ T cells in the tumour microenvironment.

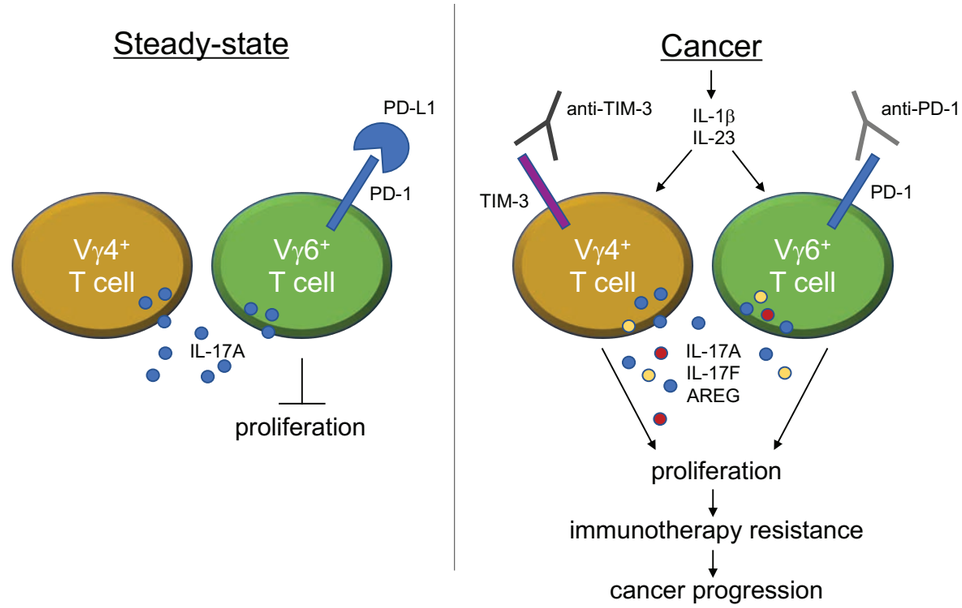

Figure 1
Phenotypic analysis of gamma delta T cells from lungs of mice shows that subsets of IL-17A-producing cells express distinct co-inhibitory molecules (i.e. PD-1 and TIM-3) that regulate cell expansion and counteract immunotherapy.

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 promoted cancer progression in mouse models driven by oncogenic β-catenin and MYC. Unlike our data in breast cancer, γδ T cells in the liver failed to express IL-17A. For the future, we will undertake a deep phenotypic analysis of these γδ T cells in the liver and perform experiments to understand how they perpetuate tumour growth.

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 over the past three years has been focused on overcovering the mechanism by which γδ T cells promote metastasis. During lockdown, we discovered that macrophages and fibroblasts were reduced in pancreatic tumours from γδ T cell-deficient mice, indicating that γδ T cells regulated these cells in some way to support metastasis. Currently, we were 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 whole-body function in health and disease.

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Figure 1
Gut-Brain Crosstalk in health and disease.
Confocal image of the adult *Drosophila melanogaster* brain stained with the neuropil marker NC82 (blue), the neuropeptide protein DH31 (magenta) and a *Dh31* gene expression reporter (yellow).

Image credit: Dr. Sofia Polcowñuk

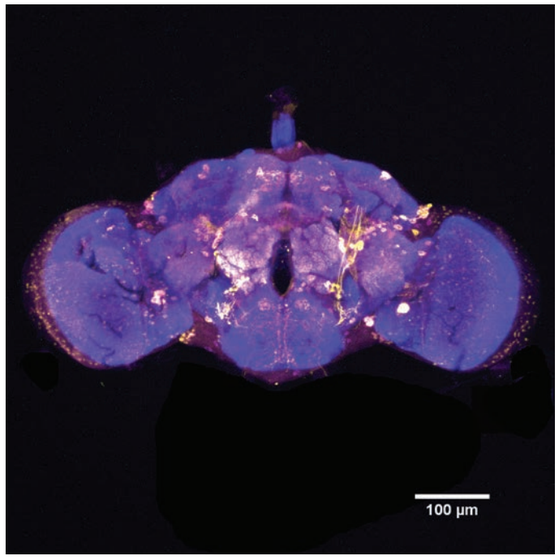
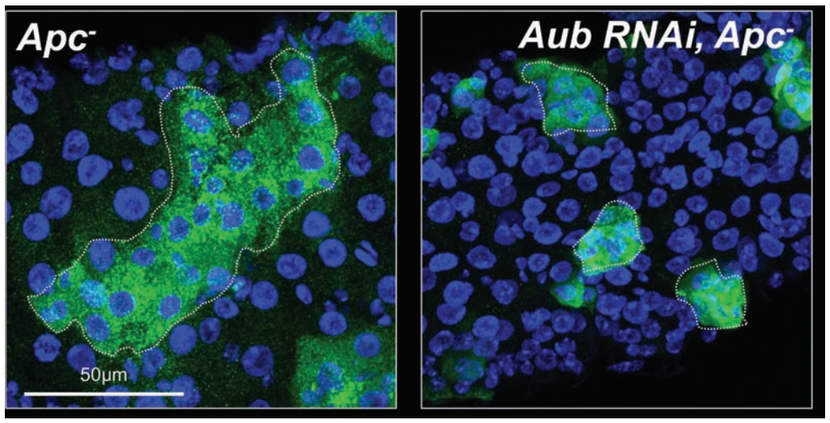


Figure 2
Elucidating the functional role of the PIWI pathway in colorectal cancer:
Clones of adult intestinal epithelial cells (green and outlined) lacking the colorectal cancer tumour suppressor gene *Apc* alone (left panel) or in combination with knockdown of the PIWI protein *PIWIL-1/ Aubergine (Aub)* (right panel). RNAi: RNA interference.

Image credit: Dr. Karen Bellec



GENE REGULATION

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Precise and responsive gene regulation directs development, immune responses and organ function. In cancer cells, gene regulatory mechanisms become altered resulting in the acquisition of deleterious features. 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 tumours and in the development of therapeutic targeting approaches. 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. This year we have reported that the cap methyltransferase, CMTR1, is recruited to its target genes via an interaction with RNA polymerase II at the initiation of transcription. We found that CMTR1 has gene-specific impacts on transcription, regulating key genes involved in DNA replication and protein synthesis. Thus, CMTR1 is critical for cell proliferation. Furthermore, we found that CK2, a kinase widely upregulated in a spectrum of tumours, phosphorylates CMTR1, increasing its interaction with RNA polymerase II. Phosphorylation of CMTR1 increases RNA capping, gene expression and cell proliferation. We are investigating the impact of this pathway in tumours and innate immune responses. 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 and the role they play in 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 CMTR1 during T cell activation, specifically defining its 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.

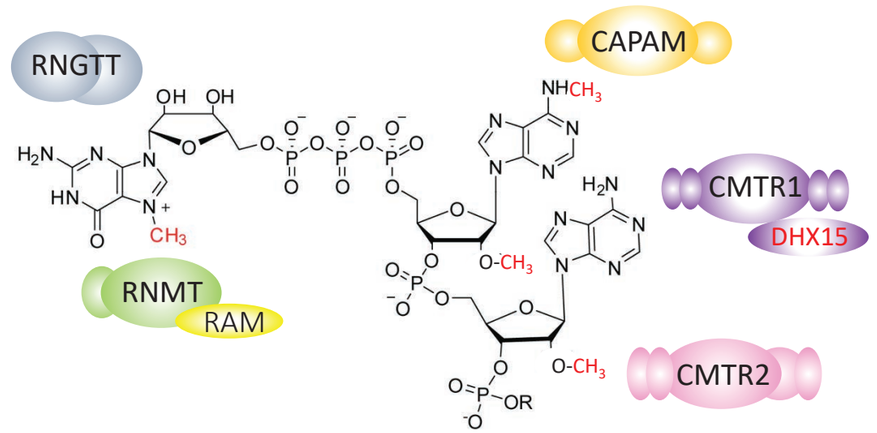

Figure 1
An RNA cap structure and capping enzymes

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

The RNA cap methyltransferases have a common methyltransferase core, but this is flanked by domains which differ in each enzyme, allowing them to be independently regulated by co-factors and post-translational modifications. We discovered that the RNA capping enzymes RNMT and CMTR1 are differentially regulated as embryonic stem cells differentiate; RNMT is repressed and CMTR1 is activated. RNMT and CMTR1 have distinct target genes. Therefore, distinct regulation of these enzymes during differentiation allows co-ordinate regulation of key genes during changes in cell identity. 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.

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. We continue to collaborate with the Dundee Drug Discovery Unit and external partners to develop tool compounds .

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PANCREATIC CANCER EVOLUTION AND THERAPEUTIC DEVELOPMENT

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Pancreatic cancer is one of the most lethal cancers and will soon become the second cause of cancer death in the UK. Working at the interface between clinical care in the NHS and laboratory research, the overall aim of our research is to improve outcomes for pancreatic cancer patients by deepening our understanding of 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 (Figure 1B).

The cell-to-cell differences that drive this cellular plasticity are determined by a complex interplay of multiple genetic and non-genetic factors (Figure 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.

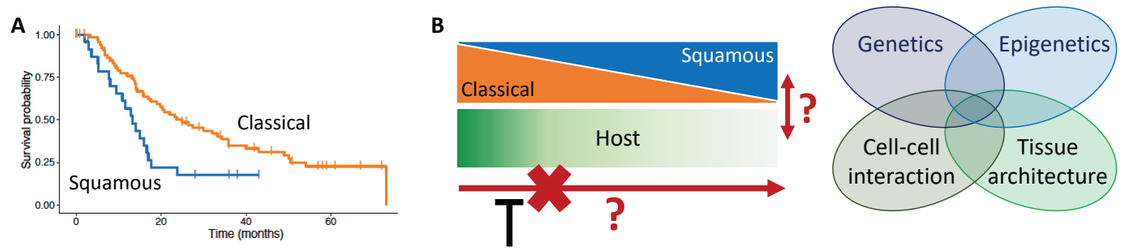

Figure 1
A Two consensus subgroups, or lineages, of pancreatic cancer (PC) are a "classical" and a poorer prognostic "squamous/basal-like" subtype. Overall survival by subtype is shown. **B** Recent research is indicating subtypes of PC exist as a continuum, with co-evolution of tumour and host cells 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.

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www.beatson.gla.ac.uk/payam_gammage

MITOCHONDRIAL ONCOGENETICS

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Mutations of mitochondrial DNA (mtDNA) are among the most common genetic events in all cancer, however their impact on disease initiation and progression is not understood. Mitochondria perform numerous metabolic functions, relying on faithful expression and maintenance of mtDNA, a small, multi-copy genome separate from the nuclear DNA that is contained exclusively within mitochondria. Mutations of mtDNA and gross changes to mtDNA copy number can lead to profound metabolic alterations - one of the earliest identified hallmarks of cancer – and these changes are observed in >60% of tumours. In order to understand the possible links between mitochondrial genetics and metabolic dysfunction in cancer, our lab studies a range of cancer models using and developing cutting edge mitochondrial genome engineering tools combined with genetic and metabolic analyses. By understanding the relationship between mtDNA and human cancer, we hope to identify new therapeutic targets for clinical application and to inform reallocation of existing treatments based on mtDNA genotype.

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.

Beyond experimental systems in the lab, using repurposed sequencing data from >40,000 tumours, we have shown that: i) mutations in mtDNA encoded genes were among the most common pan-cancer mutational events, comprising 25 of the 30 most mutated genes in all cancer (Figure 1a), that mtDNA mutational status was unaffected by nuclear DNA mutation burden or MSS/MSI state (Figure 1b,c), that recurrent hotspots defined the patterning of severe mtDNA mutations (Figure 1d) and that mtDNA mutation state offered major prognostic benefit in colorectal cancer (Figure 1e) (Gorelick *et al.*, 2021, *Nature Metabolism*). These findings illustrated 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 overlooked in cancer research to date.

Taking this knowledge forward, and using advanced mtDNA engineering techniques, we have now created the first known *in vivo* models of cancer bearing relevant mtDNA mutations across several tissue lineages.

Control of mtDNA copy number

In the nucleus, well-described mechanisms that provide tight control of genome replication are required for cellular and organismal viability. Similarly, mtDNA copy numbers are controlled in a robust, cell-type specific fashion, however, the analogous systems of control underlying regulation of mtDNA genome replication are poorly understood. Cancer cells commonly demonstrate changes in mtDNA copy number, probably due to the metabolic requirements of their tissue lineage and primary site. By developing our understanding of mtDNA copy number regulation and identifying the molecular mechanisms underlying this process, we hope to design future therapeutic strategies underpinned by manipulation of mtDNA copy number.

Genetic transformation of mammalian mitochondria

A major challenge for the field of mitochondrial genetics is the limited set of genetic tools to directly manipulate mtDNA *in situ*. Practically, this means that the experiments we can perform to determine the role of mtDNA mutations in cancer are limited in their scope. In order to develop our understanding of this area of cancer science, we aim to expand the relevant mtDNA genome engineering toolkit.

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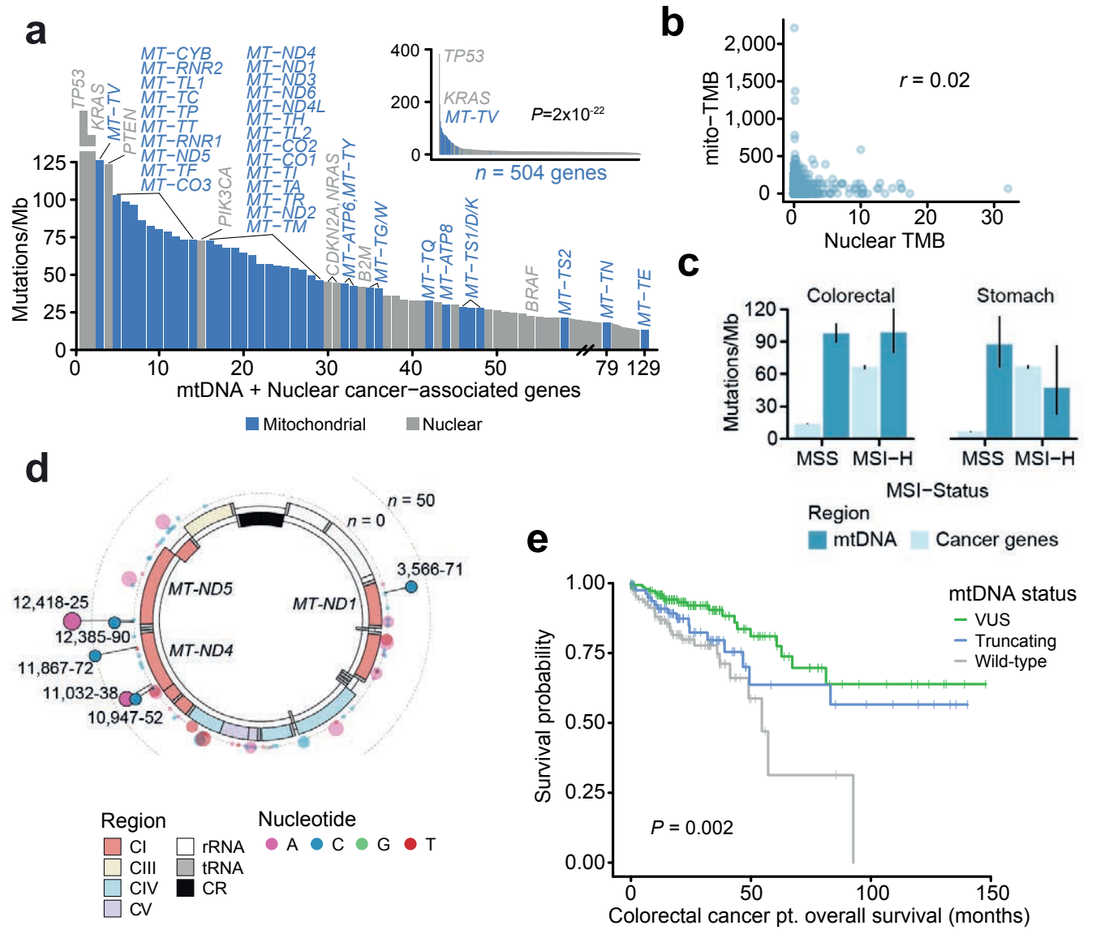

Figure 1

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).

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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.

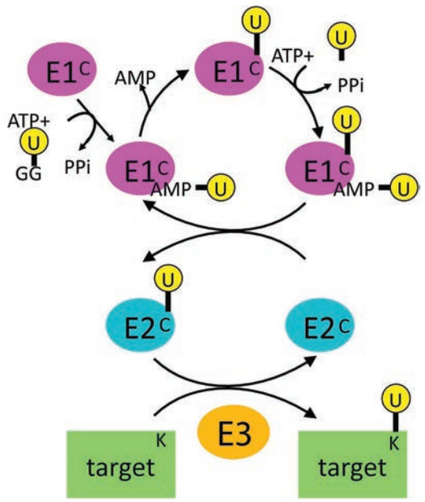


Figure 1
Enzymatic cascade for Ub modifications

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). Our ongoing work is aiming to develop therapeutics targeting CBL mutant-EGFR interaction and thereby reducing the oncogenic property of CBL mutant.

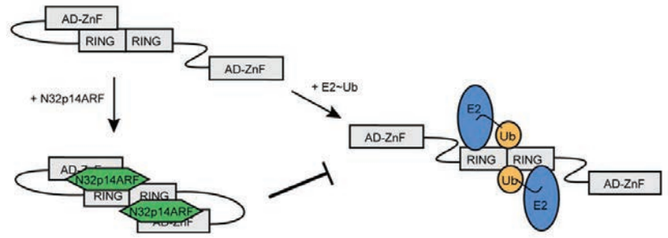
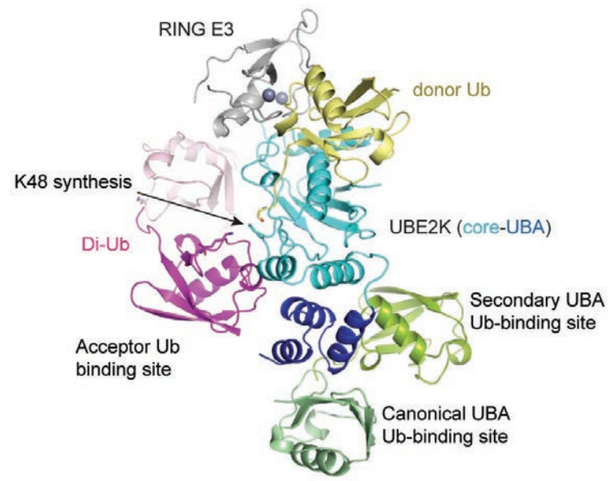


Figure 2
Regulation MDM2 E3 activity by p14ARF

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 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 2) and reveals strategies for targeting MDM2 RING domain.

Figure 3
Structure of RNF38 RING domain bound to UBE2K-Ub/K48-Ub_2 complex



Mechanism of K48-linked polyUb chain synthesis

The K48-linked polyUb chain acts as a 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 (Figure 3). We performed various NMR analyses and mutagenesis coupled with biochemical assays to validate our structure and demonstrated that our structure approximated the transition state of the K48-linked Ub chain synthesis. Our structure revealed that UBE2K active site residues and the C-terminal Ub-associated (UBA) domain bound the acceptor Ub and oriented its K48 toward the UBE2K–Ub active site for catalysis. Importantly, the UBE2K active site residues imparted K48-linked specificity whereas the UBA domain functioned to stabilise the conformational flexibility of acceptor Ub. Unexpectedly, our structure unveiled multiple Ub binding surfaces on the UBA domain (canonical, secondary and acceptor; Figure 3). We showed that this multivalent Ub binding feature served to bring UBE2K to Ub-primed substrate (substrate modified with Ub). By localising UBE2K to Ub-primed substrates, where Ub concentration was enriched, weak acceptor Ub affinity could be overcome to accelerate Ub chain extension. Moreover, we showed that UBA domain exhibited a preference for K63-linked polyUb chain as the acceptor and thereby promoted branched K48-K63 polyUb chain formation. Our work explains the molecular basis for K48-linked Ub chain synthesis and how UBA domain promotes processive polyUb chain formation (Nakasone *et al.* 2022, Nature Chemical Biology).

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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 using systems biology and biological functional approaches.

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, 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 were inactivated in 30% of sporadic cSCC and that TGFβ signalling could have potent tumour suppressive effects in the face of other mutational events *in vivo*. We are currently investigating how driver gene combinations act in concert with loss of TGFβ signalling to influence cSCC progression. Despite TGFβ's powerful tumour suppressive effects in cSCC, 70% of tumours displayed no obvious inactivation of the canonical signalling pathway. Analysis of the TCGA head and neck squamous carcinoma (HNSCC) data set revealed a similar potential loss/downregulation of canonical signalling components in ~30% of tumour samples with downregulation of TGFBR2 and SMAD4 being particularly prevalent (Figure 1). Strikingly ~70% of tumours showed overexpression of TGFβ1 and many tumours upregulated TGFBR1 expression relative to normal tissue. Taken together, these observations indicated that TGFβ signalling might also act to promote tumour progression in both cSCC and HNSCC and we are focusing our initial efforts into understanding the potential tumour promoting effects of TGFβ signalling in cSCC and HNSCC in a panel of patient derived cell lines (PDCLs).

cSCC is a life-threatening complication for patients who suffer from recessive dystrophic epidermolysis bullosa (RDEB), a skin blistering disease caused by germline mutations in collagen VII, the anchoring fibril component in the skin. Unlike in sporadic cSCC, RDEB SCC tumours do not contain inactivating mutations in TGFβ receptors (Cho *et al.,* 2018, *Sci Transl Med*) pointing to a potential tumour promotion role in these cancers. Intriguingly, we have found that exogenous TGFβ stimulation inhibited proliferation of all RDEB cSCC PDCLs but that endogenous TGFβ signalling drove proliferation, clonogenicity, migration and invasion in the majority but not all of these cell lines (Dayal *et al.,* 2021, *BJD*)(Figure 2). Targeting TGFBR1 kinase activity might 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.

The Molecular Landscape of cSCC and HNSCC

The incidence of keratinocyte skin cancers in white-skinned populations represents a rising global health burden. In SCC, development of primary tumours may be preceded by pre-malignant Actinic Keratosis. In contrast to most other epithelial malignancies, more than a third of patients develop multiple primary cSCC.

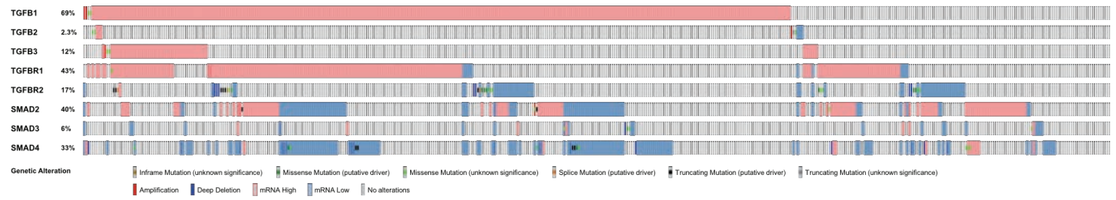

Figure 1
Oncoprint analysis of TGFβ canonical signalling components in HNSCC
Cbioportal (Cerami *et al.,* Cancer Discov. 2012, and Gao *et al.,* Sci. Signal. 2013) analysis of HNSCC (TCGA, Pancancer Atlas) reveals frequent mutational alteration and downregulation of mRNA expression of TGFBR2 and SMAD4 but overexpression of TGFB1 and TGFBR1 compared to normal samples pointing to potential tumour suppressor and tumour promoter roles of TGFβ signalling.

Metastasis occurs in ~5% of cases, and there are few effective treatments for advanced cSCC, with five-year survival of less than 30% reported for metastatic disease (Harwood *et al.,* 2016, *Acta Derm Venereol*). Cutaneous SCC is poorly understood at a molecular level. 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 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. Our whole exome-sequencing analysis of Actinic Keratosis has revealed remarkably similar complex genetic landscapes of both pre-malignant (Thomson *et al.,* 2021, *J Invest Dermatol*) and primary tumours. (Inman *et al.,* 2018, *Nat Commun*). We are now analysing whole genome, exome and bulk RNAseq profiles of human and murine cSCC samples derived from genetically engineered mouse models (in collaboration with Owen Sansom and Karen Blyth). Using systems biology approaches (driven by Peter Bailey) we are integrating these datasets and interrogating the biological pathways, processes and driver genes required for disease progression with a view to identifying therapeutic intervention approaches.

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.

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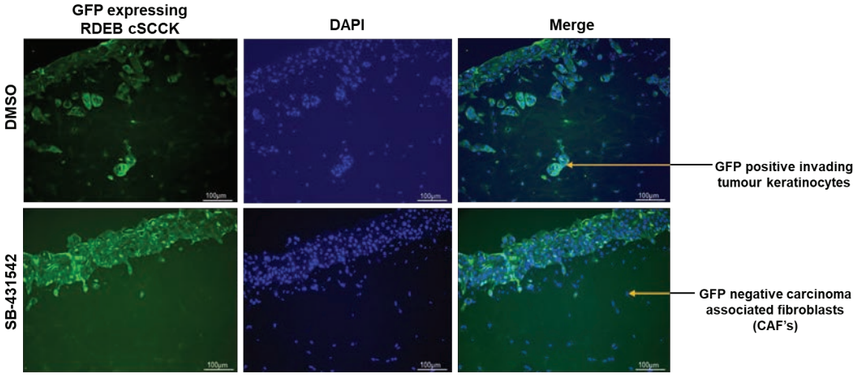

Figure 2
Organotypic assays indicate endogenous TGFβ signalling promotes invasion of RDEB cSCC tumour cells
3D organotypic assays using RDEB cancer associated fibroblasts embedded in type 1 Collagen-Matrigel gels forming a dermal component to test the invasive potential of GFP positive RDEB cSCC tumour keratinocytes. Gels containing SB-431542, a TGFBR1 kinase inhibitor, can inhibit the invasive potential of a subset of RDEB skin tumour cells compared to the DMSO control.

CELL MIGRATION AND CHEMOTAXIS

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The cell migration and chemotaxis lab studies how & why cells move, using a wide range of multidisciplinary tools, including cell biology, computer modelling and artificial intelligence techniques such as deep learning.

Metastasis, when cells spread from the tumour in which they arose and colonise other organs, is responsible for most of the damage cancer causes. In normal organs, and most benign tumours, cells do not migrate. However, when tumours become metastatic, cancer cells may start to migrate – spreading into neighbouring tissues, the blood and lymph systems to form secondary tumours. We are working to understand why cells move, and what steers them.

We ask several different questions, all aimed at the same general problem. One question is how cells are steered by external signals, a process known as chemotaxis, which is increasingly seen as a fundamental cause of cancer metastasis. We are particularly interested in a complex type of chemotaxis, in which cells steer themselves. The Insall lab are world leaders in the field of "self-generated gradients" and were recently awarded Wellcome funding to develop this area.

Another is the mechanics by which cells drive their migration. We focus on the structures that cells use to migrate, known as 'pseudopods'. Pseudopods are made by assembling fibres of a protein called actin; we try and understand what controls how actin is built, and how this leads to formation of pseudopods.

A third, and particularly relevant to cancer at the moment, is to use artificial intelligence (AI) techniques – in particular deep learning – to predict from pathology images whether tumours are metastatic. The lab contains mathematicians, computer scientists, biochemists, microscopists and geneticists. We see one of our chief jobs as spreading true multidisciplinarity – mathematicians do cell biology experiments, and biochemists use mathematical models and computational tools. However, our strategy is always based around cell migration – what drives it, and why?

Mechanisms underlying chemotaxis: Self-generated gradients

Chemotaxis is a major driver of tumour metastasis. We have found that it does not work the way we used to think it does, on many different levels. We design and build chemotaxis chambers to make experiments more informative. We can use these to show that many different types of cancer cells are exquisitely chemotactically sensitive (much more so than was previously thought), including melanoma, pancreatic ductal adenocarcinoma, glioblastoma, and of course blood cancers like lymphoma. The changes that occur as cells become malignant are more to do with speed than steering – early melanomas, for example, are slower but still highly chemotactic. We have shown that this is because the pseudopods grow and develop in a different way as cancers become more malignant.

The most interesting part of melanoma cells' response is that we find they make their own chemotactic gradients. Lysophosphatidic acid (LPA) – which appears to be present at substantial levels in the tissue surrounding tumours – is a strong attractant for all the melanoma cells we have observed. But melanoma cells also break down LPA. This leads to a self-generated gradient, in which cells move out of tumours in response to gradients they are themselves creating. Thus, tumours appear to need no external drivers to steer metastasis – they do it themselves. This appears to be a fundamental feature of many metastatic cancers.

We are now studying the details of self-generated gradients, using mathematical models to identify the range of possible behaviours, and doing experiments with a wide range of different cell types, including melanoma, glioma, pancreatic ductal adenocarcinoma, lymphoma, immune cells such as dendritic cells, cultured neutrophils and Dictyostelium. We have shown that cancer cells and Dictyostelium can use self-generated gradients to solve mazes of remarkable complexity.

We have also shown how cells can use self-generated gradients to repel one another, making cells move away from a source. This is obviously important in metastasis, where cells being driven away from a tumour results in cancer spread.

We collaborate with the Mathematics departments of the Universities of Strathclyde and Glasgow to make different computational models representing moving cells. We are now using these models to test our predictions about self-generated chemotactic gradients and the underlying mechanisms of chemotaxis. We have shown that even single cells can create their own gradients. We have also found that chemotaxis is most likely mediated by several dissimilar mechanisms acting in parallel, including regulated pseudopod growth, pseudopod retraction and the control of adhesion.

We also collaborate with the Physics and Engineering departments in Glasgow to build microscopes that will allow us to test what real cells in tissues and organs are perceiving, live and in real time. This will allow us to test which cells are responding to self-generated gradients, under realistic conditions. The microscope will combine high-resolution CMOS sensors with time-resolved SPAD sensors that allow us to measure the times when individual photons are released. This allows us to interrogate a family of intracellular probes called FRET probes, which give excellent detail about the states of living cells in 3D.

Regulators of actin and the Arp2/3 complex

Most mammalian cells use pseudopods made of polymerised actin to power migration. Our current research focuses on the proteins and pathways that control these pseudopods. We use three approaches. For genetic studies, we use Dictyostelium, taking advantage of its ease of manipulation, and prominent cell movement and chemotaxis. To apply our knowledge to cancer, we use melanoma cells cultured from tumours with different degrees of metastasis, and actual tumours from mouse models and, when possible, from fresh patient tissue.
Actin drives nearly all cell movement, and the principal driver of actin is an assembly called the Arp2/3 complex. We are particularly interested in the family of proteins that turns on the Arp2/3 complex. One such regulator is SCAR/WAVE, which is a fundamentally important regulator of cell movement. Mutants in a variety of species show that it is required whenever cells need to make large actin-based structures such as lamellipods; without SCAR/WAVE such structures are either small and malformed, or completely absent. It is found as part of a five-membered complex with the Rac-binding protein PIR121, Nap1, Abi and HSPC300. The prevailing view in the field is that all these proteins act simultaneously as a huge, homogeneous complex that couples Rac and lipid signalling to actin polymerisation. However, this view seems very simplistic considering the size of the complex and its dynamic behaviour. We were recently awarded an MRC programme grant to follow this line of research.

Deep learning from pathology images

Recently, we and others have found that deep learning can usually distinguish metastatic from nonmetastatic solid tumours, from H&E stained pathology slides alone. We are developing this technology for many reasons. It offers the prospect of faster, more accurate diagnosis for patients, but it also promises to give us new information about why cells become metastatic, how to understand it, and potentially how to stop it. Our most recent work concerns self-supervised learning, where the AI seeks to classify different regions of a tumour without training by pathologists. This offers the hope of a complementary view that bolsters what pathologists already see, rather than reproducing it.

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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 haemopoiesis 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.

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).

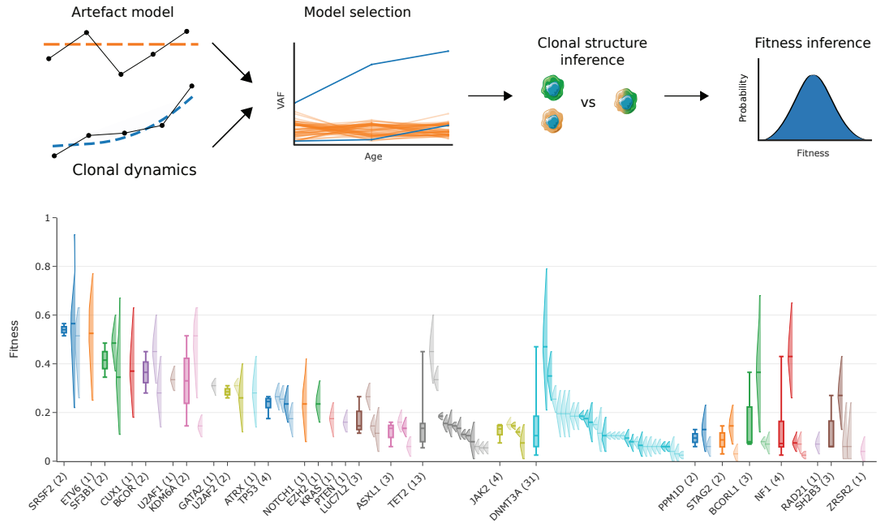

Figure 1
Mathematical modelling to interrogate stem cell fitness in clonal haemopoiesis
Mathematical models are used to estimate stem cell fitness alterations caused by mutations associated with clonal haemopoiesis. This allows us to predict outgrowth of fit clones early and progression toward leukaemia at an early stage. In addition, our data can be used to estimate when a cancer-causing mutation arose in an individual.

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). As a next step we are now linking differences in stem cell fitness to transcriptional changes longitudinally in the LBCs. In addition, we are increasing our cohort size to enable us to link stem cell fitness to outcomes, such as all-cause mortality.

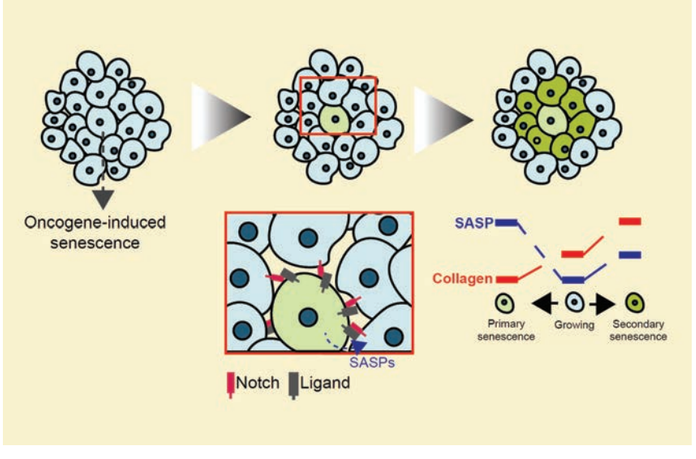

Figure 2
Model of Notch-mediated secondary senescence.
Secondary senescence *in vitro* and *in vivo* requires Notch, rather than SASP alone as previously thought, with primary and secondary senescence being distinct molecular endpoints. A blunted secretory phenotype and the induction of fibrillar collagens in secondary senescence point towards functional diversification and senescence heterogeneity.

Single cell approaches to investigate senescence heterogeneity in the tumour microenvironment

The role of secondary senescence remains 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). We found that Notch-mediated secondary senescence blunted SASP, typically seen at high levels in primary senescence. Moreover, upregulation of collagens on the transcriptional level in secondary senescence contrasted with a well reported downregulation in primary senescence (Teo *et al.,* 2019), 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:

1) Translational control in tumour cells

The dysregulation of mRNA translation is emerging as a key hallmark of malignant transformation, as tumour cells radically reprogramme their protein output by implementing translational control mechanisms associated with states such as cellular stress and altered nutrient availability. To what extent is mRNA translation regulation altered in human cells? Which hallmark behaviours are linked to which alterations in translational control? Which elements of the translational control machinery have promise as therapeutic targets?
We are examining numerous measures of translational control at the single-cell level in large collections of several common malignancies, and we are using the resulting images both to generate and to test hypotheses. For example, we have found that switching between expression of different mRNA helicases is associated with tumour cell proliferation and invasion as well as immune system evasion, and that stress signalling through eIF2 is intimately associated with tumour cell proliferation and invasion.

2) Tumour immunophenotyping

The most impactful development in cancer therapy in recent years is the introduction of immunotherapies. These treatments work by reversing the ability of tumour cells to mask themselves from the immune system which would otherwise rapidly destroy them. However, we are at present only partially successful in identifying which patients will benefit from these therapies. We believe that quantifying the degree of immune system engagement within tumour biopsy material is likely to improve our ability to do this; can we, by direct observation of complex cellular phenotypes in tissues, identify tumours which are actively evading immune system detection and/or destruction?
To achieve this, we are applying highly multiplexed panels of markers to identify tumour and immune cell phenotypes, for instance using our FUSION platform we can use upwards of 40 markers to distinguish specific cell phenotypes in the tumour microenvironment. We are then able to link the presence and relative spatial distribution of these cells to patient outcomes. We intend to apply these methods to cohorts of tissues from patients receiving immunotherapies with Glasgow's cancer treatment centre, and to see if we can improve our ability to predict patient response to immunotherapy, compared to current methods.

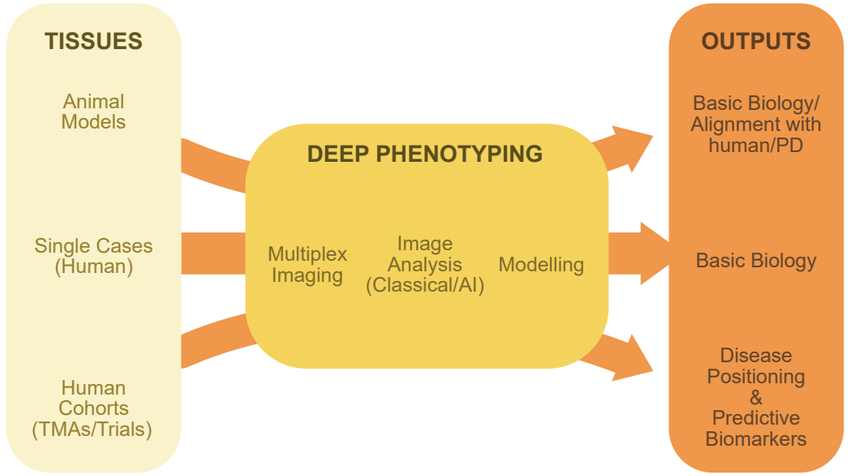

Figure 1
Workflow schematic of deep phenotyping methods. The basic pipeline (centre) is applied to a range of tissue types to achieve answers to diverse scientific questions.

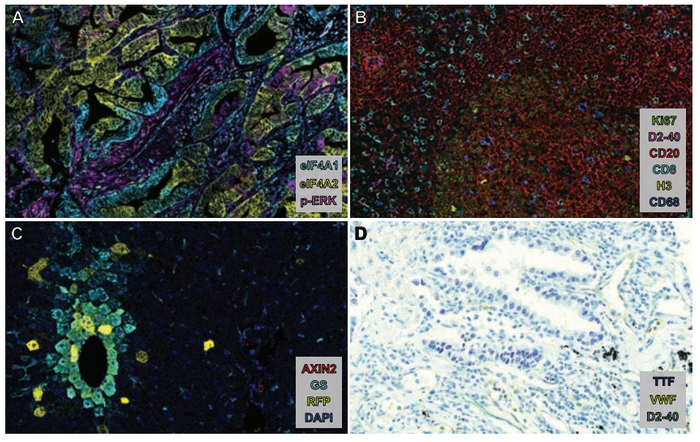

Figure 2
Example multiplex images. **A** Spectrally unmixed multiplex staining of eIF4A1, eIF4A2 and P-ERK in archival human lung adenocarcinoma tissue. **B** FUSION image of indicated protein markers on human tonsil tissue sections; only a small subset of the stained markers are shown **C** Spectrally unmixed co-ISH IHC of AXIN 2 mRNA with IF markers for red fluorescent protein and glutamine synthase in transgenic mouse liver. **D** four-colour chromogenic staining for human lung adenocarcinoma cell nuclei (TTF-1), capillaries (VWF) and lymphatics (D2-40) with haematoxylin counterstain.

3) 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.

4) Deep phenotyping of respiratory malignancies

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

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PROSTATE CANCER BIOLOGY

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Prostate cancer 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. Despite improvement in patient survival with novel androgen receptor inhibitors and taxane chemotherapy, a significant proportion of patients with advanced disease still dies within five years of diagnosis. We have a highly comprehensive cross-disciplinary programme of translational research aimed at tackling treatment (hormonal and/or taxane chemotherapy) resistance. At our disposal, we have a wide range of preclinical models and clinical resource to help us discover new treatment targets and understand the molecular mechanisms of how aggressive prostate cancer can resist current treatment.

The Prostate Cancer Biology Group applies cutting-edge technologies and innovative laboratory model systems to enhance our understanding of treatment resistance. We ultimately aim to discover new therapeutic targets and develop better treatment strategies with accompanying clinical biomarkers to support precision medicine in patients with advanced prostate cancer (Figure 1).

Castration-resistant prostate cancer (CRPC) is incurable and remains a significant challenge worldwide. Using a panel of isogenic human prostate cancer models of hormone-naïve and castration-resistant disease, we have developed matching 2- and 3-dimensional *in vitro* cultures and *in vivo* orthografts to model clinical prostate cancer. We initiated deep quantitative proteomic analysis to characterise proteins of interest in CRPC. As a result, we identified several key players in CRPC and reported our findings recently (Blomme *et al.,* 2020, 2022; Martinez *et al.,* 2021).

We further carried out multi-omic analyses (RNA sequencing, proteomics, and metabolomics) of our matched models of 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 clinical PET imaging to detect metastatic and/or recurrent disease. Here, our findings are pointing to a new research direction in understanding how PSMA-mediated functions may promote treatment resistance.

Our recent extensive research into CRPC collectively points to aspects of altered cancer metabolism to be important in treatment resistance. We were particularly intrigued by the role of abnormal cholesterol metabolism in driving lethal prostate cancer. We recently observed that an 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 an resistance mechanism (Patel, *et al,* 2018). To test the clinical relevance of this observation, we designed a proof-of-concept clinical study, 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. The primary study endpoint was the proportion of patients achieving ≥50% drop of baseline PSA levels at any time over the 6-week period of atorvastatin medication (PSA response). Exploratory endpoints included PSA velocity and mass spectrometrically identified serum metabolites (Rushworth *et al,* 2022).

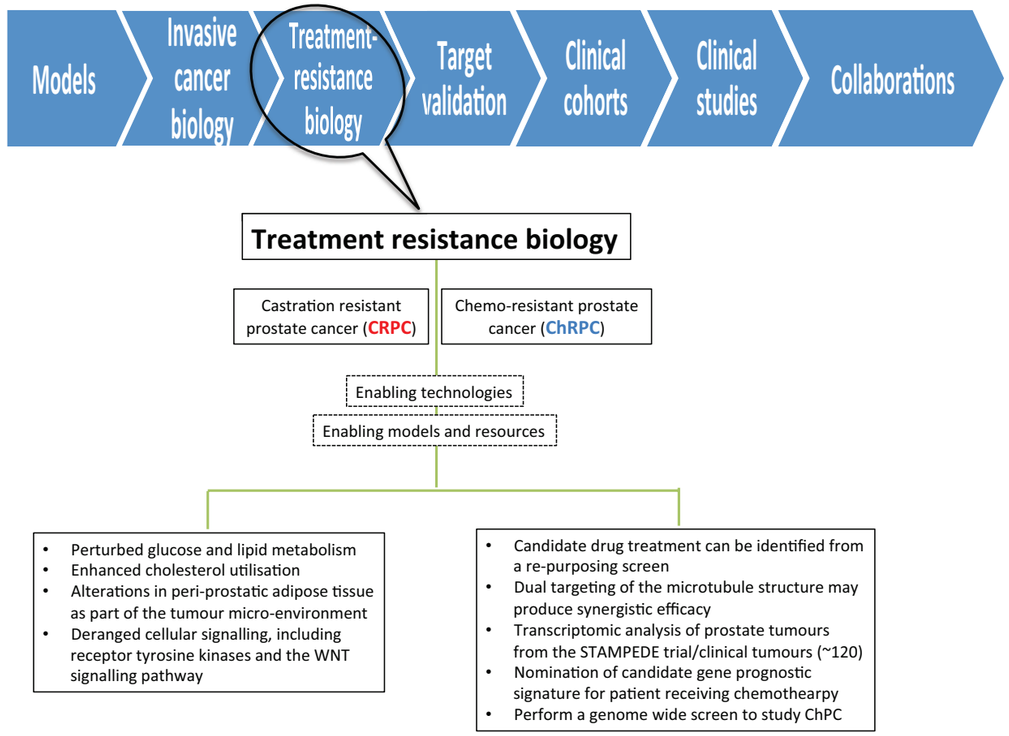

Figure 1
The research programme in the Prostate Cancer Biology Group comprehensively spans multiple aspects of translational cancer research, including basic discovery cancer biology studies, validation of novel druggable targets, collaborative drug discovery campaigns, clinical cohort studies and clinical trials of novel therapeutic agents. In this report, we highlight recent research milestones in our studies of treatment-resistant prostate cancer, particularly for castration-resistant disease. (Enabling technologies - e.g. transcriptomic, proteomic and metabolomic analyses; Enabling models and resources – e.g. genetically engineered mouse models, orthotopic xenograft models, primary cultures; Tissue microarray of clinically resected prostate tumours; Bio-repository from clinical trials and biobanks).

At scheduled interim analysis, all twelve recruited patients experienced, as expected, substantial falls in serum cholesterol levels following statin treatment. While all patients had comparable pre-study PSA velocities, 6 out of 12 patients showed decreased PSA velocities following statin treatment, suggestive of stabilised disease. Unbiased metabolomics analysis of serial weekly blood samples identified tryptophan as a dominant metabolite associated with statin response. Hence, 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.

Concluding comment

The use of a multi-omics approach on our panel of preclinical treatment-resistant prostate cancer models reveals novel treatment strategies. Based on our proof-of-concept clinical study, targeting cholesterol metabolism in CRPC warrants further investigations.

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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.

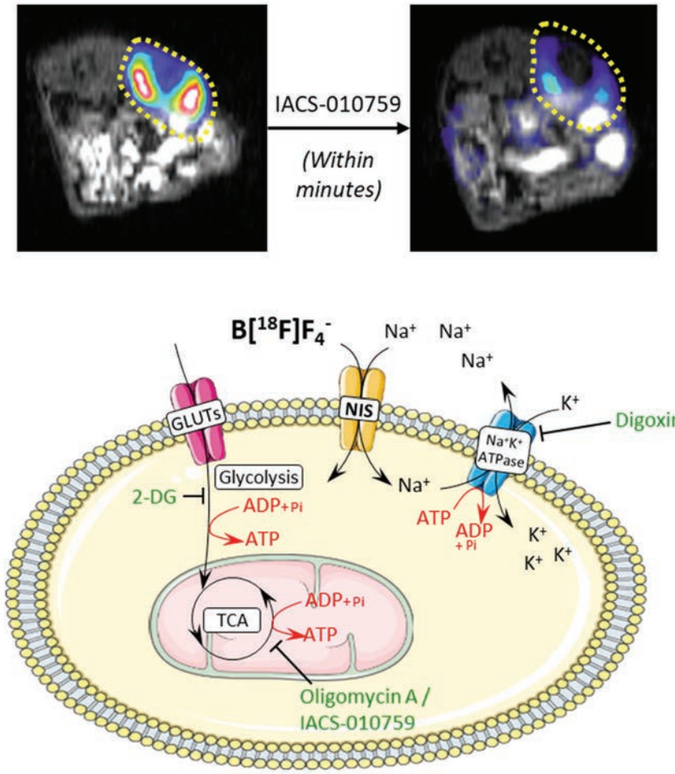

Figure 1
Metabolic sensing of energy charge *in vivo*
Rapid decrease in [^{18}F] tetrafluoroborate PET uptake within minutes of administration of drugs (2-DG, oligomycin and IACS-010759) targeting ATP production.

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-mediate 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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MIGRATION, INVASION AND METASTASIS

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The impact of the tumour microenvironment and the peri-tumour extracellular matrix on metastasis presents an opportunity to develop new therapies. Pancreatic tumours are especially fibrotic, causing starvation for nutrients and enhancing invasive behaviour. We are studying how tumour cells balance the usage of their cytoskeletal machinery to migrate and invade, with the assembly of macropinocytic structures to take up nutrients and thus survive in nutrient-depleted conditions. We aim to exploit vulnerabilities caused by the cancer microenvironment that could be targeted against metastasis and to model the metastatic niche using bioengineering.

One of the ways that tumour cells survive in the hostile tumour microenvironment is by repurposing their actin cytoskeletal migration machinery to take in large gulps of the surrounding liquid by macropinocytosis. Migration and macropinocytosis use the same basic actin machinery, and therefore can compete with each other - but the mechanisms controlling this competition are not well understood. PhD student Anh Le recently discovered an important role for the RAC1-interacting protein CYRI-A in regulating the balance between macropinocytosis and invasive cell migration (Le *et al.,* 2021, *Journal of Cell Biology*). Together with PhD student Savvas Nikolaou, they found that CYRI-A and CYRI-B were both important in resolving macropinocytic cups, by opposing actin assembly and allowing actin to disassemble for engulfment of macropinosomes. Interestingly, cells depleted of CYRI-A and CYRI-B were unable to perform macropinocytosis, but showed enhanced invasive migration, suggesting a competition between these processes (Le *et al.,* 2021, *Journal of Cell Biology;* Le & Machesky, 2022, *Bio. Protoc.*).

One of the major receptors trafficked by CYRI-B-dependent macropinocytosis was integrin alpha5 beta1 (Le *et. al,* 2021, *Journal of Cell Biology*). Postdoctoral researcher Jamie Whitelaw found that CYRI-B knockout cells displayed enlarged and less dynamic focal adhesions. He performed a Bio-ID screen and identified several differences in the composition of adhesions in the absence of CYRI-B (Whitelaw *et al.,* in preparation).

Savvas Nikolaou went on to study the effects of depletion of CYRI-B in KRas- and p53-driven pancreatic cancer and to determine how CYRI-B affected tumour progression and metastasis *in vivo*. His studies suggested that CYRI-B might function as a buffer for the effects of hyperactivation of RAC1 downstream of KRas in early tumour progression. In contrast, CYRI-B seemed to play an important role in metastatic progression, which might be due to its key function in trafficking of integrins and other surface receptors via macropinocytosis (Nikolaou *et al.,* under review).

Associate scientist Amelie Juin discovered that the MAP-kinase kinase MAP4K4, which is known to impact actin dynamics and cell migration, also had a role in the progression of pancreatic ductal adenocarcinoma. She is continuing to explore downstream signalling pathways that accelerated tumour progression upon MAP4K4 depletion (Juin *et al.,* manuscript in preparation).

Postdoctoral researchers James Drew and Vassilis Papalazarou discovered that collagen-6 acted as a mechanosensitive component of the metastatic tumour microenvironment of pancreatic ductal adenocarcinoma (Papalazarou, Drew *et al.,* J. Cell Sci., 2022). Collagen-6 is a heterotrimeric collagen, composed of up to six different alpha chains, which assembles into elastic fibres in collagen-1 rich connective tissues. Pancreatic tumours are known for their high levels of fibrous extracellular matrix and depositions of collagen-I. We found that when cancer cells experienced a mechanically soft environment

Collagen-6 was upregulated and its expression contributed to invasive migration and establishment of metastases. Collagen-6 could be produced both by cancer cells and stromal cells and our study suggested that it could be expressed early during metastasis as an important promoter of a new metastatic niche in pancreatic ductal adenocarcinoma.

PhD student Hakem Albilasi is studying the interactions between chronic myeloid leukaemia (CML) cells and mesenchymal stem cells. He found that CML cells can interact with mesenchymal stem cells and is studying the mechanisms and consequences of this interaction.

PhD students Sonia Rolo and Elaine Ma are studying the effects of mechanosensing on expression of various target genes involved in invasion and migration in pancreatic ductal adenocarcinoma. Elaine is also developing novel hydrogels for the culture of tumour cells and organoids in conditions where she can use bioengineering to control stiffness and composition of the matrix (Figure 1). These hydrogels will serve as an excellent platform to ask specific questions about the effects of physical parameters and matrix composition on the 3D growth of cancer cells. Juda Milvidaite is also exploring bioengineered materials, such as alginate hydrogels, for the growth and preservation of organoids and tumour samples in collaboration with the Biotech company Atelerix. Together, we are developing new models for the tumour and metastatic niche to build better model of the complex cancer microenvironment.

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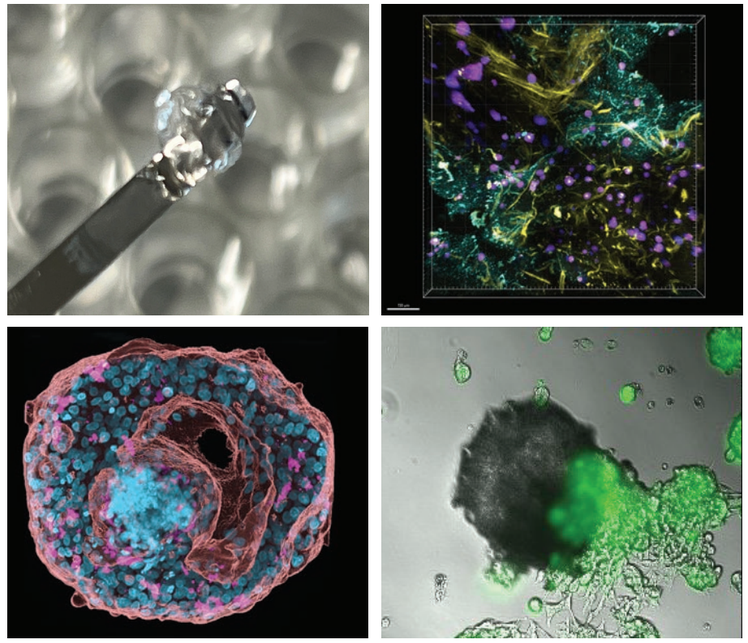

Figure 1
Polyethylene glycol (PEG) hydrogels functionalised with fibronectin and collagen 1 provides a scaffold for 3D cell culture *(top left: PEG hydrogel).* This synthetic hydrogel mimics the biochemical and physical properties of the extracellular matrix to support growth of pancreatic ductal adenocarcinoma (PDAC) spheroids *(top right: PDAC cell culture in PEG hydrogels; fibronectin (yellow), collagen 1 (cyan), phalloidin (purple), DAPI (blue).* Co-culture of PDAC cells and liver spheroids within the PEG hydrogel provides a 3D *in vitro* model of PDAC metastasis in the liver *(bottom left: co-culture of PDAC cells and liver spheroids; collagen 1 (magenta), DAPI (cyan), phalloidin (red) and bottom right: co-culture with PEG hydrogels; PDAC cells (green), liver spheroid (grey)).*

MITOCHONDRIAL REPROGRAMMING IN CANCER

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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.

During an exciting first year at the Institute, we embarked on our study of mitochondrial reprogramming in cancer cells (Figure 1). Metabolite transporters sit in the impermeable inner mitochondrial membrane and couple the metabolic reactions of the cytosol with the mitochondrial matrix. These transporters and associated regulatory components therefore represent crucial sites of cellular metabolic control. We are performing genetic screens to identify mitochondrial metabolite transporters that influence pancreatic cancer cell growth and survival under different environmental conditions. Extensive metabolic reprogramming occurs during the development of pancreatic cancer, and mitochondria are known to support pancreatic tumour proliferation upon hypoxia and nutrient deprivation. We hope that our screening results will offer new transporters and metabolic pathways as therapeutic targets to test in pre-clinical models of the disease.

The transport and metabolism of mitochondrial nucleotides

One group of metabolites that we have been particularly interested in this year are nucleotides. 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. Previous work has shown that enhanced uptake of mitochondrial pyrimidines can trigger the synthesis of mtDNA but also the activation of inflammation pathways. We are keen to understand whether mitochondrial pyrimidine transport and metabolism influences tumour immunogenicity and responses to pyrimidine analogue chemotherapies such as 5-fluorouracil and gemcitabine.

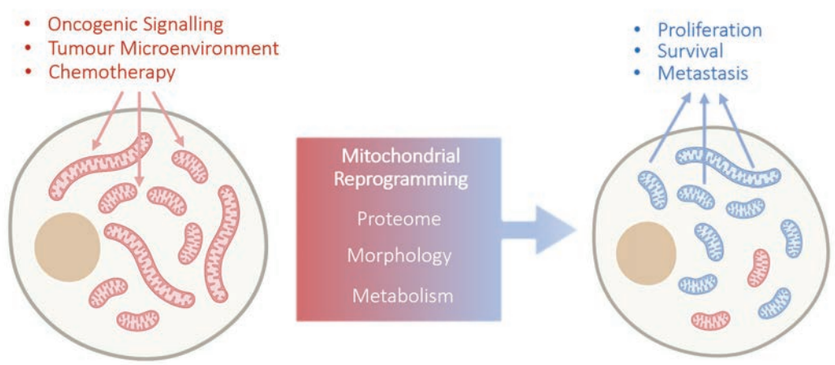

Figure 1
Mitochondria are reprogrammed to facilitate tumour progression and survival

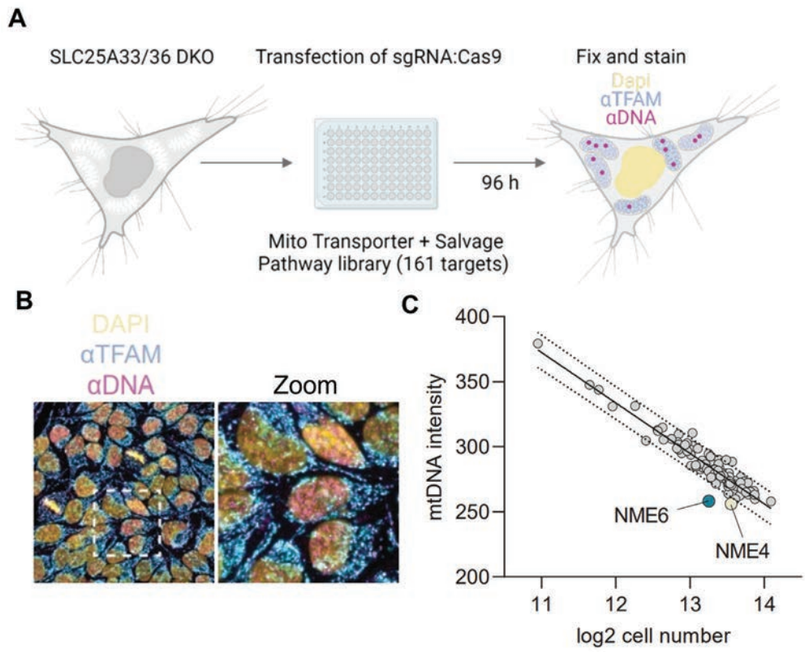

Figure 2
CRISPR-SpCas9 screen reveals regulators of mitochondrial pyrimidine metabolism
A Schematic of the screen conducted in cells lacking both mitochondrial pyrimidine nucleotide transporters, SLC25A33 and SLC25A36 (DKO – double knockout). **B** An anti-DNA antibody was used to detect and quantify mtDNA in cells transfected with a panel of sgRNAs and SpCas9. Mitochondrial nucleoids (packaged mtDNA) were also detected with an antibody against the mtDNA packaging protein, TFAM. **C** Cells transfected with sgRNA targeting the nucleoside diphosphate kinases NME6 and NME4 had reduced levels of mtDNA (data from Grotehans et al., 2022 bioRxiv doi. org/10.1101/2022.11.29.518352).

Blocking nucleotide supply to suppress mitochondrial activity

We tested what happens to proliferating cells when their mitochondrial pyrimidine import routes are blocked. We were surprised to find that depletion of the two described pyrimidine transporters, SLC25A33 and SLC25A36, had little effect on cell division or mtDNA. One challenge of studying mitochondrial metabolism in mammalian cells is an apparent redundancy in metabolite transporters. Together with the Beatson Advanced Imaging Resource and High-Content Analysis team, we performed a CRISPR-SpCas9 screen of mitochondrial transporters and nucleotide metabolism enzymes to identify genes that regulate mtDNA content when pyrimidine nucleotide import is impaired. Our screen and subsequent experiments revealed that a poorly characterised nucleoside diphosphate kinase, NME6, could preserve mtDNA in pyrimidine depleted conditions (Figure 2). Further work, in collaboration with Prof Thomas Langer (MPI Biology of Ageing, Cologne), revealed that NME6 is constitutively required for the supply of pyrimidines for mitochondrial RNA synthesis. Cells lacking NME6 were deficient in oxidative phosphorylation and could not proliferate in respiration-dependent conditions.
Recent research indicates that perturbing the transcription and translation of mitochondrial genes is a promising strategy to impair tumour proliferation and metastasis. We are therefore excited by our results as NME6 represents a novel node by which we can manipulate mitochondrial gene expression. Our next aim is to target tumours with a particularly high demand on mitochondrial activity and to test if NME6 levels are limiting for tumour progression.

COMPUTATIONAL BIOLOGY

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Boyu Yu

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

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.

Eva Freckmann a postdoc in the group is interested in regulatory sequences embedded within coding sequences, and how mutations and changes in the regulatory machinery in and around these regions can impact on protein levels. Boyu Yu, a graduate student co-supervised with the RNA and Translational Control in Cancer Group, led by Martin Bushell, 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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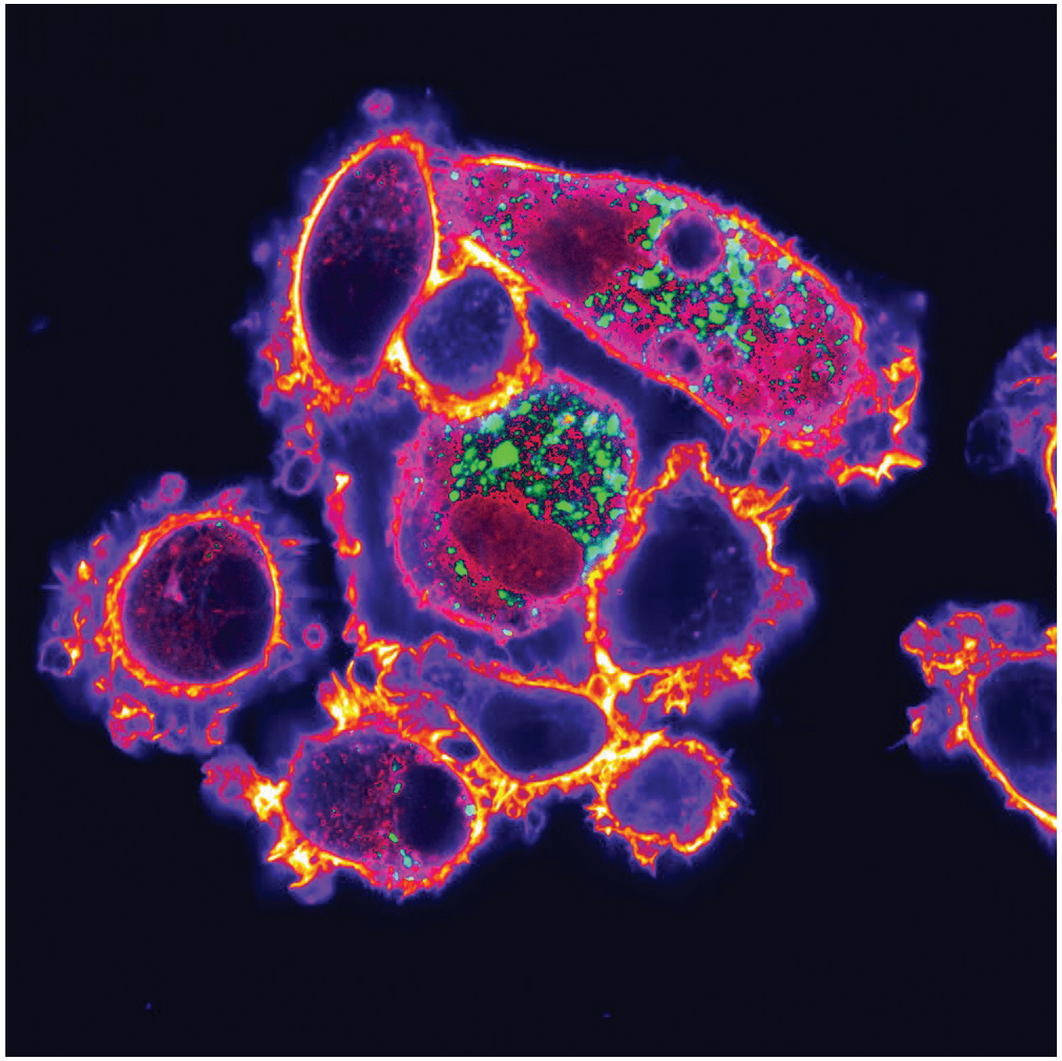

PC3 prostate cancer cell line plated in 2D, fixed and then stained with Alexa Fluor 568 Phalloidin (F-actin, shown in FIRE LUT) and anti-ARF3 (ARF GTPase shown in green).

Image by Emma Sandilands

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PRECLINICAL PANCREATIC CANCER LAB

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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.

Research has helped improve our understanding of pancreatic cancer evolution, genetic alterations, transcriptional subtypes, and the tumour microenvironment (TME). 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 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 with prominent myeloid cell infiltration and a dearth of effector T cells. Each component plays an important role in pancreatic cancer progression, influencing tumour cell proliferation and survival, metabolism, migration, immune surveillance, and response to chemotherapy (Candido *et al.,* 2018, *Cell Reports*; Steele e*t al.,* 2016,*Cancer Cell*; Vennin *et al.,*2018, *Gastroenterology*). Therefore, another aim of our work is to understand 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.

Modelling genetic subsets of patients

We have developed several models to mimic patients with mutations that may be actionable, to identify and to test therapeutic targets. Our collection of models covers many genes/pathways identified in the patient tumours. For example, *RNF43*, the gene encoding ubiquitin E3 ligase ring finger 43, has been shown to be mutated in 10-15% of cases of metastatic pancreatic cancer (Jiang *et al.,* 2013 *PNAS*). Using KPC mice as a backbone (Hingorani *et al.,* 2005, *Cancer Cell*), we have developed a genetically engineered mouse model of *Rnf43* deletion and found that *Rnf43* deletion is a strong driver of pancreatic cancer progression, with loss of even a single copy sufficient to significantly accelerate tumour progression. Mutations in DNA damage repair genes have also been reported in ~15% of pancreatic cancers (Aguirre *et al.,* 2018, *Cancer Discovery*). We have developed models of these patients, by deleting *Atm* or *Brca1* in KPC mice, and found differential sensitivities to DNA damaging agents and notably divergent immune microenvironments. We are now extending these studies to include radiotherapy, as these mutations may render tumours more sensitive to radiation. The use of radiotherapy in pancreatic cancer treatment has been limited thus far, however, this may be due to a lack of understanding of the effects of radiation on the pancreatic TME. Irradiation results in tumour cell death that can elicit a T cell response against the tumour, however, increased inflammation and fibrosis may result in an even more tumour-supporting, immunosuppressive microenvironment. Thus, we are investigating signalling in the TME to identify therapeutic combinations that could promote anti-tumorigenic immune responses while inhibiting pro-tumorigenic immune and fibrotic responses.

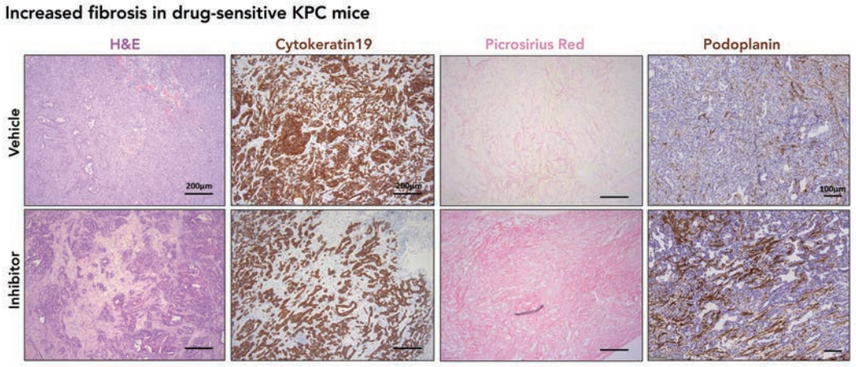

Figure 1
Representative Haematoxylin & Eosin (H&E) staining, cytokeratin 19 immunohistochemistry (IHC) for tumour cells, picrosirius red staining for collagen I, and podoplanin IHC for fibroblasts, in vehicle or inhibitor treated pancreatic tumour-bearing KPC (KrasG12D; p53R172H, Pdx1-Cre) mice, demonstrating increased fibrosis in treated tumours.

Therapeutic Resistance

By far the most common event driving pancreatic tumorigenesis is KRAS mutation. Previously believed to be "undruggable", the advent of mutant *KRAS* inhibitors has the potential to be transformative in this disease, particularly now that inhibitors are in development 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, together with results using KRAS inhibitors in other tumour types, suggested that resistance can develop quickly. In pancreatic cancer, the stroma can drive drug resistance, and we have found that drugs targeting RAS signalling can cause microenvironmental changes associated with acquired resistance. Indeed, most tumours relapsed quickly, and displayed elevated fibrosis, enhanced extracellular matrix deposition, and intriguingly, a re-wiring of signalling in the microenvironment (Figure 1). We are now investigating how signalling within the TME can help tumour cells to adapt to therapeutic intervention and influence the response to treatment. Tumour and stromal compartments display both significant heterogeneity in terms of gene expression and function, for example, discrete populations of cancer-associated fibroblasts with distinct expression profiles 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 need to investigate signalling within individual cell types (e.g., Figure 2), but also spatially link molecular changes to therapeutic responses. Building a comprehensive understanding of the relationships between signalling pathways, tumour cells and the TME following therapeutic intervention will allow us to identify the best strategies to overcome resistance.

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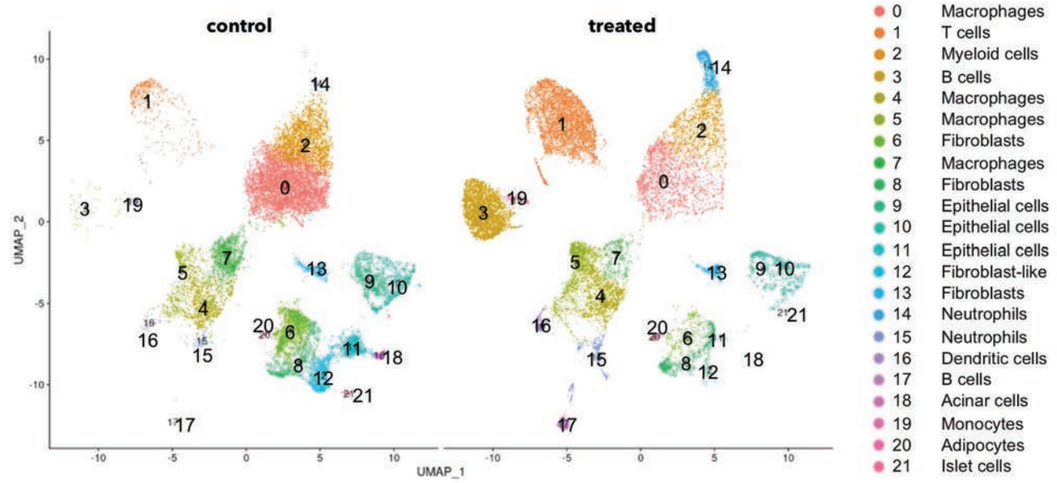

Figure 2
Example of Uniform Manifold Approximation and Projection (UMAP) of single cell RNAseq data from control and treated tumour-bearing KPC mice.

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MYC-INDUCED VULNERABILITIES/THORACIC CANCER RESEARCH

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

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

MYC in cancer

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

MYC and KRAS drive immune evasion

How tumours evade detection by the immune system defines the underlying principle behind the therapeutic success of immunotherapy across a spectrum of cancer types. MYC is known to induce expression of PD-L1, which inactivates cytotoxic T cells upon binding to PD1, but new data from multiple labs, including ours, indicated that PD-L1 expression is not the sole immune evasion strategy deployed by MYC. In 2020, we showed that MYC and KRAS combined 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 restored 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. My 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 2022

Building upon our growing strength in mesothelioma research, the year saw further successful bids for competitive funding, in the form of co-leadership of a CRUK Discovery Programme "REMIT: Reconstructing the *in vivo* Evolution of Mesothelioma for Improved Therapy" and positioning of Mesothelioma as one of six cancer themes in the CRUK Scotland Centre. We commenced work on our ambitious early detection of mesothelioma programme, IAMMED-Meso, following the extension of postdoc Pooyeh Farahmand, and the recruitment of new PhD students Danielle McKinven & Xinya Hong, along with Research Assistant, Nicola Brady. As contributors to the MRC National Mouse Genetics Network - Cancer Cluster, we also continued our work on generating a new multi-drug inducible Tandem Arrayed Regulator (TAR) allele to enable modelling sequential genetic events in mice, in contrast to current all-at-once model systems, with postdoc Sarah Laing extending her tenure in the lab to continue her work on disease positioning of immune-visible models of Lung Adenocarcinoma. These next generation models will provide exceptional platforms for further investigation of tumour progression and the dynamic interactions with anti-cancer immune responses. Postdoc George Skalka's efforts were rewarded with a 1-year funded project extension from Merck in collaboration with Cancer Research Horizons.

The year saw a welcome return to in-person conference attendance and members of the lab presented posters and/or spoke at the European Workshop on Cell Death (Fiugi Italy), EACR (Seville, Spain), CSHL Mechanisms & Models of Cancer (USA), the 5th European Workshop on AMPK & related kinases (Clydebank), and the CRUK Lung Cancer Conference (Manchester). Mice generated in the lab featured in 1 pre-print and 1 publication arising from collaborations with the labs of Beatson colleagues, Tom Bird & Saverio Tardito, while our expertise on MYC contributed to a further 2 publications with colleagues Hing Leung (Uni Glasgow) and Daniel Schramek (Uni Toronto). Former PhD student Declan Whyte submitted his manuscript on centrosome regulation by NUAK1 for publication.

We finally wish to thank local charity, Action on Asbestos, for their generous donation of £10,000 towards the cost of ongoing work on Mesothelioma. Their support is very much appreciated.

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INTEGRIN CELL BIOLOGY

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Although carcinoma initiation is driven by oncogenic mutations occurring in epithelia, cells bearing mutated oncogenes do not necessarily progress to form tumours. It is now clear that the microenvironment which surrounds mutated epithelial cells is key to determining the likelihood and rate at which they form malignant tumours, or whether they remain quiescent. Oncogenes are known to re-programme the cell's ribosomes, or protein synthesis machinery, and we have found that the way in which this occurs influences whether mutated cells remain quiescent or start growing. Moreover, this is because ribosome function has profound, and sometimes paradoxical, effects on the extracellular matrix components of the microenvironment. Importantly, our findings indicate that re-programming of ribosome function can engender fibrotic microenvironments and thus influence the ability of epithelial cells bearing oncogenic mutations to start growing uncontrollably. Therefore, it is important to understand how protein synthesis inhibitors affect extracellular matrix deposition before deploying these agents to target cancer in the clinic.

The translational repressor, eIF4A2 regulates protein synthesis landscapes to support fibrotic niche generation and hepatocellular carcinoma initiation - *an active collaboration with the Bushell, Sansom, and Bird laboratories*

To enable new strategies to target tumour growth, the Institute has an ongoing programme to map protein synthesis landscapes associated with cancer initiation and progression. We are, therefore, developing approaches to disrupt – genetically and pharmacologically – the protein synthesis machinery and determine how this influences outcomes in pre-clinical models of cancers, including hepatocellular carcinoma (HCC) and lung adenocarcinoma.

Translation of mRNA by ribosomes is a key event in protein synthesis, and translation initiation is mediated by the eIF4F complex. Integral to the eIF4F complex are the eIF4A helicases which are responsible for unwinding the 5'-UTR of mRNAs, thus influencing the ability of ribosomes to scan along mRNAs and find the 'translation start' codon to initiate translation and protein synthesis. Importantly, there are two eIF4A paralogues – eIF4A1 and eIF4A2 - and these are known to exert opposing effects on translation initiation. Whilst eIF4A1 is a 'classical' activator, eIF4A2 can act as a translational repressor by associating with the CCR4-NOT complex to mediate RNA degradation and shut-down of protein synthesis. Therefore, to determine how activation and repression of translation initiation might respectively influence cancer outcomes, we generated mice with conditional knockout (floxed) alleles of eIF4A1 and eIF4A2, and crossed these with a genetically-engineered mouse model of HCC which is driven by hepatocyte-specific expression of the β-catenin and cMyc oncogenes. Surprisingly, tumour cell-specific knockout of eIF4A2 (translation initiation repressor) markedly delayed the onset of β-catenin/Myc-driven HCC - leading to extended survival. By contrast, eIF4A1 (translation initiation activator) knockout affected neither tumour onset, nor survival from HCC.

To determine how mRNA translation initiation rates influence tumour onset and tumour-associated alterations to the liver microenvironment, we monitored changes in the liver shortly following activation of

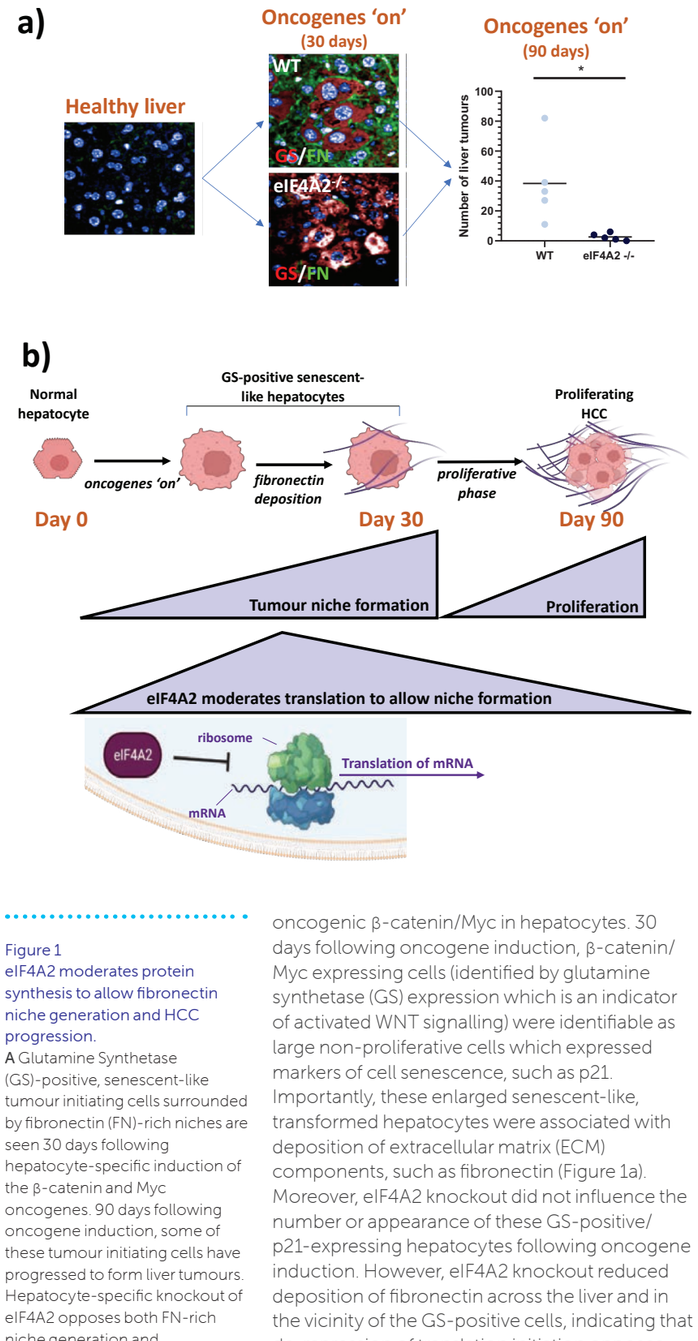

Figure 1
eIF4A2 moderates protein synthesis to allow fibronectin niche generation and HCC progression.
A Glutamine Synthetase (GS)-positive, senescent-like tumour initiating cells surrounded by fibronectin (FN)-rich niches are seen 30 days following hepatocyte-specific induction of the β-catenin and Myc oncogenes. 90 days following oncogene induction, some of these tumour initiating cells have progressed to form liver tumours. Hepatocyte-specific knockout of eIF4A2 opposes both FN-rich niche generation and establishment of liver tumours. **B** Oncogene activation drives increased translation, but this is moderated by the translational repressor, eIF4A2. This moderated level of mRNA translation fosters fibronectin-rich niche generation which allows senescent-like, growth-arrested tumour initiating cells to enter a proliferative phase to form hepatocellular carcinoma (HCC).

oncogenic β-catenin/Myc in hepatocytes. 30 days following oncogene induction, β-catenin/Myc expressing cells (identified by glutamine synthetase (GS) expression which is an indicator of activated WNT signalling) were identifiable as large non-proliferative cells which expressed markers of cell senescence, such as p21. Importantly, these enlarged senescent-like, transformed hepatocytes were associated with deposition of extracellular matrix (ECM) components, such as fibronectin (Figure 1a). Moreover, eIF4A2 knockout did not influence the number or appearance of these GS-positive/p21-expressing hepatocytes following oncogene induction. However, eIF4A2 knockout reduced deposition of fibronectin across the liver and in the vicinity of the GS-positive cells, indicating that de-repression of translation initiation opposes formation of fibrotic niches in the vicinity of recently transformed-but-not-yet-proliferative tumour initiating cells.

The assembly of soluble fibronectin into fibrils and its subsequent deposition to form insoluble/fibrotic ECM is strongly dependent on α5β1 integrin - the cell's main fibronectin receptor. We, therefore, deployed mice with floxed alleles of α5 integrin (ITGA5) to determine whether the ability of early tumour initiating cells to assemble fibronectin-rich niches might contribute to their progression to actively proliferating tumour nodules. Hepatocyte-specific ITGA5 knockout opposed fibronectin deposition following oncogene-induction, indicating that expression of α5β1 integrin by hepatocytes has a key role in generating local fibrotic microenvironments in the liver. Importantly, hepatocyte-specific knockout of ITGA5 delayed appearance of actively proliferating tumour nodules in the liver and extended survival from HCC to a similar extent as did eIF4A2 knockout. This indicated that levels of translation initiation dictated the progression of HCC by influencing α5β1 integrin function and the ensuing generation of fibronectin-rich niches in the liver.

To determine whether the increased level of translational activity resulting from eIF4A2 knockout is responsible for opposing the generation of fibrotic tumour initiation niches, we used rapamycin. Rapamycin is a drug with well-established ability to reduce mRNA translation indeed we have found it to oppose the elevated protein synthesis evoked following eIF4A2 knockout. Furthermore, because we are interested in focussing on how protein synthesis landscapes influence tumour initiation (as opposed to growth of established tumours), we administered rapamycin to mice 5 days following oncogene induction, and ceased treatment 25 days later. Administration of rapamycin in this time-window reversed the ability of eIF4A2 knockout to suppress the generation of fibronectin-rich niches associated with GS-positive, oncogene-transformed (but not-yet-proliferative) tumour initiating cells and, correspondingly, restored the ability of these cells to progress to form HCC tumour nodules.

It is now established that one of the ways in which oncogenes drive tumour growth is via their ability to promote mRNA translation and protein synthesis. Thus, agents (such as rapamycin) that oppose oncogene-driven translation, and signalling events connecting oncogenes to the protein synthesis machinery, are being evaluated as potential anti-tumour therapies. However, the present study, which involves extensive collaborative interactions between four groups at the Institute, has used sophisticated *in vivo* cancer models to demonstrate how *increased* protein synthesis can *oppose* generation of tumour initiation niches at early stages in the genesis of HCC. Therefore, we submit that it is necessary to fully evaluate the (sometimes unpredictable) consequences of protein synthesis inhibition on the microenvironment during early tumour establishment as agents targeting the mRNA translation machinery and its upstream signalling advance toward the clinic.

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IMMUNE PRIMING AND THE 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 tumour microenvironment coordinates its immune composition and how it communicates with 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.

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 aim to identify which signals attract DC precursors to migrate into the tumour. We have identified trafficking receptors on precursor DCs and are generating an assay to screen receptors individually and in combination to identify those required for DC entry to both tumours and sites of infection. We will then determine which cells are producing the signals drawing in the DC precursors both during viral infection, where immune responses are robust, and in the tumour, where the response is sub-optimal. We will finally seek to understand what induces expression of these signals and attempt to increase DC recruitment to the tumour in order to improve both initial priming in the lymph node and to augment repriming at the tumour site.

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, however, this protein is handed off to lymph node resident cells and we have shown that these stimulate T cell proliferation sub-optimally (Figure 2). We have identified a subset of DCs which are responsible for the transport and transfer of antigen to the lymph node and are now seeking to understand how this process is controlled. Furthermore, by studying this process in a viral setting we have determined a key communication channel between tissues and their lymph nodes which inform the behaviour of lymph node resident cells. In the tumour setting this process is co-opted and leads to the transfer of DC dysfunction from the tumour to the lymph node.

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 aim to use these approaches to identify organisational defects which occur in the context of tumour development which affect the quality of the anti-tumour immune response.

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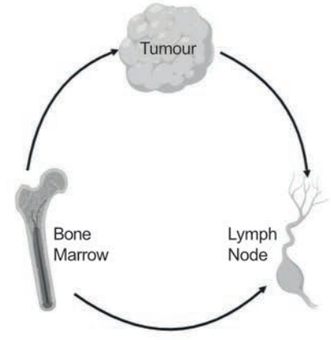

Figure 1
The DC lifecycle
DC precursors develop in the bone marrow and migrate to the tumour and the lymph node. Once within the tumour they sample proteins from the microenvironment and then mature and migrate to the lymph node. There the DC which migrated straight to the lymph node and those which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

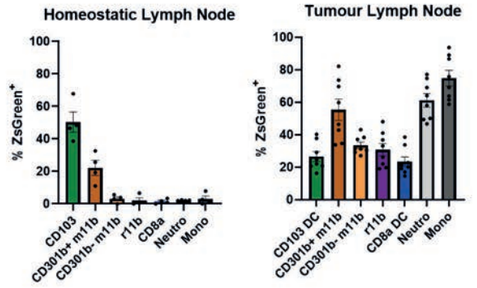

Figure 2
Tumour antigen is handled uniquely
ZsGreen expressed within the lung is carried to the lymph node by migratory DC, but the protein remains restricted to the migratory DC. When the same protein is expressed in a tumour, the protein is carried to the lymph node by migratory DC in a similar fashion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

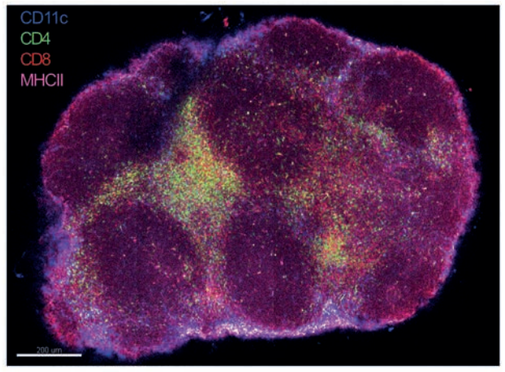

Figure 3
Lymph node organisation
A whole cleared lymph node stained for T cell, B cell and DC markers shows the organisation of a lung tumour-draining lymph node.

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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 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 characterised form of autophagy, and the focus of our work, is macroautophagy, simply referred to as autophagy. The process is characterised by the formation of unique double-membraned vesicle, termed the autophagosome. 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 catabolised 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 destabilise tumour growth and viability by interfering with autophagy, it is imperative to understand how and at what stages in different tumour types autophagy ceased to be tumour suppressive and switches role to support tumour growth and preservation, so we can decide on the appropriate intervention.

The complex role of autophagy in cancer development

Previous work by our lab, showed that p53 tumour suppressor status could determine how autophagy affected the development of pancreatic ductal adenocarcinoma (PDAC) (*Nature*, 2013). These previous studies involved activation of mutant Ras, and deletion of p53 and essential autophagy genes *in utero*, and while this is common in mouse models of cancer, it did not best recapitulate the progression of PDAC in human. In addition, in human PDAC, p53 is rarely deleted, but if often retained, but mutated. As a result, we decided to test the involvement of autophagy in a system that was more in line with normal PDAC development and utilised a tamoxifen inducible Cre recombinase to cause more focal activation of mutant Ras, mutant p53 and impairment of autophagy in adult mice. This revealed, similar to what we had observed in mice that had gene recombination *in utero* that deletion of the essential gene *Atg7*, resulted in a higher percentage of mice development PDAC, as well as pre-cancerous lesions (*PNAS* 2022). To our surprise, however, we also found that hemizygous deletion of *Atg7* also resulted in enhanced tumour development, which was not expected, as loss of one allele of *Atg7* should not, and we found did not, ablate autophagy.

We were intrigued by these observations as this indicated that ATG7 had roles in tumour development beyond its role in autophagy. Because $Atg7^{+/-}$ mice were autophagy competent, they did not undergo the pancreatic destruction observed upon loss of autophagy in $Atg7^{-/-}$ mice. This enabled us to study the further progression of mutant Ras- and mutant p53-driven PDAC tumours in $Atg7^{+/-}$ animals; and we were again surprised to observe that loss of one allele of *Atg7* in PDAC driven by mutant Ras and mutant p53 reduced the number of mice with PDAC metastasis when compared to *Atg7* wild-type animals. This therefore indicated that it could potentially be possible to partially inhibit Atg7 function to inhibit metastasis, while circumventing the detrimental effects of inhibition of autophagy in the rest of the body.

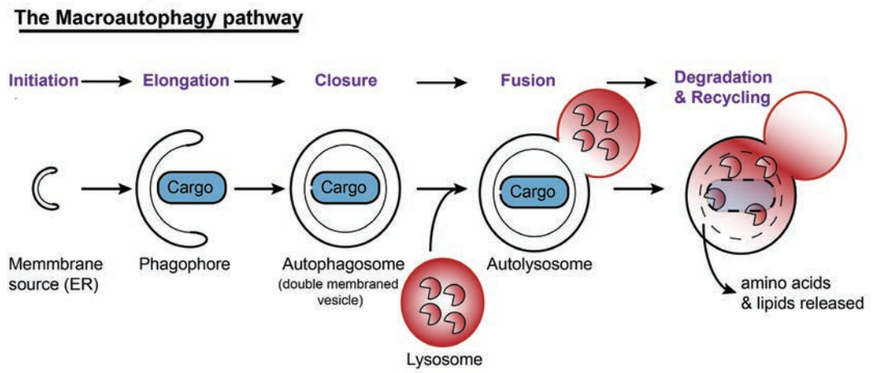

Figure 1
The autophagy pathway
The autophagy pathway is a lysosomal degradation pathway that the cell utilises to maintain cellular homeostasis and acts as an adaption mechanism for dealing with stress. Proteotoxic stress (accumulation of aggregates), reactive oxygen species (ROS), infection and nutrient depravation (starvation) all act to initiate (1) the formation of the double membrane structure, termed the autophagosome. After initiation, the phagophore undergoes an expansion (2) to engulf cellular cargo (including pathogens, damaged organelles and protein aggregates) prior to sealing (3) and closure to form the autophagosome. This then fuses (4) with the lysosome, to form the autolysosome where the contents and inner membrane are degraded (5) and recycled (6) back to the cell as basic 'building blocks' such as amino acids, lipids and other nutrients.

Identification of novel autophagy regulators

It is undisputed that autophagy has a role in the prevention of tumour development, but also in the maintenance of established tumours. As a result, we have a constant quest to identify autophagy regulators that have either a selective or a metered impact on autophagy, which potentially could be targeted therapeutically. Our entry into the autophagy field began with our discovery of the Damage-Regulated Autophagy Modulator (DRAM) as a target gene of p53 (*Cell* 2006). DRAM, now renamed DRAM-1, was subsequently found to be a member of a family, which has 5 members in human. We previously characterised DRAM-2 and DRAM-3 and more recently, we turned our interest to DRAM-4 and DRAM-5. We found that different to DRAM-1, DRAM-4 and DRAM-5 are not induced by p53 but were instead induced by nutrient deprivation. Nonetheless, we found that over-expression of either protein, like DRAM-1, resulted in induction of autophagy. Seemingly paradoxically, however, CRISPR-mediated deletion of DRAM-4 also resulted in induction of autophagy. We found, however, that deletion of DRAM-4 caused compensatory up-regulation of DRAM-5, which induced autophagy. The consequence of these effects was revealed when we examined cell survival upon nutrient deprivation. Deletion of DRAM-4 promoted cell survival upon deprivation of amino acids, glucose or serum. This effect was, however, completely reversed by concomitant deletion of DRAM-5, highlighting new inter-connected players in the regulation of nutrient-deprived conditions as occurs in the development of most solid tumours.

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Figure 2
Gene dosage of Atg7 regulates tumour development and metastasis
Studies of the essential autophagy gene Atg7 in the development of PDAC revealed autophagy-independent effects caused by its hemizygous deletion. This mono-allelic loss did not inhibit autophagy, but enhanced PDAC tumour development and conversely resulted in fewer mice with metastatic disease.

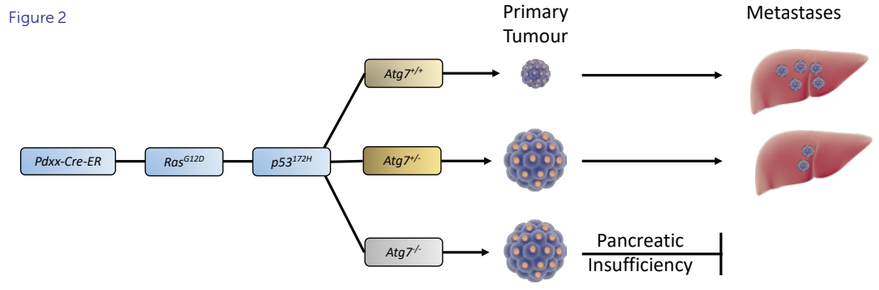
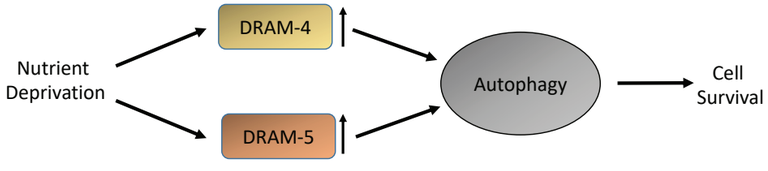


Figure 3
DRAM-4 and DRAM-5 regulate cell survival in response to glucose deprivation
DRAM-4 and DRAM-5 are novel autophagy regulators that are related to DRAM-1, and which are induced by nutrient deprivation. Over-expression of DRAM-4 or DRAM-5 induces autophagy. In addition, deletion of DRAM-4 also induces autophagy to promote cell survival under nutrient deprivation, but this occurs through compensatory up-regulation of DRAM-5.



COLORECTAL CANCER AND WNT SIGNALLING

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Colorectal cancer (CRC) is a heterogeneous disease comprising distinct molecular subgroups that differ in their histopathological features, prognosis, metastatic propensity, and response to therapy. Utilising state-of-the-art preclinical models harbouring key driver mutations, our group is interrogating the molecular mechanisms underpinning CRC. Our overarching goals are to identify early-stage diagnostic biomarkers and develop stage- and subtype-specific targeted therapies.

Most colorectal tumours develop from benign adenomas through the adenoma–carcinoma pathway, typically entailing aberrant activation of Wnt signalling, with loss-of-function mutations in the negative Wnt-regulator APC sufficient for adenoma formation. Progression to adenocarcinoma is underpinned by the accumulation of compounding mutations in oncogenic and tumour-suppressive pathways, including KRAS, TP53, and TGFβ. In this past year, we have developed tractable models of CRC ranging from early-stage adenomas through to treatment-refractory, *KRAS*-mutant CRCs, with *ex vivo* organoid cultures adding value to our suite of *in vivo* models.

The Wnt-antagonist NOTUM is a druggable mediator of cell competition in early-stage CRC

Widespread screening can detect tumours at early stages amenable to therapeutic intervention, rendering an urgent need for the validation of early-detection biomarkers and the identification of druggable targets to prevent progression of early-stage disease. We therefore sought to understand how common initiating mutations impact the dynamics of adenoma formation.

Given that the inactivation of the tumour suppressor APC is a frequent early event in adenoma initiation, we sought to identify how *Apc*-mutant intestinal stem cells (ISCs) compete with their wild-type neighbours to achieve clonal dominance and fixation (Flanagan *et al.*, 2021, *Nature*). Using gene expression profiling, we found that APC-deficient adenomas expressed an abundance of transcripts for several secreted Wnt antagonists, relative to APC-proficient tissues, with the most highly upregulated gene, *Notum*, encoding a secreted WNT deacylase that disrupted WNT ligand-binding (Figure 1A). Culture of wild-type organoids in conditioned medium, collected from *Apc*-mutant cells, curtailed growth (Figure 1B), decreased the expression of ISC-associated genes, and induced differentiation. Addition of a NOTUM inhibitor (Figure 1B), or genetic deletion of *Notum* in *Apc*-mutant organoids, abolished the effects of the conditioned medium.

In VilCre^{ER}*Apc*$^{Min/+}$ mice, genetic or pharmacological inhibition of NOTUM compromised the ability of *Apc*-mutant cells to expand and form intestinal adenomas, significantly prolonging survival (Figure 1C).

Deletion of *Notum* in *Apc*-mutant *Lgr5*-ISCs impaired their ability to outcompete wild-type counterparts. Interestingly, wild-type *Lgr5*-ISCs in the vicinity of *Apc*-mutant cells exhibited reduced expression of the WNT-regulated stemness marker SOX9, whereas cells adjacent to *Apc*-mutant *Notum*^{cKO} cells retained robust levels of SOX9, consistent with a role for secreted NOTUM in driving the differentiation of wild-type *Lgr5*-ISCs. Secreted NOTUM could therefore act in a paracrine fashion to inhibit Wnt signalling in neighbouring non-transformed wild-type ISCs, inducing their differentiation and withdrawal from the cell cycle, and ultimately driving their removal from the stem cell pool (Figure 1D). By contrast, WNT ligand-independent, APC-deficient, super-competitor cells could expand unabated with their progeny taking over the entire intestinal crypt.

Our findings identified NOTUM as a druggable mediator of cell competition and mutation fixation during the early stages of adenoma development. Bolstering the fitness of wild-type ISCs by inhibiting NOTUM might serve as a viable approach for preventing progression of early-stage disease in high-risk individuals with hereditary CRC.

Epithelial TGFβ/ALK5 engages growth-factor signalling to drive intestinal tumourigenesis with aggressive features

Building on our work with early-stage CRCs, we sought to develop means to identify so-called born-to-be-bad CRCs that are endowed with inherent metastatic potential, which enables metastasis-founder cells to disseminate before the primary tumour is clinically detectable. Indeed, histological assessment alone fell short of reliably identifying early-stage aggressive lesions destined to progress to metastatic spread. By transcriptionally profiling an early-stage human CRC cohort, enriched for born-to-be-bad lesions that went on to relapse, we correlated aggressive traits with elevated epithelial cell-intrinsic—rather than stromal—TGFβ signalling (Figures 2A–2C), alongside oncogenic *KRAS* mutations and APC deficiency (Flanagan *et al.*, 2022, *Nature Comms*).

We therefore generated VilCre^{ER}*Apc*$^{fl/+}$*Kras*$^{G12D/+}$*Alk5*^{CA} mice, where the *Alk5*CA allele encoded a constitutively active form of the TGFβ/ALK5 receptor, which instigated downstream TGFβ signalling in the intestinal epithelium. We found that, in the presence of concurrent *Apc* and *Kras* mutations, epithelial-specific activation of TGFβ signalling elicited rampant acceleration of intestinal tumourigenesis, engendering dissemination-prone tumours with born-to-be-bad transcriptomic features. Mechanistically, epithelial TGFβ signalling induced a growth-promoting EGFR-signalling module that synergised with mutant APC and KRAS to drive MAPK signalling, sensitising tumour cells to MEK and/or EGFR inhibitors and significantly prolonging survival (Figure 2D). Our data suggested that the convergence of activated Wnt, MAPK, and TGFβ/ALK5 signalling drove mitogenic and survival pathways that could be targeted therapeutically to slow the progression of intestinal tumours with aggressive behavioural traits.

Whereas tumour-suppressive roles are often ascribed to epithelial TGFβ signalling in CRC, our study found that epithelial cell-intrinsic TGFβ/ALK5 activation synergised with Wnt and MAPK signalling to drive intestinal tumourigenesis. Indeed, we identified epithelial TGFβ/ALK5 signalling as a potentially actionable, predictive biomarker in poor–prognosis, dissemination-prone early-stage CRCs that could reliably identify at-risk patients, offering an opportunity for early therapeutic intervention at a potentially curable stage. These findings were in line with the "Big Bang" model of CRC progression, which predicts that pro-invasive behaviour could be installed early in the disease trajectory. Overall, we identified epithelial TGFβ signalling both as a determinant of early dissemination and a potential therapeutic vulnerability of CRCs with born-to-be-bad traits.

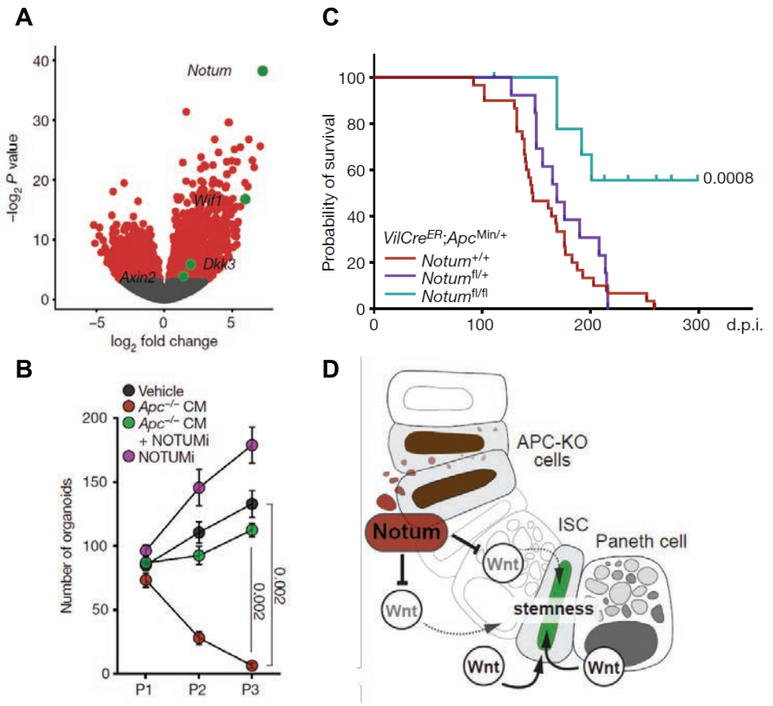

Figure 1
NOTUM is a prospective target for APC-deficient adenomas.
A Volcano plot showing genes differentially expressed between VilCre^{ER}*Apc*$^{fl/+}$ tumour tissue (n=5) and wild-type small intestine (n=3). Red, significantly altered genes; Green, Wnt antagonists. **B** Number of organoids formed over multiple passages (P1, P2, and P3) during culture in wild-type or *Apc*–/– conditioned medium (CM) supplemented with NOTUM inhibitor (NOTUMi). n = 6 mice per condition. **C** Survival of VilCre^{ER}*Apc*$^{Min/+}$*Notum*$^{+/+}$ (n=30), VilCre^{ER}*Apc*$^{Min/+}$*Notum*$^{fl/+}$ (n=13), and VilCre^{ER}*Apc*$^{Min/+}$*Notum*$^{fl/fl}$ (n=9) mice aged until clinical endpoint. **D** Schematic depicting the proposed model of NOTUM-mediated Wnt-pathway inhibition of wild-type ISCs (green) by *Apc*-mutant cells (brown). Curved arrows indicate activation; blunt-ended arrows indicate inhibition; dotted curved arrows indicate attenuation of Wnt activity.

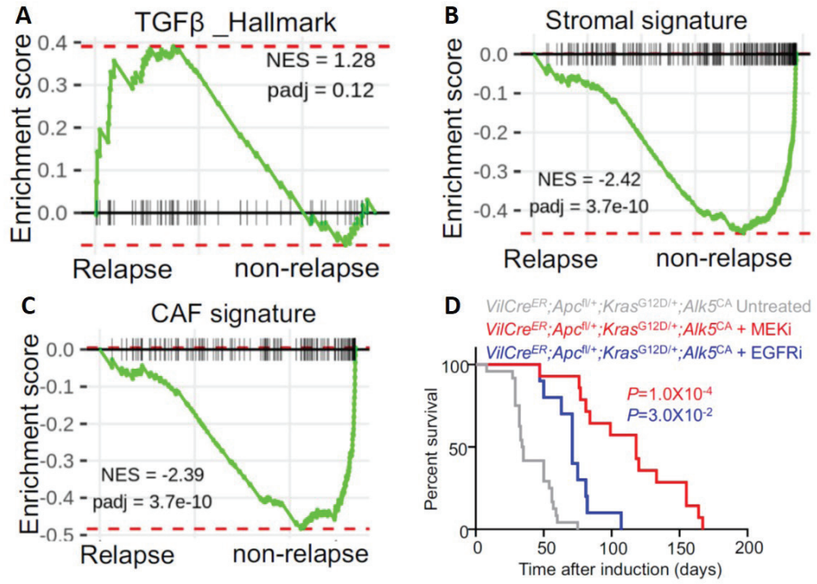

Figure 2
Epithelial TGFβ/ALK5 signalling—but not stromal content—correlates with relapsing, early-stage CRCs and sensitises to MAPK-targeted therapies.
A Hallmark gene set enrichment of TGFβ signalling in relapse cases compared with non-relapse samples. **B, C** Negative enrichment of cancer-associated fibroblast (CAF) (**B**) and stromal (**C**) signature gene sets in relapse cases compared with non-relapse samples. A–C were performed using fast gene set enrichment analysis. Benjamini–Hochberg FDR < 0.2. NES, normalised enrichment score; FDR, false discovery rate; padj, adjusted *P*-value (Benjamini–Hochberg multiple testing). **D** Kaplan–Meier survival curves for VilCre^{ER}*Apc*$^{fl/+}$*Kras*$^{G12D/+}$*Alk5*^{CA} mice treated daily with MEK1/2 inhibitor (MEKi) or EGFR inhibitor (EGFRi) and aged until clinical endpoint following tamoxifen induction. n=24 untreated (grey), n=14 MEKi (red), n=10 EGFRi (blue) mice. *P*=1.0 × 10^{-4} (MEKi), *P*=3.0 × 10^{-2} (EGFRi); log-rank (Mantel–Cox) test.

Kras allelic imbalance drives MAPK-dependent tumour initiation but sensitizes to MEK inhibition and fails to evoke metastasis

As mentioned above, oncogenic mutations in *Kras* lead to the constitutive activation of downstream effector pathways, including the MAPK-signalling cascade, and cooperate to drive colorectal tumourigenesis alongside loss of the tumour suppressor *Apc*. Oncogenic KRAS is strongly associated with therapy resistance, particularly to treatments targeting upstream or downstream signalling nodes such as EGFR, MEK, PI3K, and mTOR. Prevailing dogma holds that KRAS is a potent oncogene, with the gain of one mutant allele dominant over the remaining wild-type copy. As such, most studies to date have focused on the gain-of-function traits of oncogenic KRAS. However, accumulating evidence has argued for the existence of selective pressures that further augment oncogenic signalling through allelic imbalances that engender either focal amplifications of oncogenic *KRAS* or loss-of-heterozygosity at the wild-type allele. This implies that wild-type KRAS can influence the function of oncogenic KRAS. Yet, the role of wild-type KRAS, in the context of oncogenic KRAS, remains controversial with both pro- and anti-tumourigenic roles ascribed. We sought to better understand how wild-type KRAS impacts the fitness and drug responsiveness of CRCs, harbouring oncogenic KRAS, and to ascertain its impact on the tumour initiation and progression of *KRAS*-mutant tumours (Najumudeen *et al., in preparation*).

Towards this aim, we developed genetically engineered mouse models, which allowed the deletion of wild-type *Kras* in the context of oncogenic *Kras*^{G12D} in the phenotypically normal premalignant intestinal epithelium, the crypt-progenitor phenotype induced by acute *Apc* loss, long-term APC-deficient tumour development, and the metastatic setting. In the homeostatic small-intestinal epithelium, we found that mutant KRAS^{G12D} increased MAPK signalling, promoting enterocyte proliferation and suppressing Paneth-cell differentiation, with the deletion of the wild-type allele exacerbating these phenotypes and additionally increasing the abundance of secretory goblet cells, suggesting that wild-type KRAS restrains the activity of oncogenic KRAS^{G12D} in the premalignant setting. We further found that deletion of wild-type *Kras* potentiated oncogenic KRAS^{G12D} activity and downstream MAPK signalling, increasing the capacity of KRAS^{G12D}-mutant APC-deficient cells to dedifferentiate and initiate tumourigenesis, suggesting that wild-type KRAS functions as a tumour suppressor in the presence of oncogenic KRAS^{G12D}. In turn, however, this rendered tumours addicted to oncogenic KRAS signalling and conferred enhanced sensitivity to MEK inhibition, unveiling an exploitable therapeutic vulnerability (Figure 3A). Conversely, the presence of wild-type KRAS rendered KRAS^{G12D}-driven tumours resistant to MEK1/2 inhibition (Figure 3A) by dampening their dependence on MAPK signalling, posing a major clinical challenge. Importantly, deletion of

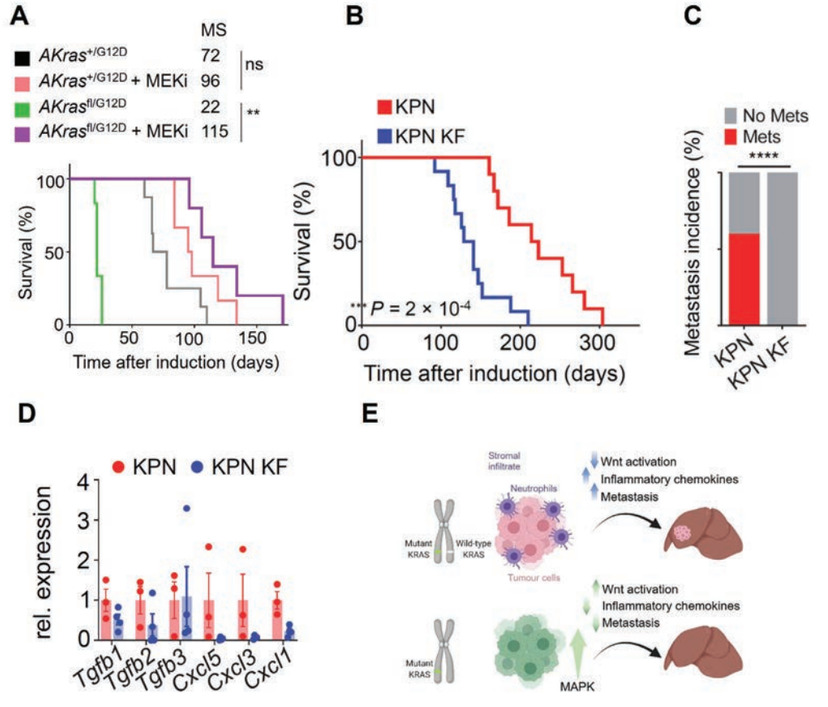

Figure 3
Loss of wild-type Kras increases sensitivity to MEK inhibition and suppresses the metastatic traits of *Kras*^{G12D} colorectal tumours.
A Kaplan–Meier survival curves for VilCre^{ER}*Apc*$^{fl/+}$*Kras*$^{+/G12D}$ (*AKras*$^{+/G12D}$) and VilCre^{ER}*Apc*$^{fl/+}$*Kras*$^{fl/G12D}$ (*AKras*$^{fl/G12D}$) mice, treated with MEK-inhibitor (MEKi) one day post tamoxifen-induction and aged until clinical endpoint. Median survival (MS) values are indicated. *Apc*$^{+/fl}$*Kras*$^{+/G12D}$, n=8; *Apc*$^{+/fl}$Kras$^{+/G12D}$ + MEKi, n=6; *Apc*$^{+/fl}$*Kras*$^{fl/G12D}$, n=6; *Apc*$^{+/fl}$*Kras*$^{fl/G12D}$ + MEKi, n=5.**P=0.0014, ns=not significant; log-rank (Mantel–Cox) test. **B** Kaplan–Meier survival curves for VilCre^{ER}*Kras*$^{+/G12D}$*Trp53*$^{fl/fl}$*Rosa26*$^{N1icd/+}$ (KPN) and VilCre^{ER}*Kras*$^{fl/G12D}$*Trp53*$^{fl/fl}$*Rosa26*$^{N1icd/+}$ (KPN KF) mice aged until clinical endpoint. KPN, n=10; KPN KF, n=12. Median survival (MS) values are indicated. ****P< 0.0001. KPN, n=10; KPN KF, n=12. **D** Relative expression of transcripts encoding *Tgfβ* ligands and chemokines in organoids derived from KPN and KPN KF tumours. KPN, n=3; KPN KF, n=4. **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.

wild-type *Kras* in oncogenic KRAS^{G12D}-driven, p53-mutant, aggressive tumours promoted initiation but significantly perturbed tumour progression and metastasis, reducing serrated morphological features, compromising invasiveness, and altering the tumour microenvironment. Furthermore, the loss of wild-type *Kras* significantly accelerated tumourigenesis and reduced survival (Figure 3B) in our aggressive NOTCH1-driven, KRAS-mutant intestinal adenocarcinoma model that metastasised to the liver (VilCre^{ER}*Kras*$^{+/G12D}$*Trp53*$^{fl/fl}$*Rosa26*$^{N1icd/+}$ and VilCre^{ER}*Kras*$^{fl/G12D}$*Trp53*$^{fl/fl}$*Rosa26*$^{N1icd/+}$ mice, designated KPN and KPN KF, respectively). Notably, however, loss of wild-type *Kras* in this model abrogated invasiveness and metastatic competence (Figure 3C). Molecularly, KPN KF tumours lacking wild-type KRAS exhibited significantly elevated Wnt-pathway activity and lacked expression of neutrophil chemoattractants (*Tgfβ2* and chemokines, such as *Cxcl1*, *Cxcl3*, and *Cxcl5*; Figure 3D) in their pre-metastatic niche, thereby blunting metastasis formation (Figures 3C and 3E). These studies provided new insights into KRAS biology and revealed a critical role for wild-type KRAS in the therapeutic resistance and metastatic proclivity of mutant KRAS-driven CRCs. These findings further suggested that, in addition to screening CRC-patients for *KRAS* mutation status, stratifying patients for *KRAS* allelic status might discern those who would derive benefit from inhibition of downstream effector signalling.

Publications listed on page 110

https://www.beatson.gla.ac.uk/colin_steele

ADVANCED COLORECTAL CANCER

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

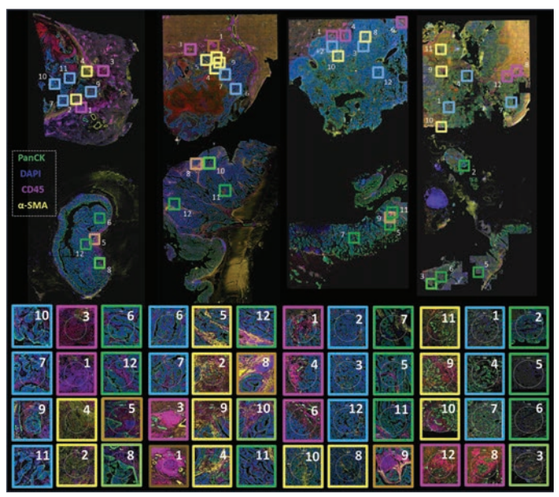
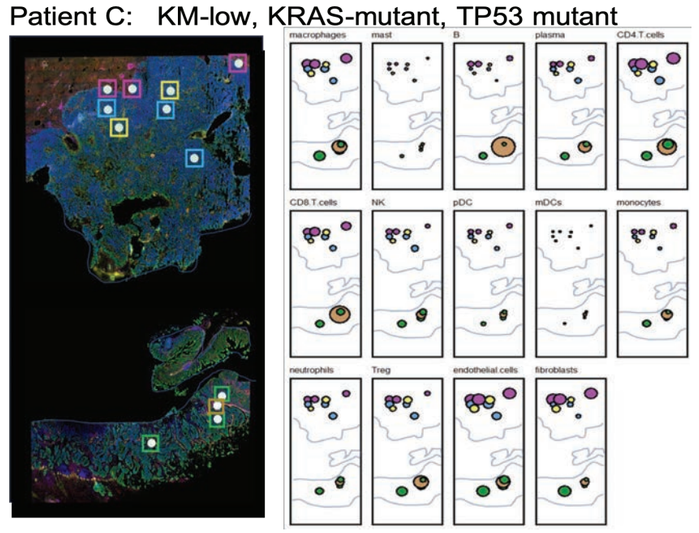
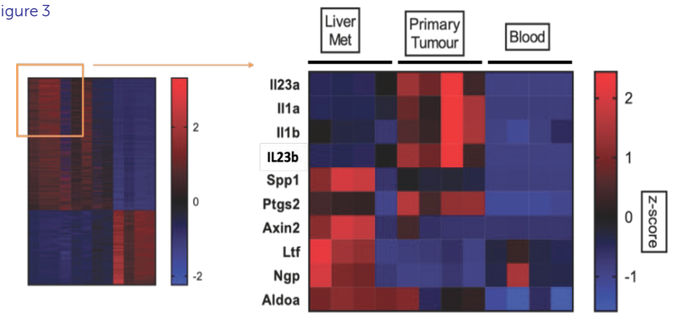


Figure 1
Transcriptomic assessment of heterogeneity, primary and metastatic sites in 4 patients.

Figure 2
Immunofluorescence. Pink CD45, Blue pan-CK, Yellow alpha-SMA. Immune cell deconvolution representing cellular component derived from transcriptome of each area.

Figure 3
Site specific differential gene expression within neutrophils





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 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.

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 (Figure 3). 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.

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 (Figure 2) 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 (*Submitted Cancer Research*), while patients with neutrophils surrounding metastatic disease had very poor survival following surgery.

Publications listed on page 113

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 ultimate 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 could trigger caspase-dependent DNA-damage having oncogenic effects. This occured through limited MOMP in a few mitochondria – what we termed minority MOMP. Nonetheless, why some mitochondria selectively permeabilised remained enigmatic. Kai Cao and Joel Riley set out to address this question finding that mitochondrial dynamics and function regulated minority MOMP. Mitochondrial fusion protected cells from sub-lethal apoptotic stress, whereas fission had the opposing effect. Moreover, we found that loss of mitochondrial function served as an intrinsic priming signal, sensitising mitochondria to permeabilization. By targeting mitochondrial dynamics and/or function these findings offered new strategies to both prevent oncogenic sub-lethal stress as well as enhance the tumour killing capacity of anti-cancer therapies.

Targeting cell death to better treat glioblastoma

Glioblastoma is an extremely aggressive type of brain tumour with limited treatment options. Anna Koessinger, Cat Cloix and others investigated whether targeting anti-apoptotic BCL-2 proteins may be an effective way to treat glioblastoma. We found genetic inhibition or drug targeting of MCL-1 and BCL-xL with BH3-mimetics could effectively kill glioblastoma cells *in vitro* and improve survival in mouse models of glioblastoma. Importantly, alternating treatment with drugs targeting BCL-xL or MCL-1 maintained potency on tumour tissue without observable toxicity in healthy brain tissue. This paved the way for further investigation of BH3-mimetics in glioblastoma treatment.

Publications listed on page 114

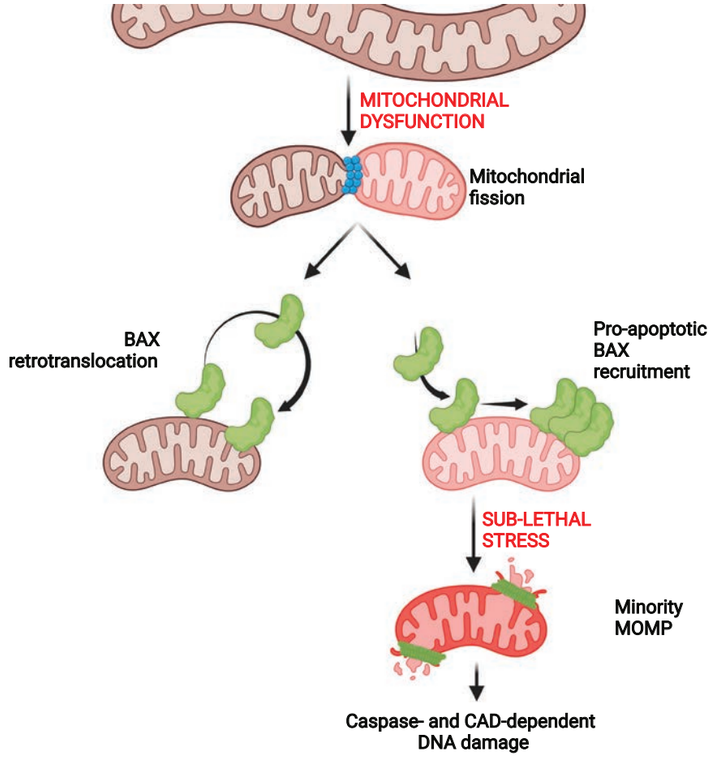

Figure 1
Mitochondrial function and dynamics regulate caspase dependent DNA-damage
Summary model: Mitochondrial dysfunction promotes mitochondrial fission and mitochondrial pro-apoptotic BAX. This facilitates mitochondrial outer membrane permeabilisation leading to caspase-dependent DNA-damage.

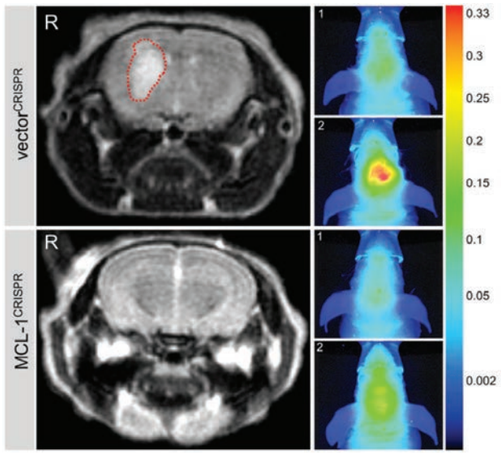

Figure 2
Anti-apoptotic MCL-1 is required for growth of glioblastoma
Glioblastoma cells expressing iRFP with or without anti-apoptotic MCL-1 (MCL-1 CRISPR) were assessed for their ability to grow in an orthotopic brain tumour model. MRI (left) or infrared imaging, demonstrated that only cells expressing MCL-1 led to tumour growth.

ONCOMETABOLISM

Group Leader
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Research Scientists
Tobias Ackermann
Victor Villar Cortes

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Maria Francesca Allega
Ruhi Deshmukh

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, 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 was the elucidation of what this enzyme is doing 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 N5-methylglutamine.

Technically, the identification of N5-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.

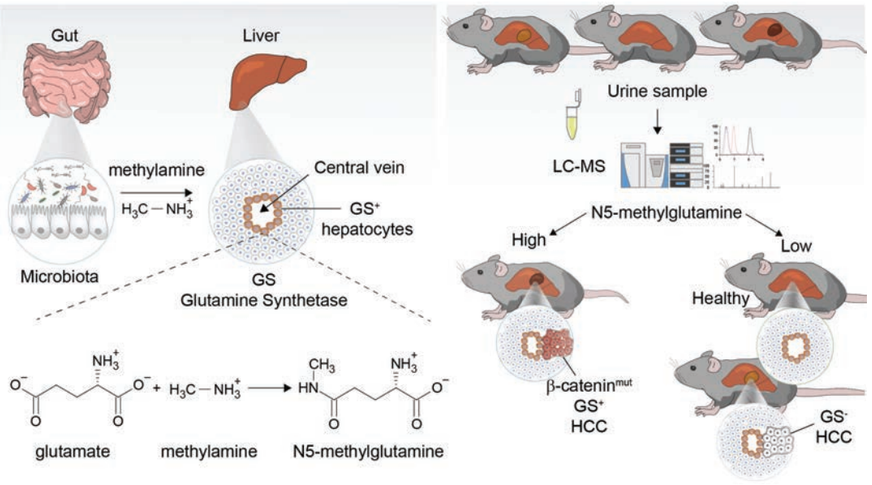


Figure 1
N^5-methylglutamine is the product of a newly identified reaction catalysed by glutamine synthetase
Hepatic glutamine synthetase accepted methylated ammonia to synthesise *N^5*-methylglutamine. In a mouse model of β-catenin mutant hepatocellular carcinoma, the urine levels of this glutamine analogue were predictive of tumour burden. Villar *et al.*, 2022, *Nature Chem Biol.* highlighted in *"A collaborative synthetase"* News & views

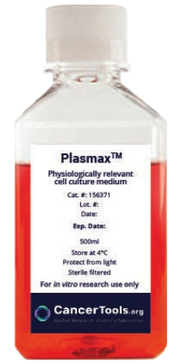
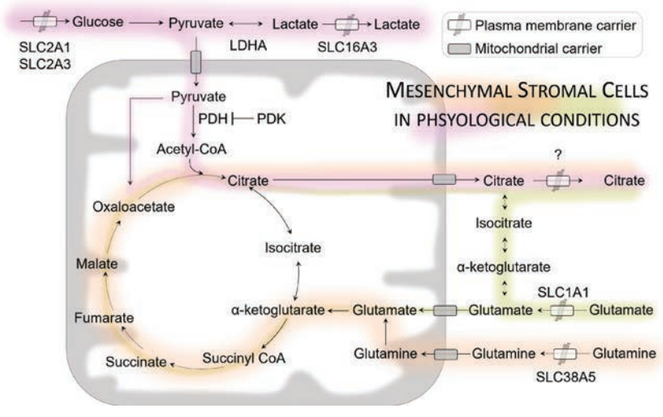


Figure 2
Plasmax™ is a physiological medium based on the levels of nutrients and metabolites found in human plasma that has been developed at the CRUK Beatson Institute. Plasmax™ is available for purchase at https://www.cancertools.org/media/156371.

Identification of metabolic vulnerabilities elicited by glucocorticoids in glioma

Glucocorticoids (e.g. dexamethasone) are part of the mainstay of treatment for glioma patients and are administered to reduce the peritumoral oedema, and to mitigate the adverse side effects of radio- and chemotherapy. As indicated by the name *(glucose + cortex + steroid)* glucocorticoids exert regulatory effects on glucose metabolism. However, the metabolic effects of glucocorticoids are not limited to systemic homeostasis of glucose and may modulate the fitness of glioma cells in the brain environment. While the anti-inflammatory action of glucocorticoids is a mainstay for the clinical management of glioma patients, the metabolic effects of these drugs on the cancer cells could be exploited to improve the prognosis of brain tumour patients. On this basis, glucocorticoids constitute excellent candidates to design novel metabolic combination therapies for the treatment of glioma.

Figure 3
A schematic of the metabolic pathways engaged by human bone marrow-derived primary mesenchymal stromal cells when cultured in Plasmax™. Taurino *et al.*, 2022, Molecular Metabolism



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 in perfecting the formulation of historic media.

Indeed, the vast majority of biomedical research employs commercially available 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 2) 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.

In 2022, we published a study (Taurino *et al.*, 2022, *Molecular Metabolism*), where we compared the response of primary bone marrow derived human mesenchymal stromal cells to Plasmax™ and DMEM. The results showed that Plasmax™ prevented the nutritional stress imposed by the skewed DMEM formulation, while sustaining mesenchymal stromal cells stemness and proliferation. Further, a panel of donor-derived cell lines were cultured in Plasmax™ at oxygen concentration (1%) relevant to the haemopoietic niche. By integrating transcriptomics, untargeted metabolomics, extracellular flux analysis, and stable isotope tracing we found that mesenchymal stromal cells consistently took up glutamate from the extracellular environment and used its carbons to support citrate synthesis and secretion. We demonstrated that this distinctive metabolic pathway was engaged even when citrate was supplied at concentrations found in human circulation, and oxygen level limits citrate production in the mitochondria (Figure 3).

Overall, our findings demonstrated that distinctive metabolic features of mesenchymal stromal cells were preserved by refined physiological cell culture conditions immediately applicable to the production of cell therapy products.

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TUMOUR MICROENVIRONMENT AND PROTEOMICS

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Clinical Research Fellow
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[1]CRUK CRH
[2]Breast Cancer Now
[3]CRUK Early Detection and Diagnosis Project Grant

High grade serous ovarian cancer (HGSOC) and triple negative breast cancer (TNBC) have limited treatment options, as only few targeted therapies effectively kill cancerous cells and patients frequently develop resistance to standard therapies. The tumour microenvironment actively supports cancer pathology and is populated by a variety of cell types that also offer alternative routes for therapy. Our research focuses on cancer-associated fibroblast (CAFs), as we and other 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, ECM 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 cancer cells as a promising strategy to hamper 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 for therapy.

CAFs can originate from normal fibroblasts resident at the site where the primary tumour develops. When a tumour starts developing, normal fibroblasts become activated. This activation induces extensive reprogramming of gene expression and protein levels, such that CAFs 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). CAFs were also shown to secrete EVs whose cargos could aid tumour progression by supporting cancer cell growth and invasion (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.

To understand how to target CAFs in tumours, it is essential that we understand how CAFs make the tumour microenvironment pro-tumorigenic and pro-metastatic, and what the molecular mechanisms are that sustain CAF function. Our major interest is the role of cell metabolism (Kay *et al.,* 2021, Front Oncol; Kay & Zanivan, 2021, *Curr Opin Syst Biol*) and extracellular vesicles secreted by CAFs. For our research model, we mostly use CAFs that we isolate from tumour tissues that were kindly donated by patients for research purposes (Hernandez-Fernaud, Ruengeler *et al.,* 2017, *Nat Commun*; Kugeratski *et al.,* 2019, *Science Signaling*). 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.

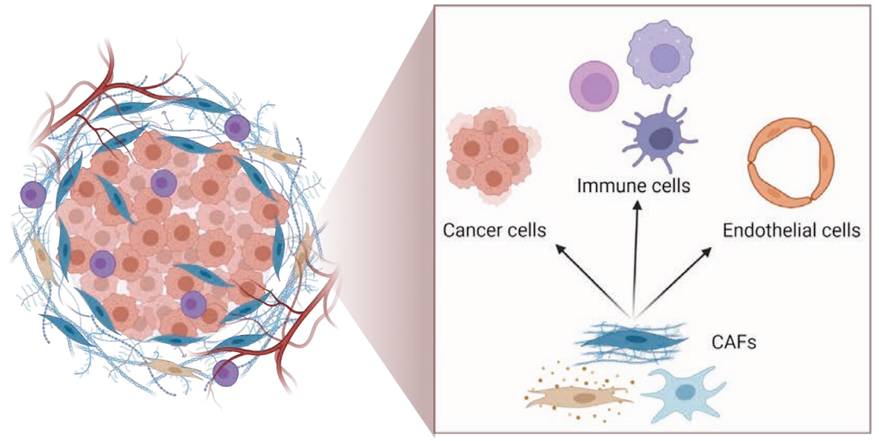

Figure 1
CAFs influence the behaviour of cancer, immune and endothelial cells
Cartoon showing that cancer associated fibroblasts (CAFs) with different phenotypes populate the tumour microenvironment and that CAFs secrete plethora of factors that influence the function of other cell types in the tumour.

CAF – tumour blood vessel interaction

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

Endothelial cells (ECs) line the inner layer of the vessel wall and regulate the functionality and growth of the vessel. Tumour blood vessels are typically embedded within a CAF-rich stroma, such that ECs directly interact with CAFs or are exposed to the factors that they secrete. Our group previously showed that CAFs secreted proteins that influenced blood vessel growth and functionality via altering endothelial cell behaviour (Hernandez-Fernaud, Ruengeler *et al.,* 2017, *Nat Commun*; Kugeratski *et al.,* 2019, *Sci Signal*). We have also shown that the ECM proteins secreted by CAFs played an active role in the metastatic dissemination through facilitating the binding of the cancer cells to the blood vessels (Reid *et al.,* 2017 *EMBO J*). We have now found that CAFs also influenced EC function by transferring functional proteins through EVs. In particular, CAFs could transfer plasma membrane and membrane-bound proteins to the surface of the endothelial cells. This process confers the ability to the endothelium to interact with other cell types, such as monocytes, which influence aspects of tumour progression, including antitumor immunity and metastasis. We therefore discovered another way through which CAFs make ECM pro-tumorigenic and we are investigating this aspect further (Santi *et al.,* 2023, *BioRxiv*).

CAFs & metabolism

Metabolic alterations are a well-established hallmark of cancer. In the last few years, it has emerged that, in addition to the metabolism of cancer cells, also the metabolism of stromal cells is an important regulator of cancer pathology (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 metabolites, such as 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 pro-tumorigenic ECM (Kay *et al.,* 2022, *Nature Metabolism*). We are now further investigating the potential of targeting acetyl-CoA production in CAFs in cancer.

News

This year, Britt Sterken has been awarded a DMM Conference Travel Grant Company of Biologists to present her work on the role of CAF metabolism in triple negative breast cancer at the EACR Cancer Metabolism meeting in Bilbao. She has also presented her work at the Cancer Metabolism Showcase and Workshop Virtual meeting. Emily Kay has also presented a poster on the immune-regulatory roles of CAFs in breast cancer at the EACR Cancer Metabolism meeting in Bilbao.

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ADVANCED TECHNOLOGIES

Hiplex staining using Akoya Phenocycler Fusion on human tonsil consisting of protein biomarkers: E-cadherin (dark green), DAPI (blue), CD20 (blue), CD68 (cyan), CD45RO (magenta), podoplanin (burgundy), CD8 (pink), CD21 (lilac), CD45 (red), pan-CK (green), keratin 14 (sage), CD31 (yellow), CD4 (orange)

Image taken by the Deep Phenotyping in Solid Tumours Group

BEATSON ADVANCED IMAGING RESOURCE

Head
Leo Carlin

Fellow of the Royal Microscopical Society (FRMS)

Scientific Officers
Ryan Corbyn
Tom Gilbey
Lynn McGarry
Ewan McGhee[1]
Claire Mitchell
Nikki Paul
David Strachan
Peter Thomason

[1]shared with Department of Physics, University of Glasgow

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

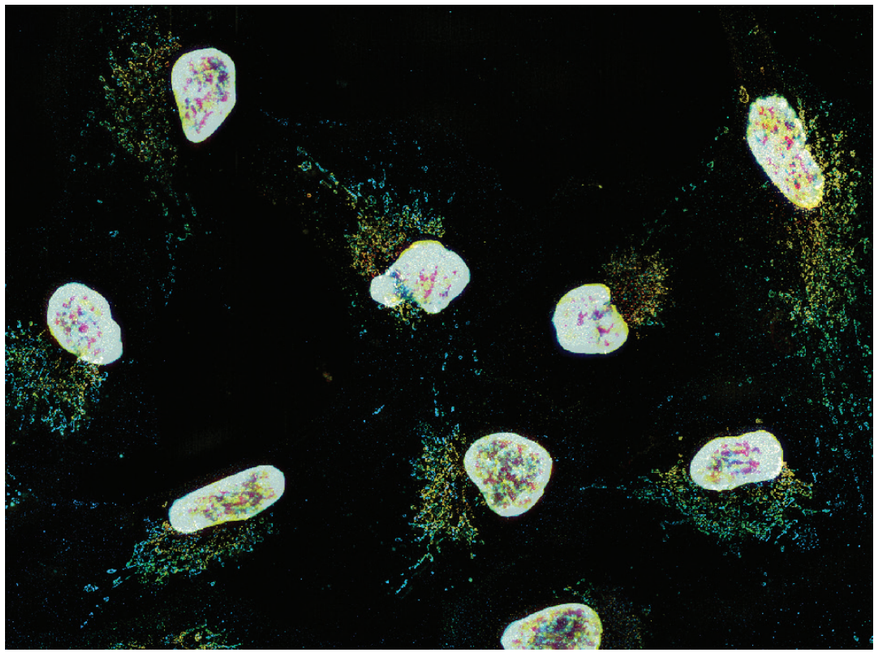
We have the expertise and instruments to:

- 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.

It was a pleasure to have two new team members join the BAIR this year. Ryan Corbyn came directly from finishing his PhD in biophysics at University of Strathclyde, focussed on developing novel microscopical approaches for sub-cellular temperature sensing. He has already developed strong skills in image analysis using Python. He works with scientists at the Institute on a plethora of image analysis tasks and will implement promising machine learning approaches from the literature. He also works closely with Lynn McGarry, our high-content imaging lead on large multiparameter datasets. Claire Mitchell joined us from Warwick where she was already working in an advanced imaging facility. She also has a background in biophysics and optical engineering with >12 years of experience developing and maintaining optical microscope systems, including 7 years specifically working in life science light microscopy facilities. Claire is training and working with users on a variety of projects. She also contributes regularly to the quality assessment and reproducibility initiative Quarep-LiMi. This means that our team is now almost 50:50 cell biology:biophysics trained allowing us to use a multi-disciplinary approach to our researchers' questions.

In terms of equipment, we were fortunate to replace a further two of our heavily used Incucyte in incubator imaging systems with the latest versions, one via external funding from the MJM Smith Trust in coordination with the University of Glasgow School of Cancer Sciences (SCS). We also received a Formulatrix Mantis high accuracy, small volume, low waste automated pipetting robot, to allow us to increase the throughput of spheroid / organoid high content imaging assays, where difficult-to-pipette, expensive matrices are used. A return to larger interactive face-to-face training allowed us to engage with researchers in additional ways this year. Tom Gilbey, with Alberto Bravas-Blas (Roberts lab) and Yi-Hsia Liu (SCS), delivered a very successful mini-course on flow cytometry which included theory, practical sessions, and analysis techniques. The light microscopy team ran a face-to-face all user forum and also short 'techbyte' sessions on imaging controls and image analysis. We look forward to continuing these throughout the next years.



Visualisation of U2OS mitochondrial network. U2OS cells were stained with Cox IV (Mitochondrial marker), and DAPI (Nuclear stain), prior to imaging with Zeiss Elyra 7 at 63x objective. Image was post processed to separately colour each z-layer and compiled into a single image.

Image was taken by Peter Thomason and processed by George Skalka (Murphy Group).

BIOINFORMATICS AND COMPUTATIONAL BIOLOGY

Head

Crispin Miller

Scientific Computing Specialist
Naveed Khan

Bioinformaticians
Ryan Kwan
Robin Shaw

The Bioinformatics unit provides numerical expertise across the Institute. A major aspect of our work continues to centre on the analysis of high throughput 'omics data, including a wide range of next generation sequencing, proteomics, and metabolomics datasets. A growing focus has been on the application of these techniques to imaging data.

Our team focuses on exploratory data analysis, and 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 Khan joined our team to lead on establishing a scientific computing platform. Naveed has begun to commission 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.

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. We have introduced an experimental design course and continue to operate a regular internal bioinformatics forum to provide a central point of contact to bring together bioinformaticians, researchers and students who are applying computational biology and numerical approaches to their data. Our team also participates in delivering part of the Cancer Research & Precision Oncology MSc programme at the University of Glasgow.

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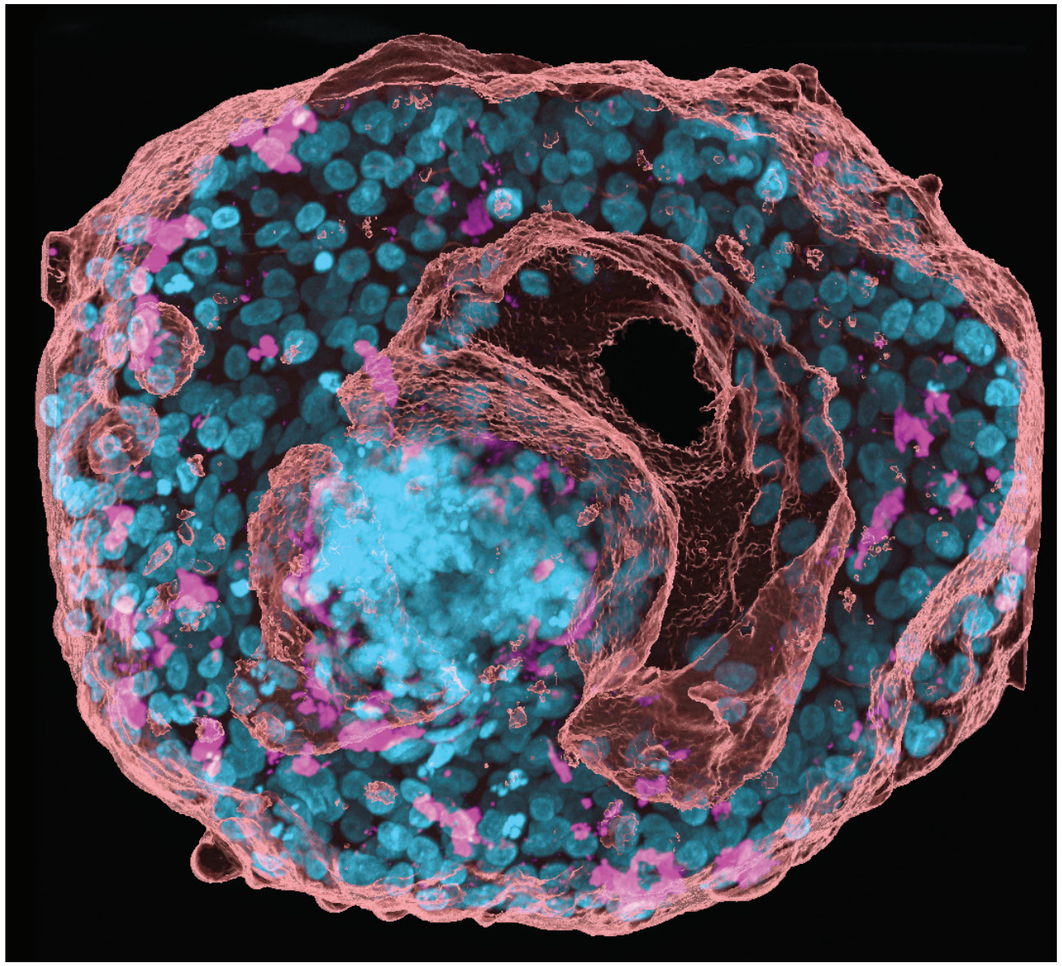


Pancreatic adenocarcinoma (PDAC) is an aggressive cancer that often metastasises to areas such as the liver. To study this metastasis, we are developing a 3D in vitro model using a co-culture system. This image shows a spheroid generated by the aggregation and culture of multiple liver cell types and PDAC cells. To study potential modelling of the extracellular matrix, the secretion of collagen 1 within the spheroid was visualised by immunofluorescence. The spheroid was also stained with DAPI and Alex Fluor 568 Phalloidin, and a z stack image was taken using a Nikon A1R confocal microscope in the BAIR facility at the CRUK Beatson Institute.

Image taken by Elaine Ma

METABOLOMICS

Head
David Sumpton

Scientific Officers
Alejandro Huerta Uribe
Engy Shokry

Graduate Student
Rachel Harris

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.

We work closely with many groups within the Institute who have interests in cancer metabolism and during 2022, we have continued to contribute to their research (see publications). Our close collaboration with Saverio Tardito's group through Rachel's ongoing PhD project, continues to investigate the therapeutic gain of inhibiting both BRAF and glutaminase in the treatment of BRAF mutant melanoma.

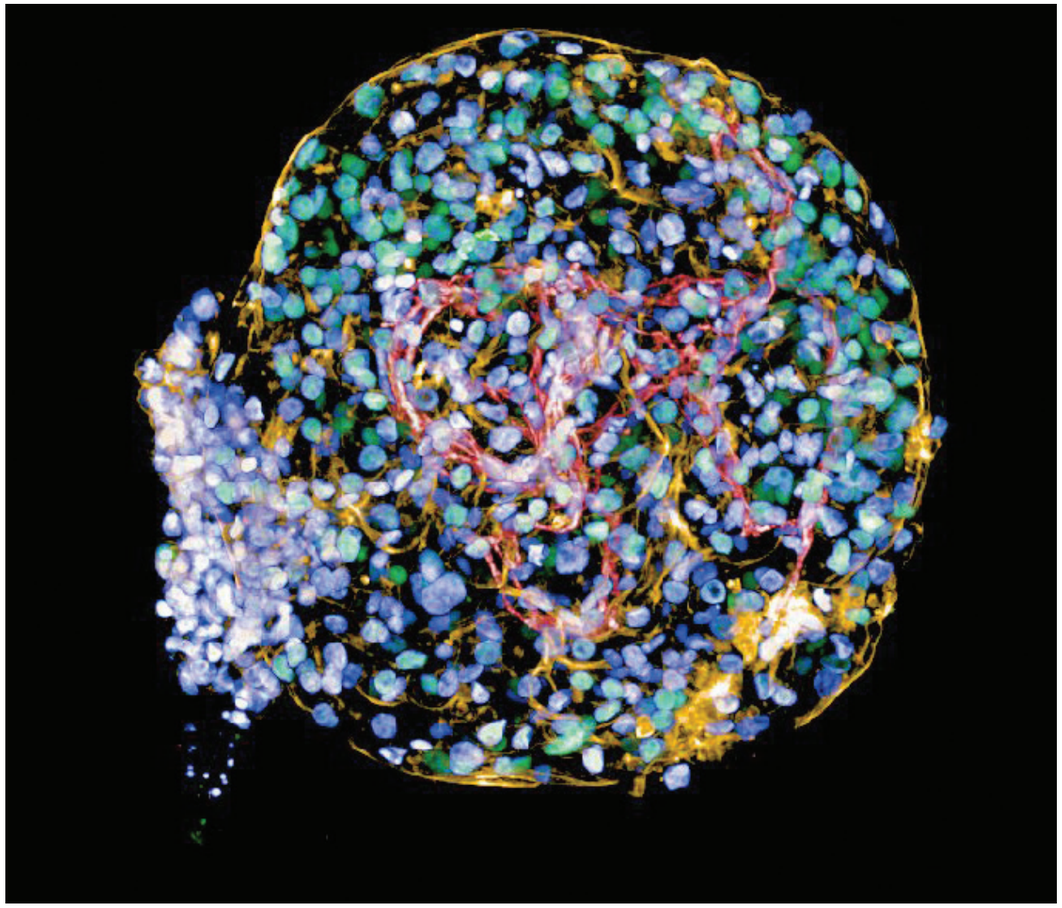
The facility's core aim is to provide access to state-of-the-art LC-MS technology that is optimised for the detection of metabolites and lipids. We maintain and operate the instrumentation, providing both standard metabolite profiling and custom analysis when needed. Through the course of the year, we have continued to develop and add new methods to the facility's portfolio. This year, these included the measurement of polyamines, acyl carnitines and fatty acids (both free and total), complementing our existing untargeted lipidomics platform.

We also offer expertise and assistance in data analysis, data interpretation and experimental design. This year, we have updated our data analysis pipeline, moving to modern analysis packages for targeted experiments and will soon offer updated in-house training for these approaches. To learn as much as possible from the data generated, we also collaborate with users to make use of more complex untargeted analysis.

In May, Alejandro Huerta Uribe joined the facility from the School of Cancer Sciences at the University of Glasgow. During his time in Oliver Maddocks' lab, Alejandro gained experience in cancer metabolism and high-resolution MS-based metabolomics which he is now employing in his role as a scientific officer in the facility.

During the summer, both David and Engy continued the long-standing association with Cold Spring Harbor labs, assisting in the organisation and practical instruction of the 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 answer fundamental biological questions.

Publications listed on page 117



Visualisation of human induced pluripotent stem cell derived liver organoids (maximum projection). Organoids were stained with HNF4-alpha (hepatocyte marker), CD31 (endothelial marker), DAPI (nuclear stain) and phalloidin (F-actin marker), prior to imaging with Opera Pheonix at 20x objective.

Image was taken by Sonam Ansel (Jim Norman's Lab, R20) with assistance from Lynn McGarry.

PROTEOMICS

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Scientific Officers
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Grigorios Koulouras
Sergio Lilla

[1]British Lung Foundation (50%)

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 unravelling the identity and function of individual proteins 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 analyses 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 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 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. Moreover, we use Skyline for the analysis of PRM data and Spectronaut for data acquired in data-independent acquisition mode. 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, and are developing approaches that allow us to study the interplay between metabolism and protein synthesis by tracing ^{13}C-labelled metabolites into newly synthesised secreted proteins (Kay EJ *et al.,* 2022, *Nature Metabolism*). We are also expert in posttranslational modifications, including cysteine oxidation. For the latter, we have developed SICyLIA, a method that enables the quantification of cysteine oxidation levels at a global scale with no enrichment steps required (van der Reest, Lilla *et al.*, 2018 *Nat Commun*) which has been fundamental to answering different biological questions (Port *et al.,* 2018, *Cancer Discov*, Hernandez-Fernaud, Ruengeler *et al.,* 2017, *Nat Commun*; Cao X *et al.,* 2020, *J Cell Sci*).

During 2022, 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 using proteomics to answer more complex biological questions and to improve the methods currently in place enriching the quality of the data that the facility can provide.

News

This year, Sergio Lilla presented a recent development of the SICyLIA technology to measure cysteine oxidation at global scale at the International Mass Spectrometry Conference 2022 in Maastricht.

Publications listed on page 117

TRANSGENIC MODELS OF CANCER

Head
Karen Blyth

Lead *In Vivo* Scientist
Louise Mitchell

Scientific Officers
Jayanthi Anand
Dimitris Athineos
Laura Galbraith
Dale Watt

Our lab strives to recapitulate human cancer in preclinical mouse models to interrogate all aspects of disease progression within a biological context, applying model systems to study early disease through to metastasis and recurrence. For 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, to study how oncogenic pathways, altered metabolism and the tumour microenvironment contribute to cancer, and how these can be exploited for earlier detection of cancer and for therapeutic gain.

Modelling cancer *in vivo*

The Beatson Institute is internationally renowned for its scientific excellence using preclinical mouse models to study cancer in a physiologically relevant way to understand these complex human diseases. This is fundamentally 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, and which can be distinct between different tumour types. Studying this multifaceted behaviour in a plastic dish has obvious 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; are exposed to metabolic limiting conditions; and have to negotiate biological barriers in order to metastasise. Furthermore, many anti-cancer drugs fail in the clinic because, although they are effective in simplified tissue culture models, the nuances of taking these drugs into the whole animal setting cannot be ignored. The Transgenic Models lab utilises genetically engineered mouse models sympathetic to the same genetic alterations in human cancers such as breast, colorectal, pancreatic and prostate cancer, and which share the same pathology and metastatic spread seen in human patients. We also have expertise in orthotopic xenograft models, and in syngeneic allograft models permitting interrogation of immune interactions with primary and metastatic tumour cells. Monopolising these state-of-the-art preclinical models, in combination with *in vivo* imaging, our lab collaborates with colleagues at the Beatson Institute and the University of Glasgow to translate *in vitro* discoveries.

Research Collaborations

It is exciting to be involved in many diverse projects and the lab enjoys the stimulating collaborations with colleagues across all the strategic themes of the Institute probing metabolism as a cancer vulnerability, studying the interplay within the tumour microenvironment that drives metastasis and recurrence, as well as modelling early disease.

Using our expertise in prostate cancer models, we have had the pleasure of contributing to various projects with David Bryant and his lab. Building on the exciting observations in 2D and 3D assays demonstrating that the ARF3 GTPase interacts with N-cadherin to control invasion of prostate cancer cells, we showed that ARF3 was instrumental in driving prostate cancer metastasis *in vivo* (Sandilands *et al., J Cell Biol.* 2023). Similarly, 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* models (Roman-Fernandez *et al, Sci Adv.* 2023). By incorporating ultrasound and sensitive fluorescence imaging in orthotopic transplant models we were able to refine these models to garner subtle information on metastatic spread of tumours at an earlier clinical endpoint by imaging the organs *ex vivo* (Figure 1). Longitudinal non-invasive imaging using a novel reporter mouse developed with Tim Humpton and Karen Vousden (Frances Crick Institute) also allowed us to delineate the activity of the Trp53 protein during normal development and liver injury (Humpton *et al., Sci Signal.* 2022).

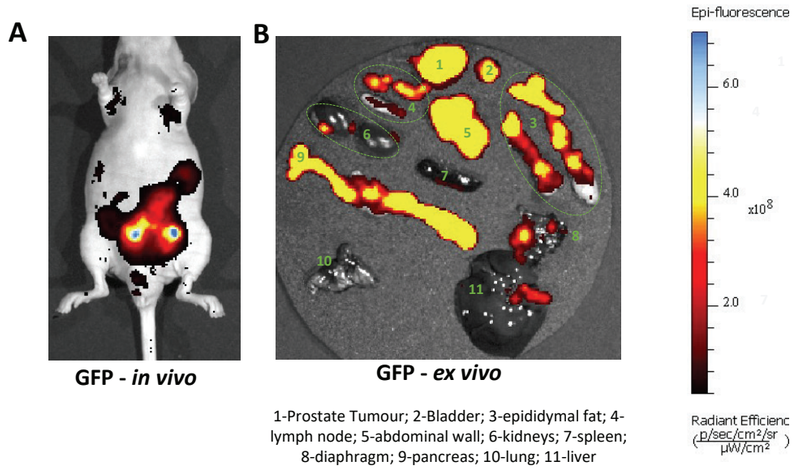


Figure 1
Fluorescence imaging reveals metastatic cells in a model of prostate cancer
A shows an *in vivo* image of a male mouse, 8 weeks post intra-prostatic injection, with human prostate cancer cell line PC3 cells (tagged with fluorescent markers). **B** Using *ex vivo* imaging from all the organs with the IVIS Spectrum, it is possible to identify fluorescent signal in the primary prostate tumour, but also identify the sites of metastasis in projects carried out in collaboration with David Bryant.

Targeting cancer cell metabolism presents an important opportunity for novel therapeutic means. Continuing a long-standing collaboration with Oliver Maddocks (University of Glasgow) exploring amino acid restriction, we showed that tumour cells could be sensitised to radiotherapy by reducing serine and glycine levels (Falcone *et al, British J Cancer* 2022). Metabolic rewiring can also be a key modulator within the tumour microenvironment. Applying *in vivo* breast cancer models, we collaborated with Sara Zanivan and her team exploring how elevated PYCR1 expression in cancer associated fibroblasts (CAFs) drives proline synthesis to regulate the extracellular matrix within the tumour microenvironment, to promote breast cancer progression (Kay *et al, Nat Metab* 2022).

We have also collaborated with Stephen Tait's group to show how specific members of the BCL2 family are important in the pathogenesis of glioblastoma and offer an avenue for therapeutic intervention (Koessinger *et al., Cell Death Differ.* 2022), and with Iain McNeish's lab on how simultaneous inhibition of epigenetic regulators G9A and EZH2 can reduce tumour growth in a model of ovarian cancer (Spiliopoulou *et al., Mol Cancer Ther.* 2022).

Resources & News

Our lab is responsible for curating and training scientists in key equipment used for preclinical modelling such as the IVIS fluorescence/bioluminescence system, the PEARL near-infrared fluorescence detector, ultrasound imaging, and the IDEXX ProCyte Dx haematology analyser. As a lab, we continue to focus on innovative technologies to refine and improve cancer models for the benefit of the Institute providing expertise in surgical procedures such as orthotopic prostate delivery and mammary intraductal delivery. In all our approaches we continually promote the 3Rs.

We were delighted to welcome Louise Mitchell to the lab this year. Louise brings a wealth of experience and as Lead *In Vivo* Scientist will drive the collaborative projects and offer advice on project design and researcher compliance. Other big news is that the lab are very excited to be part of the MRC National Mouse Genetic Network, leading the Cancer Cluster (https://nmgn.mrc.ukri.org/clusters/cancer/) where, working with the MRC Mary Lyon Centre at Harwell, we will develop and improve mouse models of cancer as accurate predictors of patient response to novel therapies.

Publications listed on page 101

TRANSGENIC TECHNOLOGY

Head
Douglas Strathdee

Scientific Officers
Cecilia Langhorne
Farah Naz Ghaffar
Eve Anderson

The Transgenic Technology Laboratory uses molecular genetic approaches to help analyse the function of genes in cancer development and progression. By using both genome editing and gene targeting, we are able to make precise genetic alterations into endogenous genes in stem cells. These methods allow us to introduce changes into cells which accurately reproduce the mutations observed in human cancers.

Making better models of clinically relevant cancers

Embryonic stem (ES) cells have some valuable attributes, which make them very useful tools to allow us to study the role of gene mutations in cancer. Firstly, ES cells have very high rates of homologous recombination, a process which enables us to introduce precise genetic changes into endogenous genes. So, by taking advantage of homologous recombination, we can copy mutations seen in human cancers directly into genes in ES cells. This makes it possible for us to study how these mutations affect the structure of the associated proteins and how these differences affect function in cells and tissues.

A second attribute that ES cells possess is that they can differentiate to form cells from a wide variety of different tissues. So, once we have stem cells carrying the genetic changes we want to study, we can then differentiate these into cells from a relevant tissue. So, for example, if we want to study the consequences of a mutation originally detected in liver cancer, we can analyse liver cells derived from the altered stem cells.

During the year, we have collaborated with other groups at the Institute to generate a variety of different kinds of alleles. These include conditional knockouts, point mutations and inducible genetic markers. In addition to making small changes to endogenous stem cell genes, where human and mouse gene differ significantly, we can now replace the entire endogenous mouse gene with its human equivalent. Consequently, using this approach, we can test the outcome of mutations directly in the appropriate context of the human gene, and this ensures that the changes we make directly imitate the mutations discovered in human cancers.

In vivo CRISPR for the study of models of Hepatocellular Carcinoma (HCC)

Over the last decade, liver cancer incidence rates have increased by almost half (45%) in the UK and are expected to reach 15 cases per 100,000 people by 2035 (Cancer Research UK). Various genetically engineered mouse models of HCC have been developed which allow the study of the multiple genetic changes occurring at each stage of progression towards tumour formation as well as provide the possibility to examine treatment strategies. However, complicated genetic models can be laborious and time-consuming to create and require extensive breeding strategies. For this reason, we attempted to test if we could generate mutations of known tumour suppressor genes directly within the liver using genome editing technology.

A Myc and β-catenin mouse model of HCC was used as a basis for the study, in which Cre recombinase is used to activate Myc expression as well as to remove exon 3 of β-catenin. These genetic changes are used to mimic the early stages of HCC. Additionally, these mice were bred to include Cre inducible expression of Cas9. 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. An Adeno-associated virus (AAV) expressing a low-dose Cre was provided to activate all three mutations in the liver. At the same time CRISPR guides designed against critical coding regions of two tumour suppressor genes (A and B) were also provided virally. These guides should direct the Cas9 enzyme to cause mutations in the two tumour suppressor genes. Following the introduction of mutations, the suppressor genes should co-operate with Myc and β-catenin to accelerate the rate of tumour progression in the model. After administration of the viruses expressing Cre recombinase and the guide RNAs, mice were then monitored for liver tumour growth and subsequent tumours were analysed for gene mutations and examined histologically.

Mice, in which guides for both tumour suppressor genes were provided, were found to reach end point significantly sooner, than those in which no CRISPR guides were provided. Median survival was around 100 days for the mice who received the A and B guide RNAs compared to 300 for the control mice without the guides (Figure 1a). Mice, in which a guide to only one suppressor gene (tumour suppressor A) was provided also had median survival which was much lower than the controls (137 days). Tumour tissue was sequenced, and various mutations identified within the tumour DNA in both tumour suppressor genes. These mutations were discovered at the exact sites where Cas9 was directed by the guide RNAs. No mutations were found at the same regions in the control mice. This suggests Cas9 works to introduce mutations directly into tissues. Histological staining was also used to identify knock-down of tumour suppressor gene A in tumour tissue of these mice (Figure 1b). Tumour growth was exhibited in all mice which were administered with CRISPR guides and mutations were also identified in all of these mice suggesting high rates of Cas9 mutation of these genes. Overall, *in vivo* CRISPR has been demonstrated to be a potentially valuable tool for cancer mouse genetics.

Publications listed on page 113

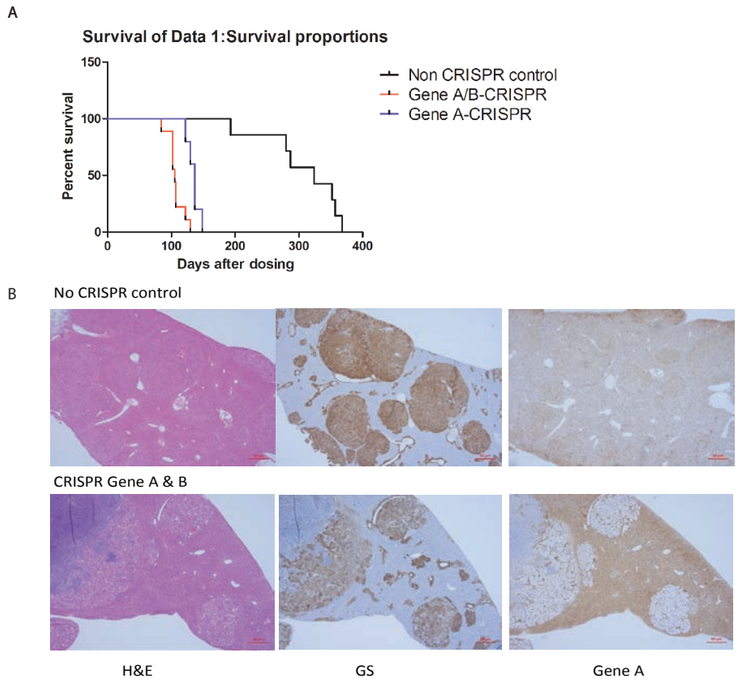


Figure 1
A Survival curve of Myc/Bcat HCC mice administered with AAV CRISPR for tumour suppressor genes A and B (Red), Gene A only (Blue) and a non-CRISPR control (Black). P Value, 0.002 ***. **B** Histological analysis of tumour samples from mice where no guide provided (top panel) H&E, GS and then stained against tumour suppressor gene A. Bottom panel shows a mouse in which guides were provided against both tumour suppressor gene A and tumour suppressor gene B, knock-down of tumour suppressor gene A can be seen within the tumours.

TRANSLATIONAL MOLECULAR IMAGING

Head
David Lewis

Staff Scientist
Gaurav Malviya

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

Medical Physicist
Caroline Findlay[2]

Senior Scientific Officer
Agata Mrowinska

Senior Image Analyst
Algernon Bloom[3]

[1]CRUK Scotland Centre
[2]Beatson Cancer Charity / Beatson Endowment
[3]CRUK RadNet

Translational Molecular Imaging (TMI) develops novel imaging technologies and acts as a hub for emerging molecular imaging research. Operating over two sites: the CRUK Beatson 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 Beatson 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, [18F]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 2022, 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 2022, Glasgow researchers (David Lewis and Oliver Maddocks) obtained Cancer Grand Challenge grant funding to study cancer cachexia within the CANCAN team. These studies require access to a wide range of carbon-11 labelled metabolic tracers, such as lactate, alanine, succinate, glutamine, fatty acids and other metabolites involved in energy, muscle wasting and the browning of white adipose tissue. To underpin the CANCAN preclinical PET imaging programme we have hired a postdoc and carbon-11 chemist, Fraser Edgar.

To expand our carbon-11 radiochemistry development capabilities we are upgrading the R&D radiochemistry laboratory in the West of Scotland PET Centre at the Gartnavel Hospital to host two SYNTHRA synthesizers, capable of carbon-11 and fluorine-18 radiotracer development.

Preclinical and translational imaging

In 2022, we participated in a collaborative KRAS-mutant colorectal cancer study with Prof Owen Sansom's laboratory. We used a SLC7A5-specific radiotracer O-(2-[^{18}F]fluoroethyl)-l-tyrosine ([^{18}F-FET) and performed [^{18}F]FET PET imaging and autoradiography to demonstrate the functional loss of SLC7A5 in mouse models *in vivo*. This analysis indicated a reduced [^{18}F]FET uptake in SLC7A5-deficient tissue compared to wild-type controls, demonstrating that expression of SLC7A5 is required for optimal amino acid uptake following KRAS mutation.

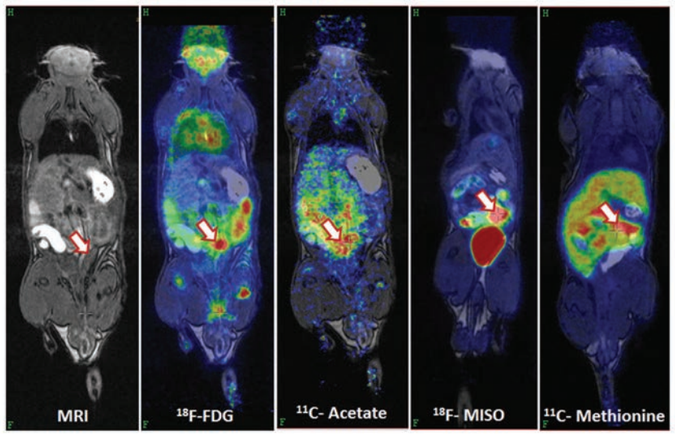


Figure 1
Multiplexed PET/MRI imaging of an *Apc*$^{fl/+}$ *Kras*$^{G12D/+}$ *Trp53*$^{fl/fl}$ *Tgfbr1*$^{-/-}$ (AKPT) orthotopic colon tumour.

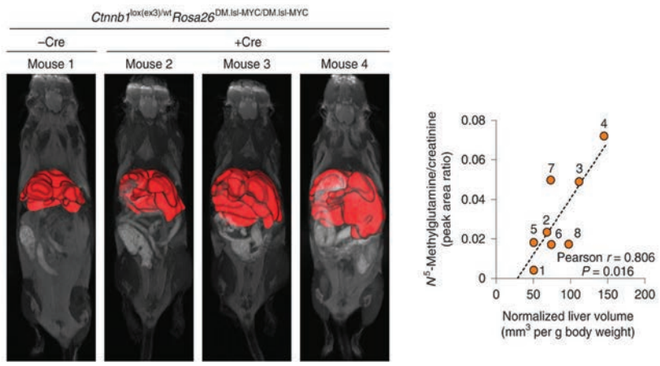


Figure 2
MRI images of *Cnntb1*$^{fl(ex3)/wt}$*Rosa26*$^{DM.lsl\text{-}MYC/DM.lsl\text{-}MYC}$ liver tumour bearing mice. Three-dimensional reconstructions of the livers are highlighted in red. Liver volumes correlated to urinary levels of the methylated glutamine analog N^5-methylglutamine, a product of liver glutamine synthetase activity (Villar *et al.*, 2023, *Nat Chem Biol*).

Glasgow is leading a European-wide consortium, ACRCelerate: Colorectal Cancer Stratified Medicine Network to enable better matching of colon cancer subtypes to therapeutic trials. In an ongoing collaboration, we are exploring the role of PET/MRI for non-invasive phenotyping of subtypes of colon cancer. Using the collection of state-of-the-art colon cancer models at the Beatson Institute, we are developing non-invasive spatial and temporal imaging biomarkers for stratification of colon cancer. Ongoing multiplexed PET imaging probing glucose, nucleotide, amino acid and fatty acid metabolism has shown subtype specific differences in imaging phenotypes. We aim to validate this work in autochthonous genetically engineered mouse models, representing the spectrum of human colon cancer subtypes (Figure 1).

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 will facilitate 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 2).

In 2022, we supported Stephen Tait's group imaging a mouse model of glioblastoma for investigating the therapeutic potential of targeting anti-apoptotic BCL-2 proteins. We are collaborating with the research groups of Daniel Murphy (University of Glasgow) and Kevin Blyth (Macmillan Scottish Mesothelioma Network) to image genetic models of malignant pleural mesothelioma.

Publications listed on page 118

HISTOLOGY

Colin Nixon

Barbara Cadden
Shauna Currie Kerr
Saira Ghafoor
Mark Hughes
Wendy Lambie
Sophie Mclaughlin
Denise McPhee
Vivienne Morrison
Gemma Thomson

Histology performs processing of tissue and cellular materials 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 disease mechanics.

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 materials 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 block sections 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 in order 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 the researcher at any time. Immunohistochemical training can be provided in order that an individual scientist can understand the rationale and techniques available allowing them to perform the staining to an acceptable and consistent standard.

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

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

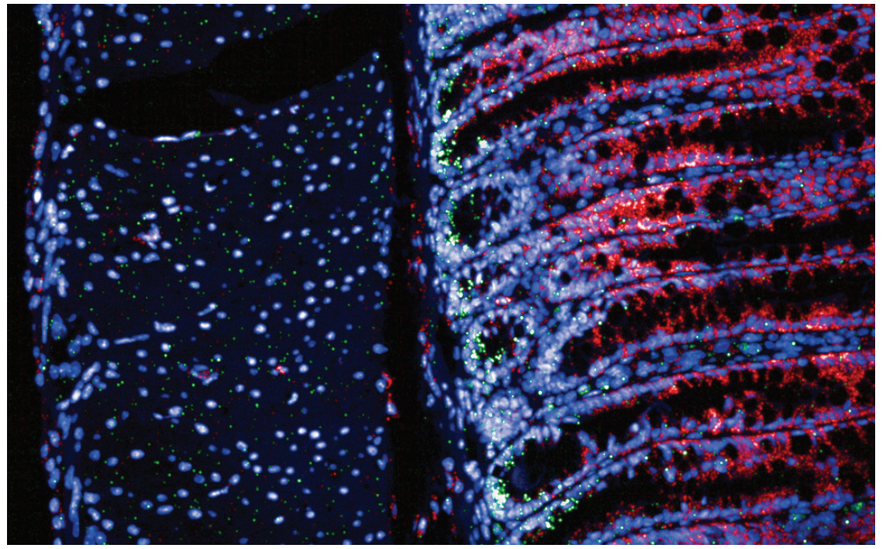
A recent advancement in *in situ* hybridisation technique now means where a probe is available or one can be specifically designed to the meet the researcher's needs, we can label and visualise much smaller targets, around 50 base pairs in size.

A number of specialised histological stains are available 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.

New development for 2023: Dual RNAScope immunofluorescent staining

Ly6a (Red); Lgr5 (Green)



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.

LABORATORY OPERATIONS & PUBLICATIONS

Visualisation of U2OS mitochondrial network. U2OS cells were stained with Cox IV (Mitochondrial marker), and DAPI (Nuclear stain), prior to imaging with Zeiss Elyra 7 at 63x objective. Image was post processed to separately colour each z-layer and compiled into a single image.

Image was taken by Peter Thomason and processed by George Skalka (Murphy Group).

LABORATORY OPERATIONS

Head
Scott Kelso

Laboratory operations cover a number of different functions with the remit to ensure the smooth operation 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.

In the last year, the Institute saw a welcome return to more normal ways of working after a challenging couple of years of COVID restrictions. The operational teams have been focusing on delivering first class services to the researchers in the Institute, whilst ensuring that we continue looking for opportunities to improve how we deliver them. This vision of continuous improvement in operations was aided by further training of some of our operational staff in Lean Six Sigma, which is an incredibly powerful set of tools in developing and delivering on improvement projects. We had some excellent projects delivered in stores, lab management and facilities which realised good savings for the Institute.

Complementing our commitment to improvement, we continued replacing some ageing equipment with renewal of some of our buildings de-ionised water systems and autoclaves, whilst we also reviewed our procedures for building access cards and induction, significantly improving the experience for our new researchers and staff joining the Institute.

The coming year will present some real challenges, most notably the increasing energy costs. However, initiatives around moving to LED lighting across the Institute, as well as several suggestions from our recently formed Eco committee, will help us to try and mitigate these increases where we can, and I look forward to continuing to work with our incredible team to address these challenges.

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 in compliance with appropriate regulatory standards. The use of our online helpdesk facility continues to be an effective means of logging reactive calls for breakdowns.

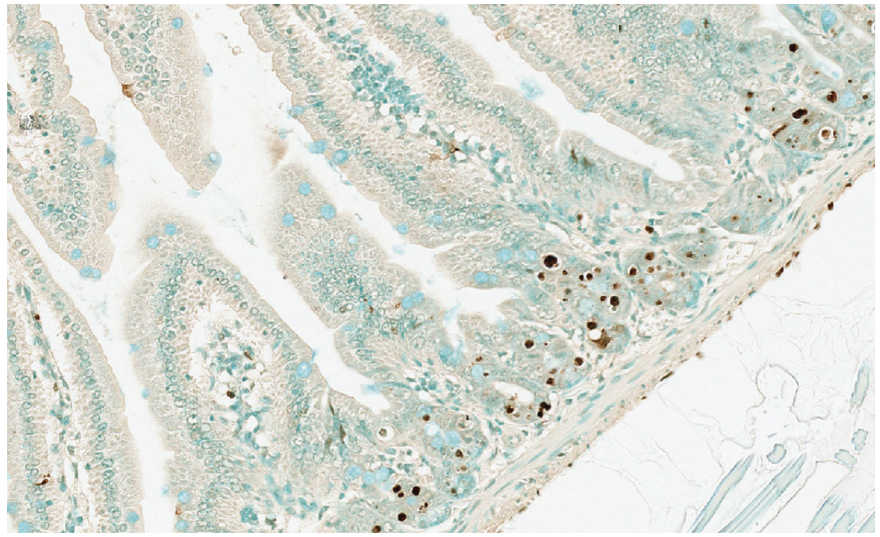
This year has seen us complete thermographic studies of electrical systems in our BSU area, as well as the completion of the statutory 5 year fixed wire testing in this area. There has also been significant planning in this same area to prepare for the replacement of our autoclaves, as well as the co-ordination required to make significant progress in the roll out of LED lighting across the Institute.

We undertook a project to rationalise our 3rd party service and maintenance agreements to try and ensure we received better service support which has been a great success so far. Alongside further increasing our resilience of IT infrastructure with UPS upgrades, as well as managing various moves of laboratory groups in the building, its has continued to be a varied and rewarding year.

Laboratory Management & Health and Safety

Caroline O'Connell, John Kinsella, Karen Thomas, James Dyball

The Laboratory Management team (Caroline O'Connell, Karen Thomas and James Dyball) is responsible for providing a number of vital support roles to the Institute and ensures the smooth running of the laboratories, aiding researchers to perform their research as efficiently and effectively as possible.



TUNEL staining on mouse gut tissue allowing the recognition of apoptotic nuclei in paraffin sections; Apoptotic material – brown, Background – green, x20 magnification

Image by Histology

Laboratory Management coordinates the service and maintenance of communal core equipment and of any systems that these require. We maintain the containment level 1 and 2 tissue culture rooms, and the Cryostore, as well as the management and co-ordination of the provision of gas supplies such as carbon dioxide or nitrogen, to equipment. We are proactive in ensuring that equipment breakdowns are kept to a minimum and, when they do occur, are being dealt with as quickly as possible. We identify key areas of improvement and oversee the replacement and purchase of equipment to facilitate the needs of researchers. We also engage with sales and technical representatives of companies to arrange demos and training of new equipment. We maintain our laboratory equipment database and asset register and, alongside this, Lab Management also ensures the safe disposal and compliance of laboratory waste including chemical, clinical and Institute WEEE waste.

The Laboratory Management team works very closely with John Kinsella, the Health and Safety manager, to ensure that all staff, students and visitors work in a safe laboratory environment.

As safety plays an integral part of everyday life in the laboratory, we review health and safety processes regularly and identify training needs. One of the primary roles of the team is the provision of advice, training, and information to all staff on health and safety issues so we often advise on risk assessments and appropriate containment and control measures necessary for laboratory work involving biological, chemical, radiation and genetic modification processes. 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.

We maintain a good relationship with our suppliers to ensure we achieve best prices and discounts for goods. Here, we work closely with the stores team to ensure costs for service contracts and laboratory consumables are kept as low as possible. In addition, assistance is given to researchers to enable smooth processing of their orders, to ensure best prices are used and to ensure orders comply with any requirements for import and with any regulatory requests.

Laboratory Support Services

Angela Miller, Tracy Shields, Abbie McFarlane, Dilhani Kahawela, Jessica Reynolds, Jonny Sawers, Kirstie McPherson, 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, *Drosophilia* 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, Emma Walker and Michael McTaggart.

Stocks are kept of a wide range of frequently used consumables from a variety of renowned scientific suppliers to ensure quality, high-use materials are available at all times. 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 a number of high-use tissue culture items.

Stores has introduced a Supply Centre with Thermo Fisher Scientific, holding consignment stock which is the property of the company until requested by the end user. A total of 46 research items are currently part of this, many of which are new products held by the Stores department, reducing the numbers of external orders to this supplier. The new items available are more research appropriate and 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 a number of items being purchased in bulk, in advance. By holding a set quantity in the supply centre, there is replenishment when required and this can be modified in line with research and project requirements.

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 make best use of the space available within the department, introduce new cost saving methods and ensure the items held within the Institute are essential to the world-renowned research that is ongoing at the Beatson.

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.

High-throughput plasmid (mini-prep) DNA purifications are currently performed on a Qiagen® Universal BioRobot®. Researchers provide pelleted overnight bacterial cultures that are processed by the facility. Sample numbers are consistently in the region of 17,000–19,000 per year. We also provide lower throughput, large-scale DNA purification (maxiprep) service from bacterial cultures. The majority of the samples processed through Mini/Maxi-prep are subsequently processed through our Sanger sequencing service.

Sanger sequencing is employed routinely to sequence and screen plasmids and PCR products for a large number of service users. Sanger sequencing is currently performed on an in-house Applied Biosystems® 3130xl (16 capillary) Sequencer which is capable of processing approximately 200 samples per day.

Human cell line authentication using the Promega GenePrint® 10 Kit is available as an internal service. The samples are run on the Applied Biosystems® 3130xl Sequencer (Gene Fragment Analysis) and analysed using Genemapper® v4.0 software (Applied Biosystems®). Regular cell line authentication is important to confirm integrity of data and is increasingly requested by journals as a requirement prior to publication.

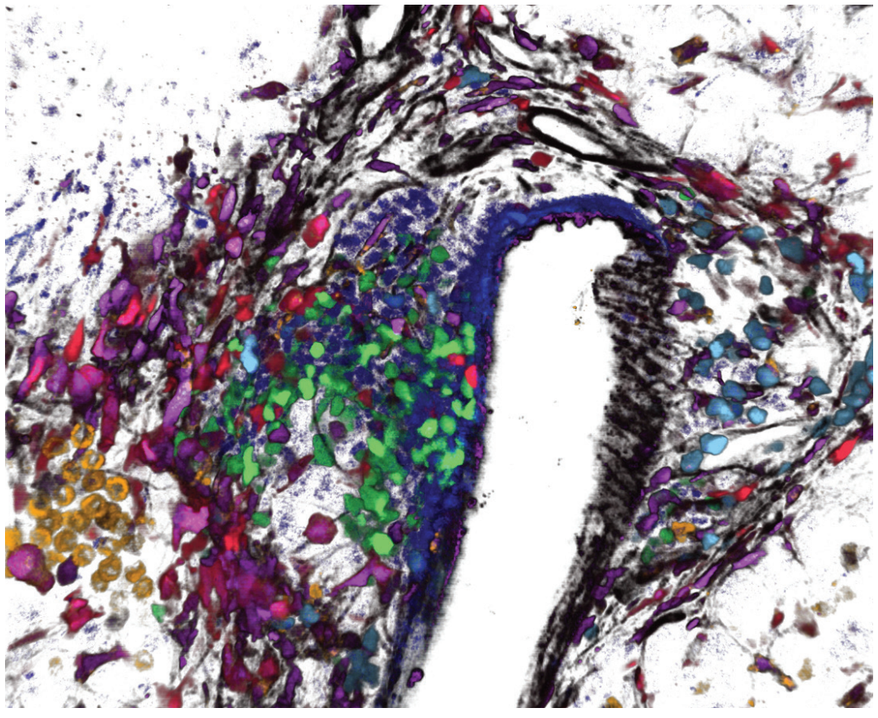
Mycoplasma screening is offered on a weekly basis, with researchers encouraged to have newly imported cell lines tested as soon as possible after arrival, in order to detect any infected cell lines early. Supernatant from cell lines are tested using the Venor GeM qONEStep Mycoplasma detection kit for qPCR (Cambio). They may also be tested by Hoechst staining to detect the presence of mycoplasma DNA.

The Molecular Technologies Service provides Next Generation Sequencing (NGS) services to all Beatson 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, with ancillary support provided by means of a Beckman Biomek FxP liquid handler, Tapestation 2200 for automated electrophoresis and a Qubit Flex fluorometer. A strong NGS footing has been established primarily in the area of bulk RNAseq, however this has recently been expanded to include Whole Exome Sequencing (WES) – human & mouse, Whole Genome Sequencing (WGS), custom DNA panels, and small RNA sequencing. We also support researchers performing their own library preparations, and regularly provide a sequencing only service to these researchers/groups.

Tell me what attracts you

Pulmonary meet up of leukocytes expressing different chemokine receptors in a tumour-associated perivascular area. Nuclei (blue), endothelia (black), macrophages (orange), chemokine receptors (red, purple, cyan and green)

Image by Lindsey Arnott & Ximena Raffo Iraolagoitia



PUBLICATIONS

Imran Ahmad (page 10)
Models of Advanced Prostate Cancer

Primary Research Papers

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Negative mpMRI rules out extra-prostatic extension in prostate cancer before robot-assisted radical prostatectomy. ***Diagnostics (Basel)***. 2022;12:1057.

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Tom Bird (page 12)
Liver Cancer, Disease and Regeneration

Primary Research Papers

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p53-mediated redox control promotes liver regeneration and maintains liver function in response to CCl4. ***Cell Death Differ.*** 2022;29:514-526.

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CXCR2 inhibition enables NASH-HCC immunotherapy. ***Gut***. 2022; 71:2093-2106.

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Neutrophils as potential therapeutic targets in hepatocellular carcinoma. ***Nat Rev Gastroenterol Hepatol***. 2022; 19: 257–273

Karen Blyth (page 14)
In Vivo Cancer Biology

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Hernandez-Fernaud JR, Athineos D, Dhayade S, Stepanova E, Gjerga E, Neilson LJ, Lilla S, Hedley A, Koulouras G, McGregor G, Jamieson C, Johnson RM, Park M, Kirschner K, Miller C, Kamphorst JJ, Loayza-Puch F, Saez-Rodriguez J, Mazzone M, Blyth K, Zagnoni M, Zanivan S.
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David Bryant (page 16)
Epithelial Polarity

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Traject3d allows label-free identification of distinct co-occurring phenotypes within 3D culture by live imaging. ***Nat Commun***. 2022;13:5317.

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Martin Bushell (page 18)
RNA and Translational Control in Cancer

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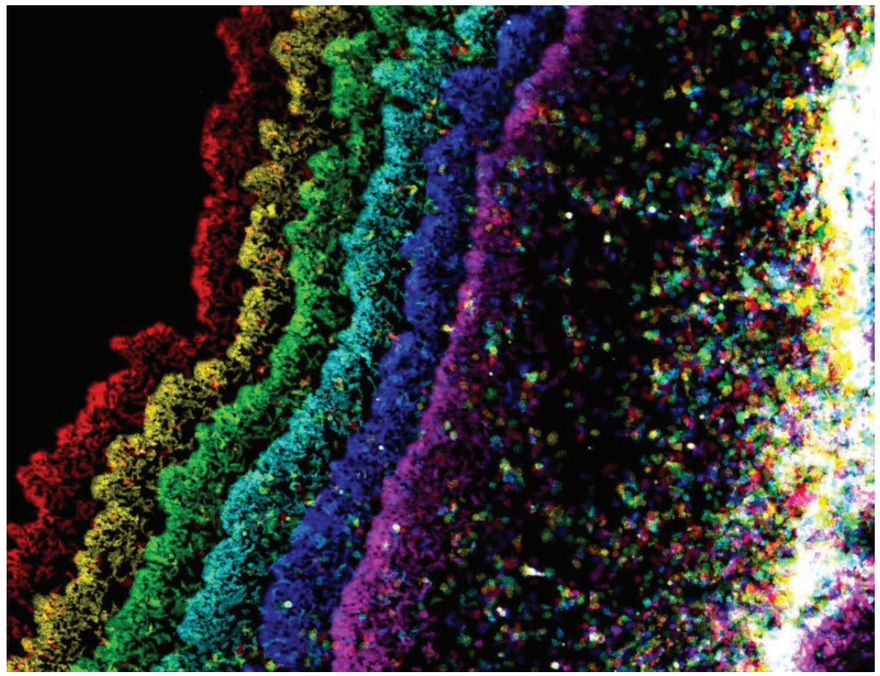
Pantaleao LC, Inzani I, Furse S, Loche E, Hufnagel A, Ashmore T, Blackmore HL, Jenkins B, Carpenter AAM, Wilczynska A, Bushell M, Koulman A, Fernandez-Twinn DS, Ozanne SE.
Maternal diet-induced obesity during pregnancy alters lipid supply to mouse E18.5 fetuses and changes the cardiac tissue lipidome in a sex-dependent manner. ***Elife***. 2022;11.



Vegetative amoebas expressing the cAMP sensor Flamindo2 were pulsed with cAMP for 4h hours (one pulse every 6 min) and settled in a well cut into the middle of a thin layer of agarose. The agarose contained the attractant cAMP too. Motivated by the presence of cAMP the cells crawl under the agarose. While moving forward the cells are secreting PDE which breaks down cAMP – this way forming a self-generated gradient. The result is the formation of a clear wave – only leader cells are moving forwards while other cells are left behind because there is not enough attractant left behind for them to sense.

The rainbow captures 6 distinct time point of the experiment as an overlay – displayed in 6 different colours.

Zeiss 880 Airyscan -fast mode – tile scan/ timelapse; 10x objective; 488 nm; snap of a single tile. LUT: gray – later coloured as a rainbow.

Image by Peggy Paschke

Ross Cagan (page 20)
Biology of Therapeutics

Other Publications
Cagan R, Shokat K.
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Leo Carlin (page 22)
Leukocyte Dynamics

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Humpton TJ, Hock AK, Kiourtis C, De Donatis M, Fercoq F, Nixon C, Bryson S, Strathdee D, Carlin LM, Bird TG, Blyth K, Vousden KH.
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CXCR2 inhibition enables NASH-HCC immunotherapy. ***Gut***. 2022; 71: 2093–2106

Seth Coffelt (page 24)
Immune Cells and Metastasis

Primary Research Papers
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Julia Cordero (page 26)
Local and Systemic Functions of the Adult Intestine in Health and Disease

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Vicky Cowling (page 28)
Gene Regulation

Primary Research Papers
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Jeff Evans

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Fieke Froeling (page 30)

Pancreatic Cancer Evolution and Therapeutic Development

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Payam Gammage (page 32)

Mitochondrial Oncogenetics

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Danny Huang (page 34)

Ubiquitin Signalling

Primary Research Papers

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Gareth Inman (page 36)

Growth Factor Signalling and Squamous Cancers

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Robert Insall (page 38)

Cell Migration and Chemotaxis

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Kristina Kirschner (page 40)

Stem Cell Ageing & Cancer

Primary Research Papers

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John Le Quesne (page 42)

Deep Phenotyping of Solid Tumours

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Hing Leung (page 44)
Prostate Cancer Biology

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αSmooth muscle actin/IL6 combined immunohistochemistry/ *in situ* hybridisation (IHC/ISH) staining on mouse PDAC tissue. αSMA IHC staining - brown, IL6 ISH staining – red, Nuclear stain – blue, x20 magnification

Image by Histology

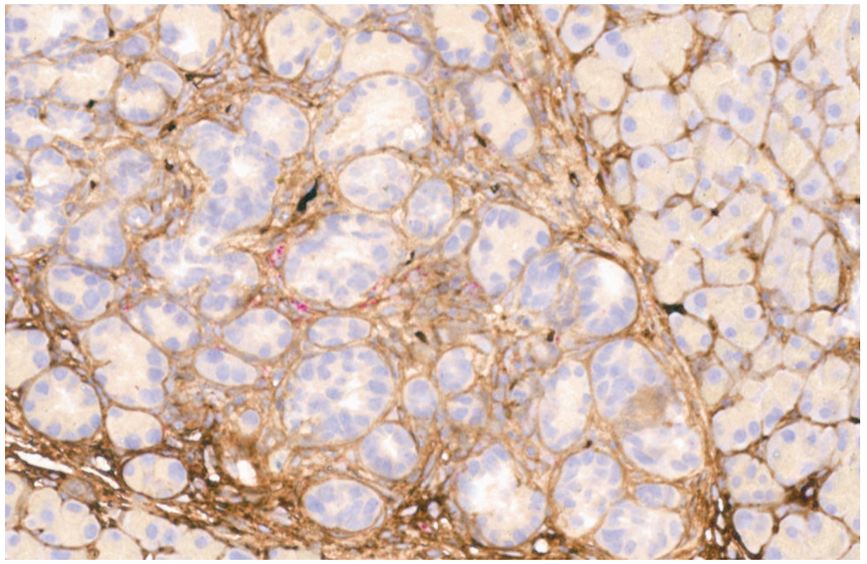

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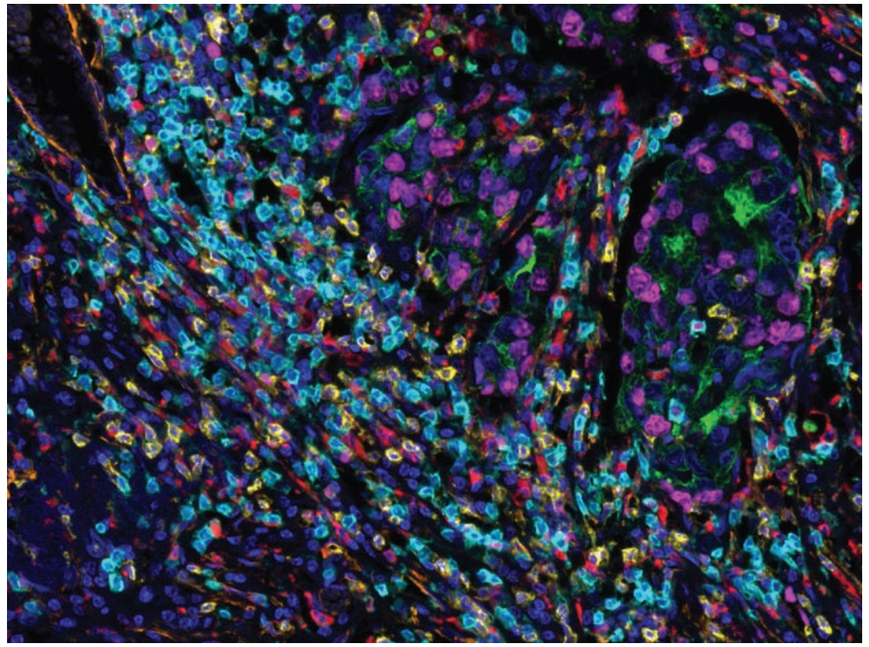
Image taken by Deep Phenotyping in Solid Tumours Group



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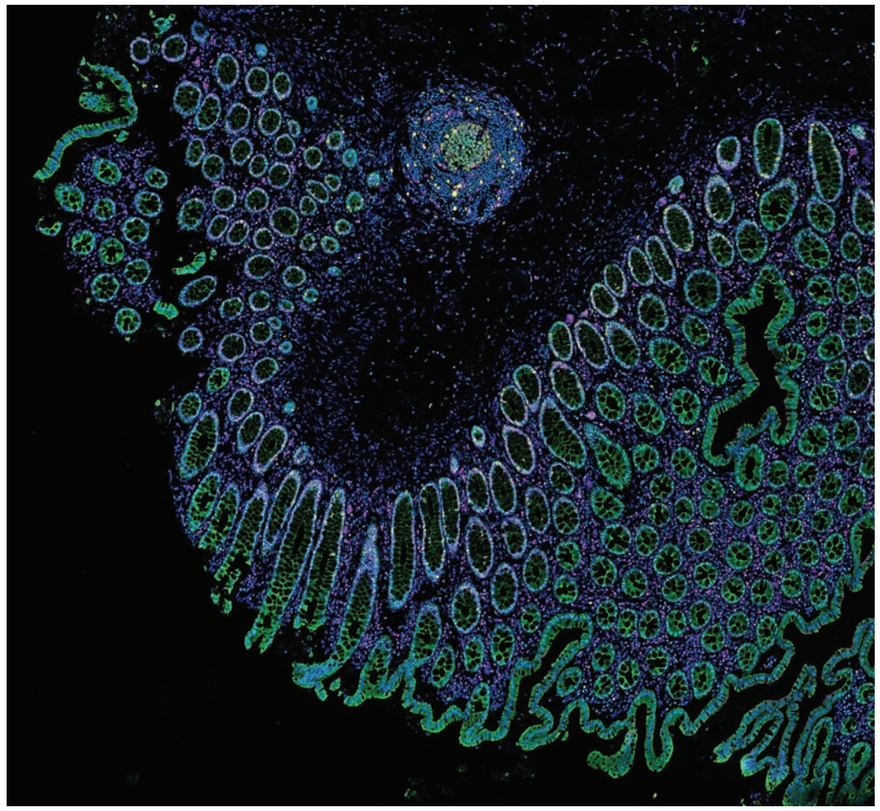

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Advanced Colorectal Cancer

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Pennel KA, Quinn JA, Nixon C, Inthagard J, van Wyk HC, Chang D, Rebus S, Group G, Hay J, Maka NN, Roxburgh CS, Horgan PG, McMillan DC, Park JH, Roseweir AK, Steele CW, Edwards J.
CXCL8 expression is associated with advanced stage, right sidedness, and distinct histological features of colorectal cancer. ***J Pathol Clin Res***. 2022;8:509-520.

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Douglas Strathdee (page 88)

Transgenic Technology

Primary Research Papers

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Glycan degradation promotes macroautophagy. ***Proc Natl Acad Sci U S A***. 2022;119:e2111506119.

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Epithelial TGFbeta engages growth-factor signalling to circumvent apoptosis and drive intestinal tumourigenesis with aggressive features. ***Nat Commun***. 2022;13:7551.

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Stephen Tait (page 70)
Mitochondria and Cancer Cell Death

Primary Research Papers
Cao K, Riley JS, Heilig R, Montes-Gomez AE, Vringer E, Berthenet K, Cloix C, Elmasry Y, Spiller DG, Ichim G, Campbell KJ, Gilmore AP, Tait SWG.
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Increased apoptotic sensitivity of glioblastoma enables therapeutic targeting by BH3-mimetics. ***Cell Death Differ***. 2022;29:2089-2104.

Zacharioudakis E, Agianian B, Kumar Mv V, Biris N, Garner TP, Rabinovich-Nikitin I, Ouchida AT, Margulets V, Nordstrom LU, Riley JS, Dolgalev I, Chen Y, Wittig AJH, Pekson R, Mathew C, Wei P, Tsirigos A, Tait SWG, Kirshenbaum LA, Kitsis RN, Gavathiotis E.
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Saverio Tardito (page 72)
Oncometabolism

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Kay EJ, Paterson K, Riera-Domingo C, Sumpton D, Dabritz JHM, Tardito S, Boldrini C, Hernandez-Fernaud JR, Athineos D, Dhayade S, Stepanova E, Gjerga E, Neilson LJ, Lilla S, Hedley A, Koulouras G, McGregor G, Jamieson C, Johnson RM, Park M, Kirschner K, Miller C, Kamphorst JJ, Loayza-Puch F, Saez-Rodriguez J, Mazzone M, Blyth K, Zagnoni M, Zanivan S.
Cancer-associated fibroblasts require proline synthesis by PYCR1 for the deposition of pro-tumorigenic extracellular matrix. ***Nat Metab***. 2022;4:693-710.

Oberkersch RE, Pontarin G, Astone M, Spizzotin M, Arslanbaeva L, Tosi G, Panieri E, Ricciardi S, Allega MF, Brossa A, Grumati P, Bussolati B, Biffo S, Tardito S, Santoro MM.
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Mesenchymal stromal cells cultured in physiological conditions sustain citrate secretion with glutamate anaplerosis. ***Mol Metab***. 2022;63:101532.

Sara Zanivan (page 74)
Tumour Microenvironment and Proteomics

Primary Research Papers
Baudot AD, Wang VM, Leach JD, O'Prey J, Long JS, Paulus-Hock V, Lilla S, Thomson DM, Greenhorn J, Ghaffar F, Nixon C, Helfrich MH, Strathdee D, Pratt J, Marchesi F, Zanivan S, Ryan KM.
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Blomme A, Peter C, Mui E, Rodriguez Blanco G, An N, Mason LM, Jamieson LE, McGregor GH, Lilla S, Ntala C, Patel R, Thiry M, Kung SHY, Leclercq M, Ford CA, Rushworth LK, McGarry DJ, Mason S, Repiscak P, Nixon C, Salji MJ, Markert E, MacKay GM, Kamphorst JJ, Graham D, Faulds K, Fazli L, Gleave ME, Avezov E, Edwards J, Yin H, Sumpton D, Blyth K, Close P, Murphy DJ, Zanivan S, Leung HY.
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Neuropilin 1 and its inhibitory ligand mini-tryptophanyl-tRNA synthetase inversely regulate VE-cadherin turnover and vascular permeability. ***Nat Commun***. 2022;13:4188.

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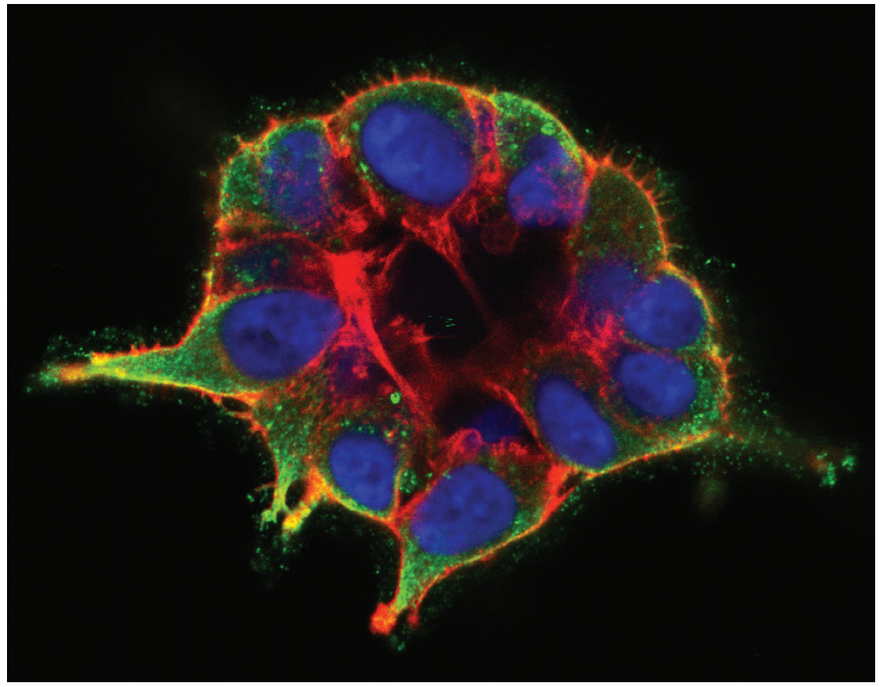
Cancer-associated fibroblasts require proline synthesis by PYCR1 for the deposition of pro-tumorigenic extracellular matrix. ***Nat Metab***. 2022;4:693-710.

McGarry DJ, Castino G, Lilla S, Carnet A, Kelly L, Micovic K, Zanivan S, Olson MF.
MICAL1 activation by PAK1 mediates actin filament disassembly. ***Cell Rep***. 2022;41:111442.

Narva E, Taskinen ME, Lilla S, Isomursu A, Pietila M, Weltner J, Isola J, Sihto H, Joensuu H, Zanivan S, Norman J, Ivaska J.
MASTL is enriched in cancerous and pluripotent stem cells and influences OCT1/OCT4 levels. ***iScience***. 2022;25:104459.

Confocal image of PC3 prostate cancer cell spheroid grown in extracellular matrix for 4 days. Fixed and stained with Alexa Fluor 568 Phalloidin (F-actin, shown in red), anti-ARF5 (ARF GTPase shown in green) and Hoechst (nuclei, blue).

Image by Emma Sandilands



Salji MJ, Blomme A, Dabritz JHM, Repiscak P, Lilla S, Patel R, Sumpton D, van den Broek NJF, Daly R, Zanivan S, Leung HY.
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Extracellular vesicles as central regulators of blood vessel function in cancer. ***Science Signaling***. 2022;15:eaaz4742.

Crispin Miller (page 52)

Bioinformatics & Data Science

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Epithelial TGFbeta engages growth-factor signalling to circumvent apoptosis and drive intestinal tumourigenesis with aggressive features. ***Nat Commun***. 2022;13:7551.

Freckmann EC, Sandilands E, Cumming E, Neilson M, Roman-Fernandez A, Nikolatou K, Nacke M, Lannagan TRM, Hedley A, Strachan D, Salji M, Morton JP, McGarry L, Leung HY, Sansom OJ, Miller CJ, Bryant DM.
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p53-mediated redox control promotes liver regeneration and maintains liver function in response to CCl4. ***Cell Death Differ***. 2022;29:514-526.

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Cancer-associated fibroblasts require proline synthesis by PYCR1 for the deposition of pro-tumorigenic extracellular matrix. ***Nat Metab***. 2022;4:693-710.

Leslie J, Mackey JBG, Jamieson T, Ramon-Gil E, Drake TM, Fercoq F, Clark W, Gilroy K, Hedley A, Nixon C, Luli S, Laszczewska M, Pinyol R, Esteban-Fabro R, Willoughby CE, Haber PK, Andreu-Oller C, Rahbari M, Fan C, Pfister D, Raman S, Wilson N, Muller M, Collins A, Geh D, Fuller A, McDonald D, Hulme G, Filby A, Cortes-Lavaud X, Mohamed NE, Ford CA, Raffo Iraolagoitia XL, McFarlane AJ, McCain MV, Ridgway RA, Roberts EW, Barry ST, Graham GJ, Heikenwalder M, Reeves HL, Llovet JM, Carlin LM, Bird TG, Sansom OJ, Mann DA.
CXCR2 inhibition enables NASH-HCC immunotherapy. ***Gut***. 2022; 71:2093–2106

Taurino G, Deshmukh R, Villar VH, Chiu M, Shaw R, Hedley A, Shokry E, Sumpton D, Dander E, D'Amico G, Bussolati O, Tardito S.
Mesenchymal stromal cells cultured in physiological conditions sustain citrate secretion with glutamate anaplerosis. ***Mol Metab***. 2022;63:101532.

David Sumpton (page 82)

Metabolomics

Primary Research Papers

Blomme A, Peter C, Mui E, Rodriguez Blanco G, An N, Mason LM, Jamieson LE, McGregor GH, Lilla S, Ntala C, Patel R, Thiry M, Kung SHY, Leclercq M, Ford CA, Rushworth LK, McGarry DJ, Mason S, Repiscak P, Nixon C, Salji MJ, Markert E, MacKay GM, Kamphorst JJ, Graham D, Faulds K, Fazli L, Gleave ME, Avezov E, Edwards J, Yin H, Sumpton D, Blyth K, Close P, Murphy DJ, Zanivan S, Leung HY.
THEM6-mediated reprogramming of lipid metabolism supports treatment resistance in prostate cancer. ***EMBO Mol Med***. 2022;14:e14764.

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Multi-omics & pathway analysis identify potential roles for tumor N-acetyl aspartate accumulation in murine models of castration-resistant prostate cancer. ***iScience***. 2022;25:104056.

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Mesenchymal stromal cells cultured in physiological conditions sustain citrate secretion with glutamate anaplerosis. ***Mol Metab***. 2022;63:101532.

Sara Zanivan (page 84)

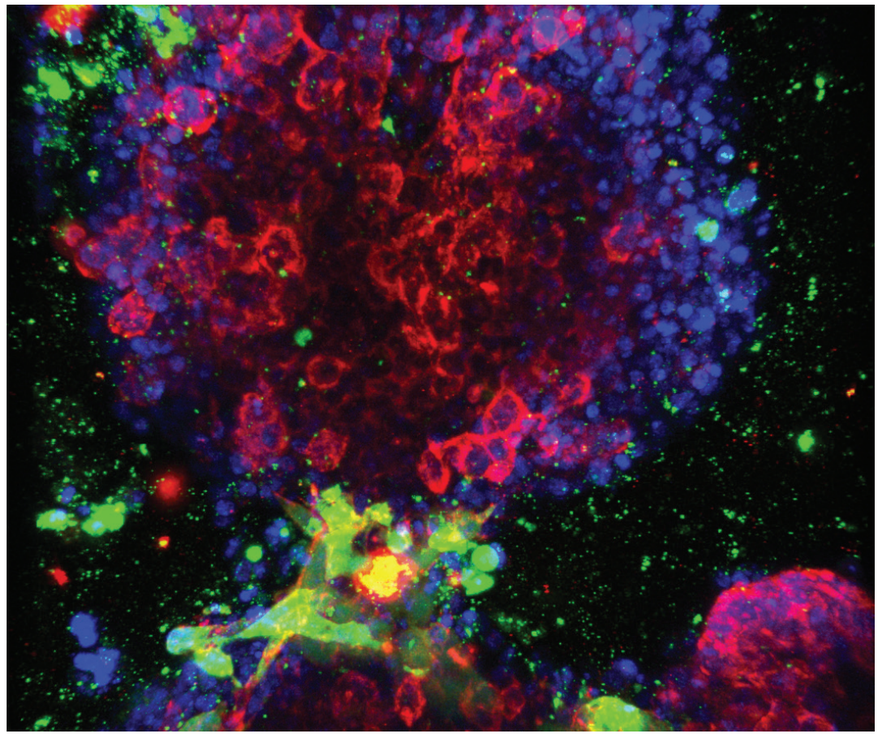
Proteomics

Primary Research Papers

Baudot AD, Wang VM, Leach JD, O'Prey J, Long JS, Paulus-Hock V, Lilla S, Thomson DM, Greenhorn J, Ghaffar F, Nixon C, Helfrich MH, Strathdee D, Pratt J, Marchesi F, Zanivan S, Ryan KM.

This image shows GFP-tagged pancreatic ductal adenocarcinoma (PDAC) cells (green) interacting with liver spheroids in a synthetic fibronectin-poly (ethylene glycol) hydrogel. The liver spheroids are stained with Phalloidin (red) and DAPI (blue). The PDAC cells appear to interact with the liver spheroids and with some artistic licencing you could say this image shows colourful chemistry between the cell types. This image was taken using a Nikon A1R confocal microscope.

Image taken by Elaine Ma



Glycan degradation promotes macroautophagy. ***Proc Natl Acad Sci U S A***. 2022;119:e2111506119.

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MASTL is enriched in cancerous and pluripotent stem cells and influences OCT1/OCT4 levels. ***iScience***. 2022;25:104459.

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Multi-omics & pathway analysis identify potential roles for tumor N-acetyl aspartate accumulation in murine models of castration-resistant prostate cancer. ***iScience***. 2022;25:104056.

David Lewis (page 90)

Translational Molecular Imaging

Primary Research Papers

Koessinger AL, Cloix C, Koessinger D, Heiland DH, Bock FJ, Strathdee K, Kinch K, Martinez-Escardo L, Paul NR, Nixon C, Malviya G, Jackson MR, Campbell KJ, Stevenson K, Davis S, Elmasry Y, Ahmed A, O'Prey J, Ichim G, Schnell O, Stewart W, Blyth K, Ryan KM, Chalmers AJ, Norman JC, Tait SWG.
Increased apoptotic sensitivity of glioblastoma enables therapeutic targeting by BH3-mimetics. ***Cell Death Differ***. 2022;29:2089-2104.

Soloviev D, Dzien P, Mackintosh A, Malviya G, Brown G, Lewis D.
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Taralli S, Quartuccio N, Malviya G.
Editorial: Case reports in PET imaging. ***Front Med (Lausanne)***. 2022;9:1087583.

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Fasting improves therapeutic response in hepatocellular carcinoma through p53-dependent metabolic synergism. ***Sci Adv***. 2022;8:eabh2635.

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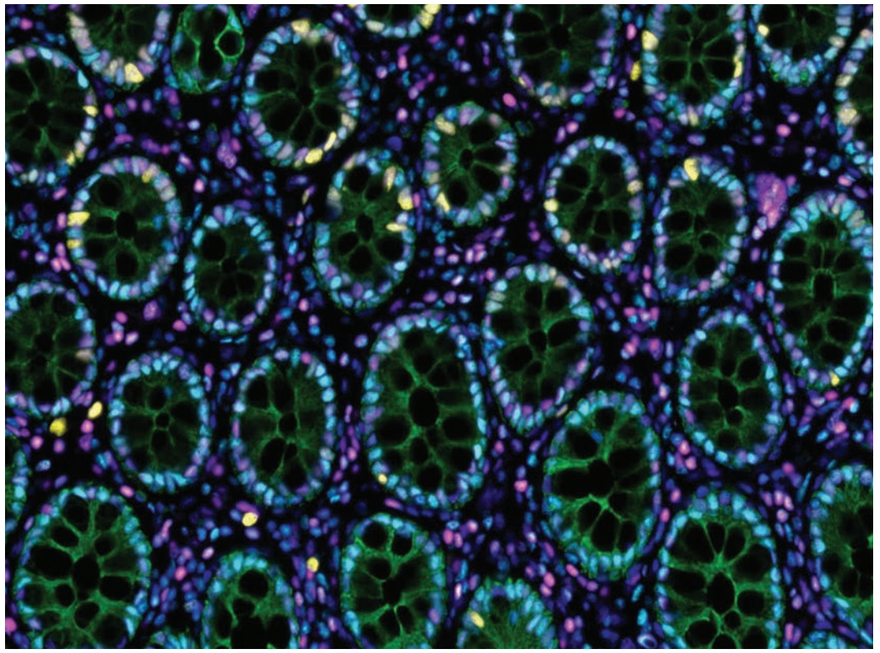
Protocols for understanding the redox behavior of copper-containing systems. ***ACS Omega***. 2022;7:45057-45066.

Razis E, Escudero MJ, Palmieri C, Mueller V, Bartsch R, Rossi G, Gampenrieder SP, Kolberg HC, Zdenkowski N, Pavic M, Connolly RM, Rosset L, Arcuri J, Tesch H, Vallejos C, Retamales J, Musolino A, Del Mastro L, Christodoulou C, Aebi S, Paluch-Shimon S, Gupta S, Ohno S, Macpherson I, Ekholm M, Zaman K, Vidal M, Chakiba C, Fumagalli D, Thulin A, Witzel I, Kotecki N, Gil-Gil M, Linderholm B.

Multiplex staining on a normal human colon section using antibody markers: nucleoli (cyan), Ki67 (yellow), mina (magenta), and pan-CK (green).

Image taken by the Deep Phenotyping in Solid Tumours Group



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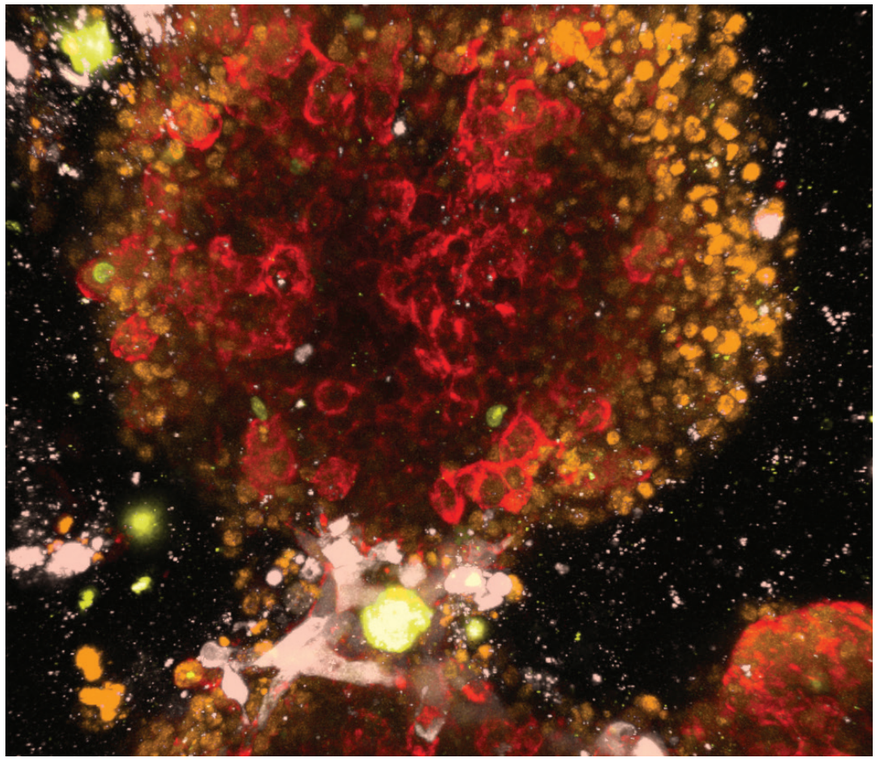
Winder ML, Campbell KJ.
MCL-1 is a clinically targetable vulnerability in breast cancer. ***Cell Cycle***. 2022;21: 1439-1455

This image shows co-culture of pancreatic ductal adenocarcinoma cells (pink) and hepatocyte spheroids. Fibronectin (red) outlines the spheroids and positive ki67 staining (orange) shows the spheroids contain healthy proliferating cells. The aim of this co-culture is to develop a 3D in-vitro model of pancreatic ductal adenocarcinoma metastasis to the liver. This image was taken using a Nikon A1R confocal microscope.

Image taken by Elaine Ma



John Paul Career Award

All final year PhD students at the Beatson 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 Michalis Gounis from Jim Norman's group. He has been investigating the relationship between cell metabolism and exosome release in metastatic breast cancer.

THESES

Allega, Maria Francesca (2022) Identification of dexamethasone-induced metabolic vulnerabilities in glioblastoma [PhD thesis, University of Glasgow, Beatson Institute]

Dowdell, Adam (2022) Controlling chemotaxis: A study on novel migration behaviours in multi-signal systems [PhD thesis, University of Glasgow, Beatson Institute]

Freckmann, Eva (2022) Building the tools for identifying heterogeneity in 3D [PhD thesis, University of Glasgow, Beatson Institute]

Gounis, Michalis (2022) An investigation into the relationship between cellular metabolism and small extracellular vesicle release in metastatic breast cancer [PhD thesis, University of Glasgow, Beatson Institute]

Harris, Rachel (2022) Investigating the metabolic and therapeutic effects of combined glutaminase and mutant-BRAF inhibitors in melanoma [PhD thesis, University of Glasgow, Beatson Institute]

Hartley, Andrew (2022) Investigating ARID1A in Prostate Cancer [PhD thesis, University of Glasgow, Beatson Institute]

Khan, Adiba (2022) Investigating the role of CBFB in breast cancer [PhD thesis, University of Glasgow, Beatson Institute]

Lawrence, Mark (2022) Understanding the role of γδ T cells in pancreatic cancer metastasis. [PhD thesis, University of Glasgow, Beatson Institute]

Medina, André (2022) Characterization of Drosophila enteroendocrine cells and their neuroendocrine role in intestinal health and disease [PhD thesis, University of Glasgow, Beatson Institute]

Nikolatou, Konstantina (2022) The role of the small GTPase ARF6 in Pten-null ovarian cancer [PhD thesis, University of Glasgow, Beatson Institute]

Puoti, Ilaria (2022) Deciphering tumour-stroma crosstalk signalling driven by Trp53 and Pten loss in high grade serous ovarian cancer [PhD thesis, University of Glasgow, Beatson Institute]

Rink, Curtis (2022) Investigating PI3K/AKT/mTOR Signalling in Pancreatobiliary Cancer [PhD thesis, University of Glasgow, Beatson Institute]

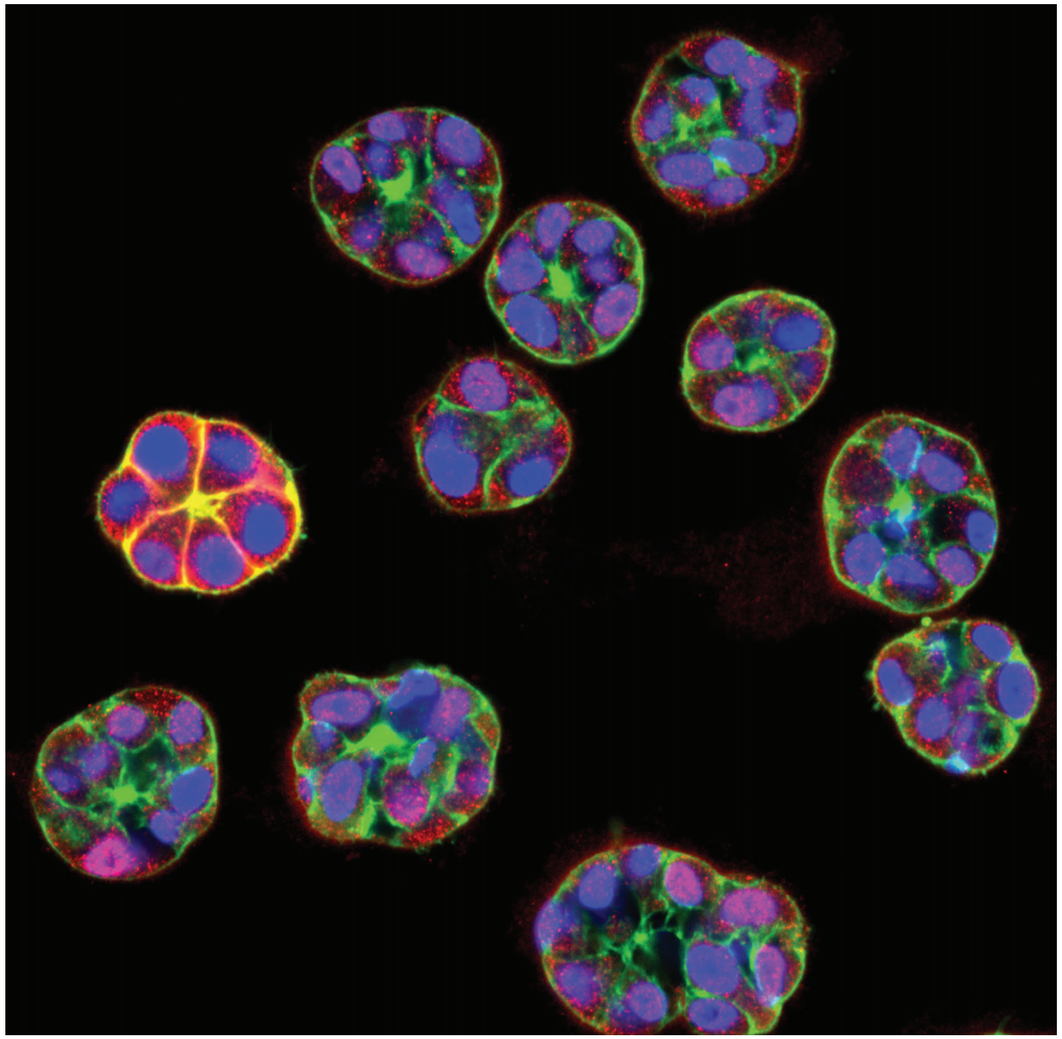
Samarakoon, Youhani (2022) Ciliary regulators of cytotoxic T cell signaling and their roles in T acute lymphocytic leukemia [PhD thesis, University of Glasgow, Beatson Institute]

Sweeney, Kerri (2022) Investigating the tumour suppressor function of RUNX1 in breast cancer [PhD thesis, University of Glasgow, Beatson Institute]

Vringer, Esmee (2022) Understanding the immunogenicity of caspase-independent cell death. [PhD thesis, University of Glasgow, Beatson Institute]

Whyte, Declan (2022) The role of NUAK1 in pancreatic cancer. [PhD thesis, University of Glasgow, Beatson Institute]

Zeiger, Lucas (2022) Targeting PI3-Kinase Signalling in Genetically Engineered Mouse Models of Intestinal Cancer [PhD thesis, University of Glasgow, Beatson Institute]



Maximum intensity projection of PC3 prostate cancer cell line forming spheroids when embedded in extracellular matrix. Fixed and stained with Alexa Fluor 488 Phalloidin (F-actin, shown in green), anti-E-cadherin (red) and Hoechst (nuclei, blue).

Image by Emma Sandilands

CONFERENCES AND WORKSHOPS

An immunological lens for cancer

7 – 8 July 2022
CRUK Beatson Institute, Glasgow
Organisers: Megan Macleod, Ed Roberts

Immuno-oncology is of considerable interest to a number of groups at the Institute as well as within the University of Glasgow. The focus of this meeting, led by junior group leader Ed Roberts and colleague Megan Macleod from the School of Infection & Immunity, lined up a high calibre of scientists whose work offers novel immunological understanding relevant to cancer. The invited speakers spanned the spectrum of development, control and treatment of cancer and included keynote speakers Jessica Strid (Imperial College London) and Guido Kroemer (INSERM).

Beyond the exciting scientific exchange, a key aim of the workshop was to reflect on research culture as a whole. Invited speakers were encouraged to share their slot with either a PhD student or postdoc providing more opportunities for Early Career Researchers to present their work and network with attendees. In a session of guided round table discussions attendees talked about challenges around diversity in research, alternative careers, work/life balance and career progression.

The workshop was generously sponsored by BMG Labtech, Miltenyi Biotech, PCR Biosystems, Amsbio, Beckman Coulter, Proteintech. We are also grateful for additional support by The Company of Biologist and Cancer Research UK.

Problems in the Powerhouse: Mitochondrial Dysfunction in Cancer

24 – 25 October 2022
CRUK Beatson Institute, Glasgow
Organisers: Payam Gammage, Tom MacVicar, Stephen Tait

The second workshop of the year focused on mitochondria as crucial regulators of cancer. Organisers junior group leaders Payam Gammage and Tom MacVicar together with Prof Stephen Tait brought together an exciting programme of speakers, including Christian Frezza (University of Cologne), Nora Kory (Harvard University), Laura Greaves (University of Newcastle) and Eyal Gottlieb (Israel Institute of Technology), to address the role of the organelle in metabolism, cell death, inflammation and genetic dysregulation.

Thank you! - to Abcam for the generous sponsorship of this workshop.

Scottish Biomedical Postdoctoral Researcher Conference
Left - Keynote lecture by Ian Ganley from the University of Dundee
Right - the conference was generously supported by the Scottish Universities Life Sciences Alliance

Scottish Biomedical Postdoctoral Researcher Conference
Top - Hannah Donnelly who received the prize for People's Choice Presentation
Bottom - this year's organising committee

Beatson International Cancer Conference 2023

Interorgan Communication in Cancer

16-19 July 2023
Scientific Committee: Kevin Ryan, Julia Cordero (Co-Chair), David Lewis, Jim Norman, Ed Roberts, Saverio Tardito

While significant advances in cancer biology have been made from research on individual and collective properties of cancer cells, studying tumours within a multiorgan setting is essential to identify tumour intrinsic and systemic principles involved in cancer initiation, progression and spread. In 2023, we will see the return of the Beatson International Cancer Conference to the Institute on the Garscube Estate. We have developed a strong programme of world-leading researchers themed around Interorgan Communication in Cancer. We will discuss how the use of *in vivo* model organisms combined with genetic, metabolic, physiology, immune and behavioural studies is necessary to recapitulate the full complexity of cancer as well as to understand whole body responses to tumours. Alongside an exciting line-up of invited speakers, we are looking forward to a keynote lecture by Eileen White, Rutgers Cancer Institute of New Jersey. Four thematically themed sessions invite the speakers and delegates to connect on a scientific basis which is complemented by a fantastic social programme including a barbecue dinner and a traditional Scottish Ceilidh at the scenic Kelvingrove Museum. We are looking forward to welcoming you back to Glasgow!

Visit www.beatson.gla.ac.uk/conf for more details and to register

Scottish Biomedical Postdoctoral Researcher Conference

Postdoctoral scientists at the Institute, in particular Nuray Gunduz, together with their colleagues at the Institute of Genetics and Cancer (University of Edinburgh) and MRC Protein Phosphorylation and Ubiquitylation Unit (University of Dundee), were delighted to host the 7th edition of this symposium on the 11th Nov 2022. The meeting included short research talks and poster presentations as well as a keynote lecture by Ian Ganley (University of Dundee). Congratulations go to Beatson postdocs Hannah Donnelly and Stephanie May for People's Choice Presentation and the runner-up poster prize, respectively.

We would like to thank SULSA, Proteintech, Stratech, Starlab, Thistle Scientific and Transnetyx for supporting this meeting.

SEMINARS

The following seminars were held at the Cancer Research UK Beatson Institute during 2022.

February

Mariam Jamal-Hanjani, UCL Cancer Institute, London, UK

Philip Dunne, Patrick G Johnston Centre for Cancer Research, Queen's University Belfast, Belfast, UK

March

Hind Medyouf, Institute for Tumour Biology and Experimental Therapy, Frankfurt, Germany

Audrey Gerard, The Kennedy Institute of Rheumatology, University of Oxford, UK

April

Kathryn Lilley, Department of Biochemistry, University of Cambridge, UK

Massimiliano Mazzone, VIB Center for Cancer Biology, University of Leuven, Belgium

Anne Willis, MRC Toxicology Unit, University of Cambridge, UK

May

Jason Lewis, Memorial Sloan Kettering Cancer Center, New York, USA

Kendle Maslowski, Institute of Immunology and Immunotherapy, University of Birmingham, UK

Sarah Garnish, Walter & Eliza Institute, University of Melbourne, Australia

June

Claudio Cantù, Wallenberg Centre for Molecular Medicine, Linköping University, Sweden

Anne Kiltie, Rowett Institute, University of Aberdeen, UK

Dan Davis, Department of Life Sciences, Imperial College London, UK

August

Claus Jørgensen, CRUK Manchester Institute, UK

Mark Ashe, Division of Molecular and Cellular Function, University of Manchester, UK

Maria Libera Ascierto, Saint John's Cancer Institute, Santa Monica, USA

September

Alan Parker, Division of Cancer and Genetics, University of Cardiff, UK

Gail McConnell, Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, UK

October

Sara Sigismund, Department of Oncology and Hemato-Oncology, University of Milan, Italy

Steven Pollard, Edinburgh Cancer Research, University of Edinburgh, UK

Rob Bristow, Manchester Cancer Research Centre, UK

November

Zoi Diamantopoulou, Swiss Federal Institute of Technology, Zürich, Switzerland

Patrycja Kozik, MRC Laboratory of Molecular Biology, Cambridge, UK

Adam Sharp, Institute of Cancer Research, London, UK

Eugenia Piddini, School of Cellular and Molecular Medicine, University of Bristol, UK

Irene Roberts, Department of Paediatrics, University of Oxford, UK

Jerome Korzelius, School of Biosciences, University of Kent, UK

Marine Collart, Department of Microbiology and Molecular Medicine, University of Geneva, Switzerland

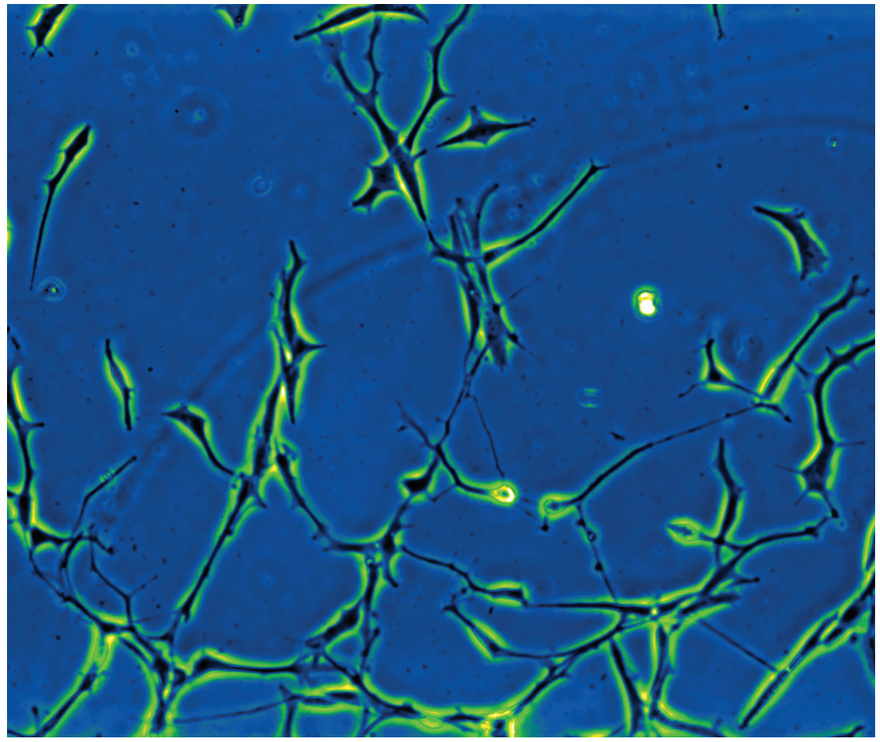
December

Dek Woolfson, School of Chemistry, University of Bristol, UK

Pat Caswell, Division of Cell Matrix Biology & Regenerative Medicine, University of Manchester, UK

GBM E2 cells were imaged in an Insall chamber. Plated on fibronectin, the cells form long protrusions to move away from the well they were seeded. On top of this, cells are connecting each with the protrusions what results in a net like structure. Nikon TE timelapse. 10x objective; phase contrast; snap of a timelapse movie. LUT: biop electric indigo.

Image taken by Peggy 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

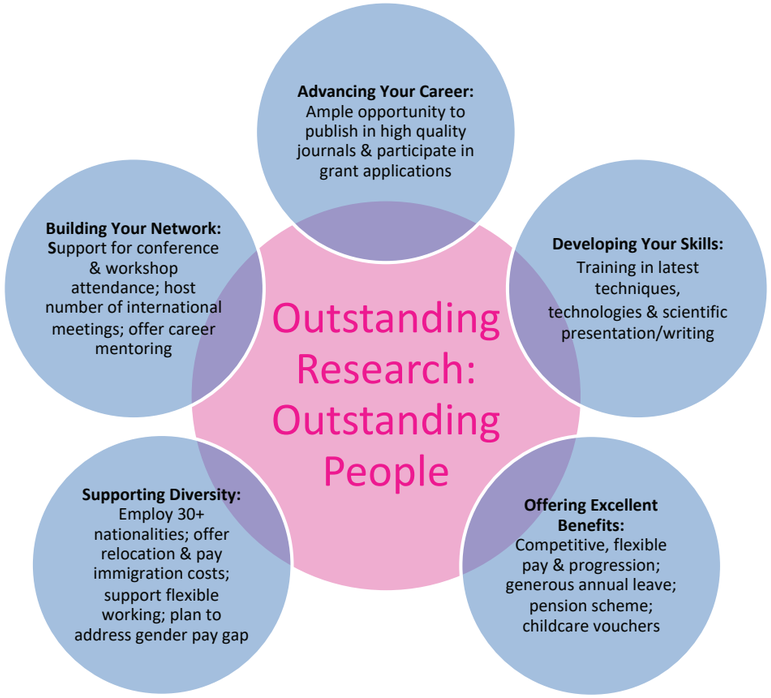
We see postdocs as pillars of the research and intellectual activities of their own groups and of the Institute as a whole. Our postdoctoral training, which is overseen by a senior member of the Institute (Professor Jen Morton), is designed to promote the development of outstanding and dedicated early 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.beatson.gla.ac.uk.

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

POSTDOC OPPORTUNITIES AT THE BEATSON



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.

2022 continued to be a challenging year as the Institute started to return to normal levels of activity following the devastating impact of Covid-19. This has been against a backdrop of reduced funding and increased competition for grants. However, the Institute is in a healthy financial position through careful management of costs and the McNab legacy received in 2019/20.

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.

Human Resources

Angela Stuart FCIPD, Elaine Marshall ACIPD, Selina Mungall ACIPD, Barbara Laing

Our vision is to be a Human Resources team that is professional, open, inclusive and collaborative. Our professionally qualified Human Resources team provides support and advice across a wide spectrum of issues, including recruitment, performance management, learning and development, pay and grading, absence management, employee relations and employee engagement. They also play a vital role in providing managers with the necessary budgetary and legal information with the aim of helping managers to more effectively manage their key resources – our people. In 2022, much of HR's focus was on managing staff back to work after the pandemic, including the introduction of a hybrid working policy. We recruited close to 70 new staff and students in 2022 making it our busiest year ever for recruitment of talented research and support staff. We also continued to work with colleagues focusing on the Institute's Equality, Diversity and Inclusion agenda with the training of our EDI Advocates so that they can best advise and represent staff and students across the Institute. Against a backdrop of continuing difficult financial circumstances, we reviewed our pay and grading structure – in collaboration with CRUK colleagues in our Manchester and Cambridge Institutes and offered a pay increase of 4%. In addition we were able to offer a one-off payment to staff in December to recognise the difficult financial climate that we are living in at present.

Administration

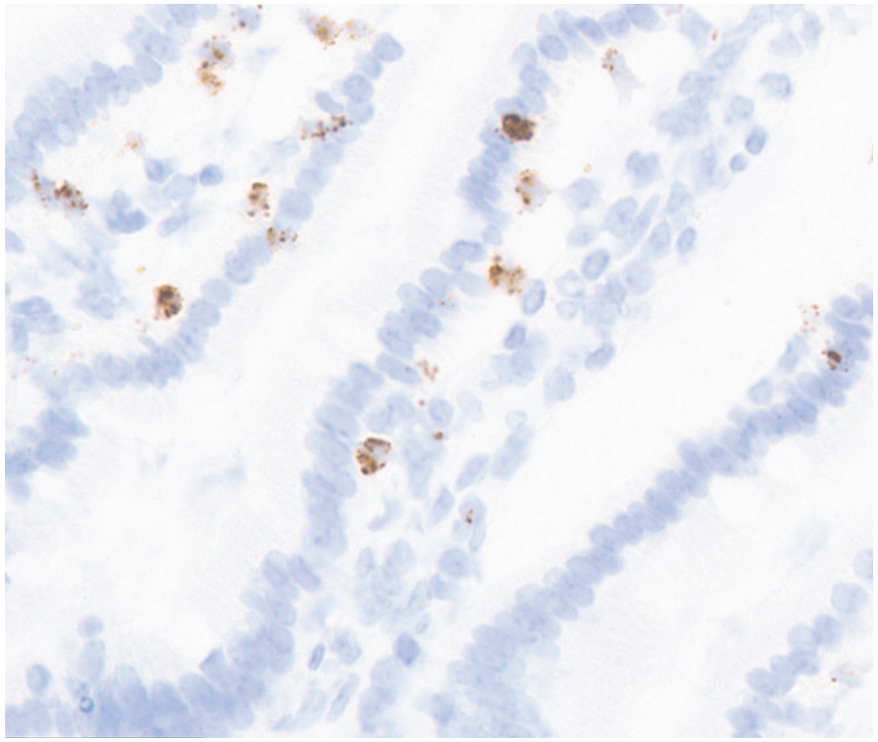
Carole Mumford (Senior Administrator), Catriona Entwistle (Receptionist/Administrator), Rebecca Gebbie (PA to Director), Shona McCall (PA to Director)

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

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.

EQUALITY, DIVERSITY AND INCLUSION

The Beatson is committed to promoting equality, diversity and inclusion (EDI) within our community. We value equity in our actions and deeds, diversity and inclusion within our workforce and collaborators, and the diversity of thought this brings. We already offer a range of leading family-friendly, inclusive employment policies, including up to 6 months full pay for those on maternity leave and 6 weeks full pay for shared parental leave. We also offer flexible and hybrid working opportunities, support for staff through various forums and mentoring and coaching opportunities and have strong links with the School of Cancer Sciences, University of Glasgow VOICE (Athena Swan) Committee. This includes hosting frequent seminars and events aimed at giving all staff the opportunity to develop and have a voice in how we enact our commitments to EDI.

We provided training for our EDI Advocates so that they are now equipped to advise and listen to any issues or ideas that staff and students wish to make with regard to our EDI. Along with the University of Glasgow VOICE Committee, we continued to hold a series of seminars and events for LGBT and Neurodiverse colleagues and their allies. We also continued to use search committees for more senior appointments, resulting in the appointment of 2 female junior research fellows at the Institute and we recruited Prof. Victoria Cowling as a Senior Group Leader. Finally, we published our gender pay gap report for 2022 , which is under constant review by our senior management team and Board of Directors, and details of our EDI vision and aims:

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

Addressing the gender pay gap at the Cancer Research UK Beatson 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 Beatson 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 Beatson 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. Table 1 shows that compared to 2021, the mean hourly pay gap between females and males increased by 1.2 percentage points and the median hourly pay gap increased by 2.16 percentage points in 2022.

Table 1: Pay Gender from April 2020 to April 2022

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.

Pay Gender			Difference		
(£/hour)	Female	Male	**Apr-22**	Apr-21	Apr-20
Mean	16.87	19.05	**11.44%**	10.24%	13.20%
Median	16.71	19.04	**12.24%**	10.08%	11.10%

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 Beatson, 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?

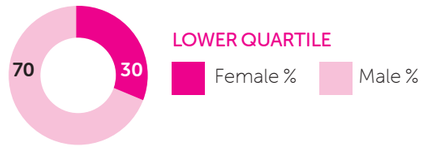
In 2022, our gender pay gap increased slightly with the mean difference between female and male salaries increasing 1 percentage point and the median increasing 2.16 percentage points. To understand this increase, it is important to reflect on our recruitment activities over that period. In the past year, we have recruited an equal split of males and females. However, the majority of newly appointed females (59% of all females appointed during this period) were recruited into lower-level positions in quartiles 1 and 2. The majority of newly appointed males were recruited into more senior positions in quartiles 3 and 4 (59% of all males appointed during this period). We continue to work to improve our gender pay gap for staff already employed by us, with the number of females in quartiles 3 and 4 increasing each year. In 2022, the %age of females in Quartiles 3 and 4 increased by 18% and 4% respectively.
In addition, we continue to take a deep dive into our grades to review any discrepancies in pay between males and females and make adjustments to female salaries accordingly. In 2022, 58% of all promotions were women and 76% of advancements in grades (salary increases above our cost-of-living increase) were also women.

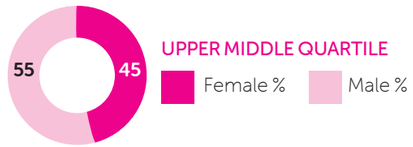
It is important to note that our senior faculty e.g. Group Leaders, are not reflected in our gender pay gap analysis. This is because they are employed on hybrid contracts and are technically employed by the University of Glasgow. However, the CRUK Beatson Institute determines their pay and grading, and we think it is important to highlight our pay gender ratios with them included. You will find these in Table 2 below.

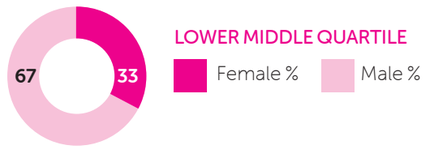
Table 2: All staff pay gender April 2022

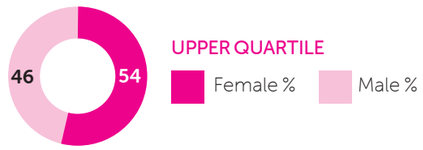
Pay Gender April 22 All staff, including Group Leaders on hybrid contracts			Calculations
(£/hour)	Female	Male	**Apr-22**
Mean	17.60	22.05	**20.16%**
Median	17.20	19.52	**11.88%**

In 2022, our workforce was 40% male and 60% 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 recruited female staff have been recruited to the lower and lower middle quartiles (70% and 67% respectively of all staff recruited within these quartiles). Compared to 2021, the number of females in the upper middle quartile has increased by 18 percentage points and the upper quartile by 4 percentage points. This is a result of career progression and promotion of women already employed at the Institute, with 58% of all promotions and 76% of all advancements in grade awarded to women in 2022.









Comparison of Quartiles 2020 to 2022

	M-2020	F-2020	M-2021	F-2021	M-2022	F-2022
Lower Quartile	28%	72%	34%	66%	30%	70%
Lower Middle Quartile	42%	58%	37%	63%	33%	67%
Upper Middle Quartile	48%	52%	48%	52%	45%	55%
Upper Quartile	61%	39%	59%	41%	54%	46%

What are we doing to close our gender pay gap?

The CRUK Beatson 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 Beatson 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 is 51.5% (WISE Campaign Report June 2022).

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. Between 2020 and 2022, two thirds of our newly appointed postdocs were 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 Group Leader. We have introduced search committees for senior research positions such as Group Leader and Chair appointments in order to spread our net wider when recruiting to include more women and people with other protected characteristics and more recently, we had a 60% appointment rate of women to Junior Faculty positions.

In recent years, an increasing number of female postdocs at the Institute have taken maternity leave and we have been able to support their return to work through extension of their temporary contracts and cover for their project during their maternity leave. This has provided them with more time to develop their scientific track record and potentially compete for a PI position.

Review of areas for improvement from 2021

We will conduct another detailed review of our grades to identify where any PDR2G issues exist and take what financial and other measures we can to address these.

- In 2022, 58% of all promotions were of women and 76% of advancements in grades (salary increases above our cost-of-living increase) were also of women.

We will continue to breakdown attitudes to flexible working patterns for more senior scientific researchers.

- We continue to offer flexible working and hybrid working arrangements, with a small increase in the number of senior staff working more flexibly or in hybrid working arrangements.

We will continue to review our senior level recruitment practices and aim for 50% female applicant shortlists.

- In 2022, we advertised for Junior Faculty positions. The results of this campaign are shown below.

	Application Stage	Shortlisting Stage	Offer/ Acceptance Stage
Male	75%	55%	40%
Female	25%	45%	60%

We will report more widely with respect to Equality, Diversity and Inclusion by collating the necessary data, identifying gaps and initiating an action plan to ensure equity in our recruitment, retention and development practices. We believe this will be part of a cultural shift at this Institute, which will encompass bridging our gender pay gap.

- We have reported on the results of our 2021 EDI survey and are currently holding focus groups to involve staff in our EDI plan for the future.
- We now capture EDI data during recruitment, and this has led to better compliance in the provision of data to allow us to identify gaps, benchmark and make improvements.
- We now have established and fully trained EDI advocates with a remit to raise awareness, promote best practice, and support and advise staff and students across the Institute.

In Summary

It is encouraging that the percentage of women in the upper quartile has risen in 2022 by 4% points, though there is still more to do in terms of female appointments at this level. As we do every year, we will also be reviewing salaries at all levels in 2023 to ensure equity by job and grade.

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 Beatson 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 Beatson Institute

Tom Bird
Aligos Therapeutics, Tenovus, University of Edinburgh

Karen Blyth
Breast Cancer Now, MRC, Omideon

Martin Bushell
BBSRC, Celgene, MRC

Kirsteen Campbell
Prostate Cancer Research

Leo Carlin
Breast Cancer Now, Medical Research Scotland

Vicky Cowling
ERC, MRC, Wellcome Trust

Payam Gammage
EPSRC, NIH

Danny Huang
AstraZeneca, BBSRC, FogPharma, Glasgow Children's Hospital Charity

Gareth Inman
British Skin Foundation, DEBRA, SANOFI

David Lewis
Beatson Cancer Charity/Beatson Endowment, NIH

Laura Machesky
ESPRC Physics of Life, Saudi Government Scholarship

Tom MacVicar
Medical Research Scotland

Jen Morton
Pancreatic Cancer UK, UCB Biopharma

Jim Norman
Chief Scientist Office, MRC

Kevin Ryan
The Kay Kendall Leukaemia Fund, Wellcome Trust

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

Douglas Strathdee
Scenic Biotech

Saverio Tardito
University of Bergen

Sara Zanivan
Breast Cancer Now

A big thank you to Irene Young and Irene Mitchell who organised a Cancer Research Dance at Kilmaronock Village Hall Gartocharn in order to raise funds for the Beatson Institute

School of Cancer Sciences, University of Glasgow

David Bryant
EssenBio, UKRI

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

Julia Cordero
China Scholarship Council, Wellcome Trust

Robert Insall
MRC, Wellcome Trust

Kristina Kirschner
MRC, Saudi Government Scholarship, Turkish Government Scholarship

John Le Quesne
Celgene, Jean Shanks Foundation

Daniel Murphy
Asthma + Lung UK, British Lung Foundation, Merck, Mick Knighton Mesothelioma Research Fund, 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.

- Abbotsford Chapter No 14
- S Armour
- Bearsden Bowling Club
- BICR Staff – Sale of BICR Cookbook
- Lesley Bryans – *In memory of Lesley's Mother, Mrs Annie Bryans*
- Charities Aid Foundation
- Charities Trust
- Mrs Lindsey Coverdale – *In memory of Lindsey's mother, Anne McRoberts*
- Mrs Senga Dempster
- Mr Gordon Dunn – *In memory of Mr Fred McKerchar*
- Fair City Stampers – Catherine Drummond
- A K Haddow
- The Kilt Walk
- Michael & Janet Lyke – *in lieu of Christmas presents*
- Margaret McIntyre – *In memory of her son Scott*
- Mr Graeme McLeish – *in memory of his wife Isobel*
- Mrs Fiona McNeill & Family – *In lieu of sending Christmas cards*
- Mrs M Muir
- Mull Camera Club – Mr Whenman
- Mr Patrick O'Kane – *In memory of his wife, Mrs Margaret O'Kane; to purchase a Tissue Culture Microscope*
- R Pearson – *in memory of family member*
- E Simpson
- Dr Tom Slimming NBE & Mrs Mary Slimming – *In loving and respectful memory of lives which have enriched ours*
- John Smith Sharp – *Norma Sharp Fundraiser*
- Mrs Rose A Soutar – Donation Precision-Panc
- Mrs Strachan
- Strathmore Funeral Directors – *donated at the funeral of the Late Mr Eric Watson J Teevan (Darrows)*
- West of Scotland Women's Bowling Association
- Anonymous donations via JustGiving, PayPal, Sharegift

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The Rt Hon. Lord Mackay of Clashfern
The Viscountess Weir

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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.

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The Beatson Institute for Cancer Research is a registered charity in Scotland (SC006106) and registered as a company limited by guarantee in Scotland (84170).
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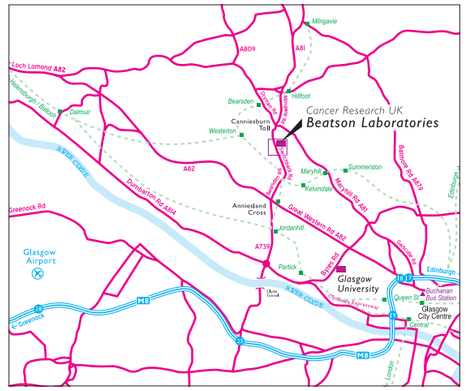
Electronic version of this report can be found at:
www.beatson.gla.ac.uk/annual_report

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

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