SCIENTIFIC REPORT 2021

cruk.org

CANCER RESEARCH UK

BEATSON INSTITUTE

COVER IMAGE

Spectral imaging of a murine lung section where you can see a bronchus and pulmonary arteries surrounded by a flock of leukocytes stained with CD45 (in cyan). Green shows pulmonary capillaries stained with CD31, while red and magenta are immune adhesion molecules CD54 and CD102, respectively which are expressed on different types of pulmonary vascular beds.

Image by Marco De Donatis

SCIENTIFIC REPORT 2021

BEATSON INSTITUTE

University of Glasgow

CANCER RESEARCH UK | BEATSON INSTITUTE

Cancer Research UK Beatson Institute building. *Image by Jim Norman*

CONTENTS

DIRECTOR'S INTRODUCTION

Director of the Cancer Research UK Beatson Institute

Professor Owen Sansom FRSE, FMedSci, FRCPS(Glasg)

Driven by CRUK's research strategy, our mission continues to be to increase our understanding of cancer by being a lead centre of excellence for discovery research focusing on three key areas: **biology of early disease** & **early detection; energetic stress** & **cancer metabolism**; and **microenvironment** & **metastasis**.

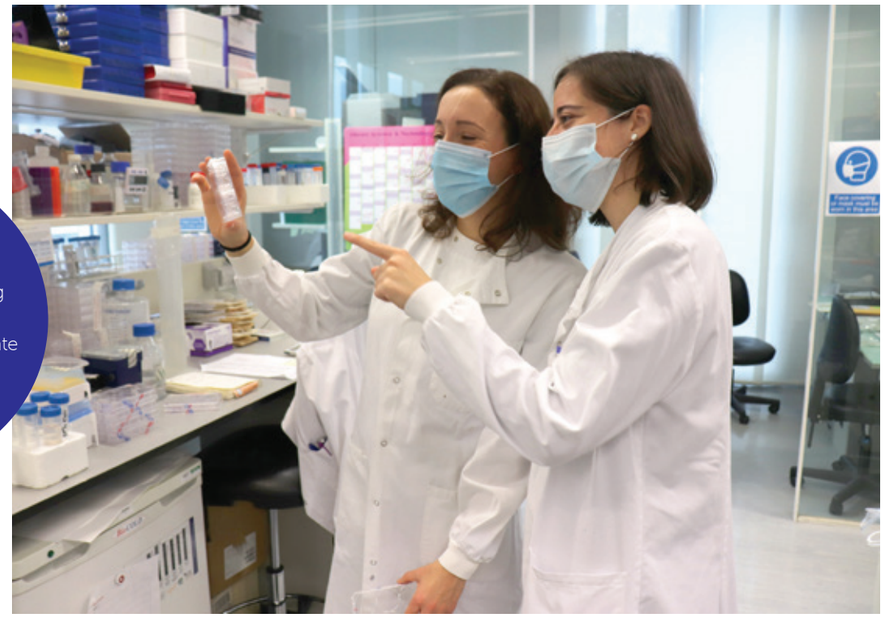
Sophisticated cancer models remain central to our research and this year, we continued to maintain our leadership in the advance and application of mouse models of cancer. The National Mouse Genetics Network, of which I am Director, received final approval from the MRC Strategy Board and will consequently receive £20 million in funding over the next 5 years, including £3 million for the Director's Core and £3 million for the Cancer Cluster led by Professor Karen Blyth and Professor Louis Chesler (Institute of Cancer Research, London).

Closely aligned with our strategic theme of understanding and targeting metastatic disease, we are utilising part of the McNab legacy to create a 'McNab Centre for Cancer Innovation', a collaborative laboratory initially consisting of 6-7 researchers plus collaborators. The principal scientific focus will be to develop human organoid and organ-on-a-chip approaches to study how KRAS–driven colorectal cancers (CRC) and pancreatic adenocarcinoma (PDAC) instruct the microenvironment of the liver, thus enabling us to identify the metabolic vulnerabilities of the metastases of these cancers.

Dr Tom MacVicar joined the Beatson to start his group 'Mitochondrial Reprogramming in Cancer'

One of our key priorities continues to be to support and build on our investment in **disease positioning** and **spatial imaging** led by Professor John Le Quesne. Now the equipment for this is in place, we are pump-priming some 'exemplar' projects that are central to our research themes. Several of our faculty have also been successful in obtaining external funding to help support the rollout of this technology, including Professor Jen Morton (Pancreatic Cancer UK), Dr Seth Coffelt (Cancer Research Institute) and Dr Nigel Jamieson, University of Glasgow (College of Surgeons).

We have made progress this year in targeting additional **external funding** to support our key research areas. Professors Jen Morton and Sara Zanivan were awarded CRUK Early Detection and Diagnosis Project Awards, while Professor Daniel Murphy was awarded a CRUK Early Detection and Diagnosis Programme Award with Dr Marion MacFarlane (Cambridge). These will focus on early detection in pancreas, liver and lung cancer, respectively. Project grants were awarded to Dr Seth Coffelt (Worldwide Cancer Research), Professor Daniel Murphy (British Lung Foundation) and Dr Kirsteen Campbell (Prostate Cancer Research), while Junior Group Leaders Dr Payam Gammage and Dr Seth Coffelt were successful in important fellowship applications to the ERC and CRUK, respectively. In terms of our wider network activities, we were delighted that the CRUK Scotland Centre bid was successful and will be funded in full (£12 million over 5 years) as this aligns closely with our ambition to maximise the translation of our findings for the benefit of patients and focuses on several of our key tumour types: CRC, liver, PDAC and mesothelioma.



Dr Kirsteen Campbell and Dr Laura Martinez Escardo are investigating MCL-1 protein as a new treatment option in prostate cancer funded by Prostate Cancer Research

We also continue to make strategic **appointments** where possible. We have recruited Professor Vicky Cowling (from Dundee) as a Senior Group Leader. 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. She will move to the Beatson in May 2022. Dr Tom McVicar (previously Max Planck Institute for Ageing Research, Cologne) also joined us as a Junior Group Leader with a CRUK Career Development Fellowship in December. Tom is an expert in mitochondrial metabolism and a key addition to our energetic stress theme.

The Institute 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 and have strong links with the Institute of Cancer Sciences, University of Glasgow VOICE (Athena Swan) Committee through which we host various seminars and events aimed at giving our staff the opportunity to develop and have a voice in how we enact our commitments to EDI. More details of our current EDI action plan and gender pay gap report can be found on page 137.

In conclusion, we have continued to build strength and depth in our key areas in the past year and are well-positioned to continue to advance our understanding of cancer and to translate our findings for the benefit of cancer patients. This is thanks in very large part to the hard work of our researchers and the operational teams that support them.

RESEARCH HIGHLIGHTS

Despite the challenges of COVID-19, we continued to make considerable advances in our key research themes and to be highly collaborative. This is highlighted by the large number of ground-breaking papers we published this year, including:

Biology of early disease and early detection

In a seminal study, Owen Sansom's lab collaborating with Pekka Katajisto (Helsinki) revealed key events in very early tumourigenesis. The findings of this study have extremely important ramifications for identifying the early signs and causes of intestinal cancers. This paper has consequently been highlighted by several new-and-views articles in leading journals (Flanagan *et al.* NOTUM from Apc-mutant cells biases clonal competition to initiate cancer. Nature 2021).

Julia Cordero's fly lab worked with Owen Sansom, Dave Bryant and Jim Norman's groups to demonstrate a key link between the control of receptor endocytosis and tumourigenesis (Nászai *et al.* RAL GTPases mediate EGFR-driven intestinal stem cell proliferation and tumourigenesis. ELife 2021).

Sara Zanivan's group collaborated with Raghu Kalluri (MD Anderson) to identify a key component of circulating exosomes that may pave the way for early detection of aggressive cancers (Kugeratski *et al.* Quantitative proteomics identifies the core proteome of exosomes with syntenin-1 as the highest abundant protein and a putative universal biomarker. Nature Cell Biology 2021).

Kevin Ryan's group with Tom Bird progressed our understanding of the cell of origin in liver cancer. In a preclinical model driven by the loss of macroautophagy and PTEN, mature liver cells developed into stem cell-like progenitors giving rise to cancerous cells. The activation of YAP together with TAZ also highlighted possible new avenues for treatment intervention in liver cancer (Barthet et al. Autophagy suppresses the formation of hepatocyte-derived cancer-initiating ductular progenitor cells in the liver. Science Advances 2021).

Energetic stress and cancer metabolism

A paper from Martin Bushell's lab along with Sara Zanivan defined the key mRNA features that determine how the human Ccr4-Not complex differentially regulates mRNA fate and protein synthesis (Gillen *et al.* Differential regulation of mRNA fate by the human Ccr4-Not complex is driven by coding sequence composition and mRNA localization. Genome Biology 2021).

John le Quesne, Daniel Murphy, Owen Sansom, David Sumpton and Martin Bushell's groups collaborated with Anne Willis' lab (Cambridge) to demonstrate important links between altered energy metabolism and translational control in mesothelioma, a devastating lung disease, which has its epicentre in the West of Scotland. This study provides key leads that will help to expose therapeutic vulnerabilities of mesothelioma (Grosso *et al.* The pathogenesis of mesothelioma is driven by a dysregulated translatome. Nature Communications 2021).

Owen Sansom's lab and Martin Bushell used the Rosetta team's tumour mapping tools to identify SLC7A5 as a novel therapeutic target for colorectal cancer. The paper featured in the Cancer Grand Challenges' annual celebration of scientific progress (Najumudeen *et al.* The amino acid transporter SLC7A5 is required for efficient growth of KRAS-mutant colorectal cancer. Nature Genetics 2021).

Stephen Tait's group with Yaron Fuchs (Technion) and Karen Blyth's team described an FGF2 pathway by which cells under apoptotic stress can increase resistance to cell death in surrounding cells. Beyond mediating cytotoxic drug resistance, this process also provides a potential link between tissue damage and repair (Bock *et al.* Apoptotic stress-induced FGF signalling promotes non-cell autonomous resistance to cell death. Nature Communications 2021).

Microenvironment and metastasis

Payam Gammage, Stephen Tait and Jim Norman's groups revealed a novel pathway leading to the packaging of mitochondrial DNA (mtDNA) into exosomes, their release into the circulation and how they activate toll-like receptor signalling to prime metastatic niches. This study provides opportunities for developing new approaches to using circulating mtDNA for early detection of aggressive cancers. This paper has been selected for inclusion in a special collection by JCB on Cancer Cell Biology for the AACR meeting in New Orleans in April 2022 (Rabas *et al.* PINK1 drives production of mtDNA-containing extracellular vesicles to promote invasiveness. Journal of Cell Biology 2021).

Jim Norman's group, in association with computational biologist Matthew Neilson, identified a new signalling pathway linking receptor tyrosine kinase activation to gene expression in pancreatic adenocarcinoma (PDAC). This pathway represents a route into the nucleus for antisense oligos targeting KRAS, and thus provides an opportunity to inhibit this key cancer driver in metastasising cells (Marco *et al.* EphA2 enables nuclear capture of endosomes to promote SRF/MRTF gene expression and cancer cell invasion. Nature Communications 2021).

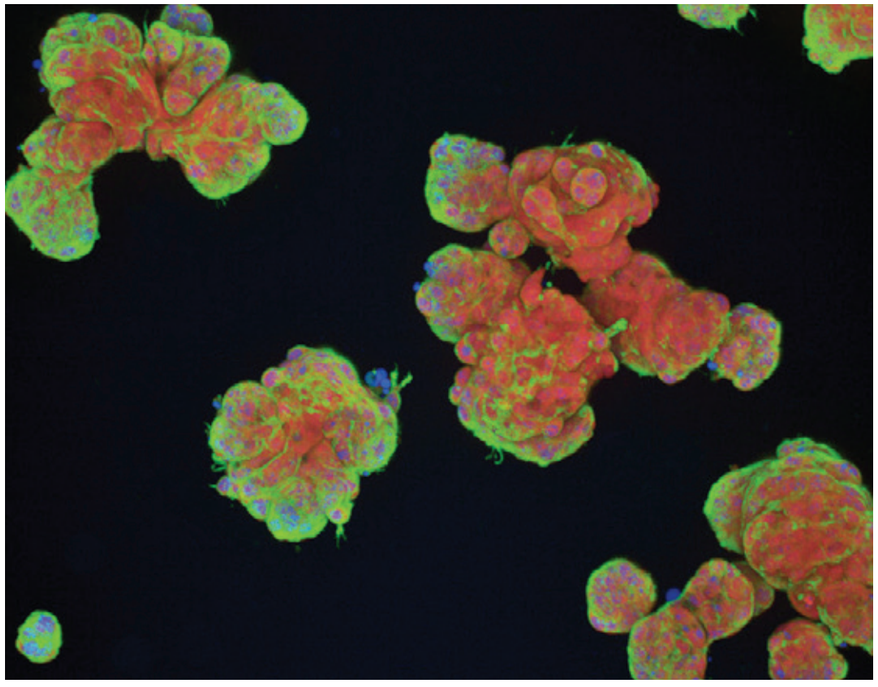
Key tumour types

Owen Sansom's group have found that right-sided colorectal cancers, which have particularly poor prognosis, exhibit a foetal-like progenitor phenotype, and this will assist in the use of agents targeting TGFβ in treating colon cancers (Leach *et al.* Oncogenic BRAF, unrestrained by TGFβ-receptor signalling, drives right-sided colonic tumorigenesis. Nature Communications 2021).

Jen Morton and Owen Sansom's groups have collaborated with Claus Jorgenson (CRUK Manchester Institute) in a key study revealing how the complexity of the pancreatic cancer stroma can define tumour growth. This paper is a key example of an active and fruitful ongoing collaboration between CRUK Institutes (Hutton *et al.* Single-cell analysis defines a pancreatic fibroblast lineage that supports anti-tumor immunity. Cell 2021).

Pancreatic Spheroids
Pancreatic cancer cells grown in 3D Matrigel and imaged using an Opera Phenix HCS microscope. Blue: Nuclei, Green: Actin, Red: Whole Cell Stain. Maximum intensity projection of Z-stack.

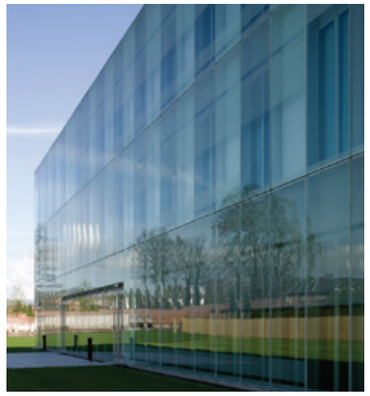
Image by Nikki R. Paul



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 Institute 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

Experimental compound designed by Drug Discovery Unit undergoing purification by chromatography

Image by John Taylor

www.beatson.gla.ac.uk/imran_ahmad

MODELS OF ADVANCED PROSTATE CANCER

Group Leader

Imran Ahmad

CRUK Clinician Scientist
Clinical Senior Lecturer
(University of Glasgow)
Consultant Urological Surgeon
(NHS Greater Glasgow & Clyde)

Research Scientist
Amy Tibbo

Graduate Student
Andrew Hartley[1]

[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.

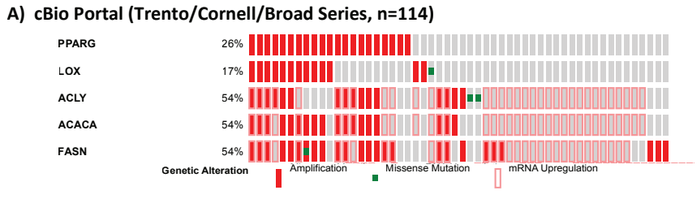
Our current research interest lies in understanding the mechanisms of treatment resistance in advanced prostate cancer. Work in our lab together with the Leung group uses state-of-the-art *in vivo* models in conjunction with patient samples to interrogate the disease processes in advanced and treatment-resistant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

As an Honorary Consultant Urological Surgeon based at the Queen Elizabeth University Hospital in Glasgow, I have one of the highest-volume robotic prostatectomy practices in the UK for patients with aggressive 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.

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)



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

Our data suggests that prostate cancer patients could be stratified in terms of PPARγ/FASN and PTEN levels to identify patients with aggressive prostate cancer who may respond favourably to PPARγ/FASN inhibition (low PTEN/high pAKT expression); a finding that has potential to guide the design of future clinical trials. Ongoing research by our group has demonstrated that this lipid synthesis phenotype may be driven through alterations in mitochondrial function and AKT3 activations.

In addition, to our knowledge, we were the first to demonstrate the strength of the Sleeping Beauty transposon model system in successfully determining low-frequency somatic mutations that may drive prostate tumorigenesis. We are further investigating and validating other novel and clinically relevant 'hits' from this screen. Galbraith *et al.*, Oncogene. 2021;40:2355-66

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.

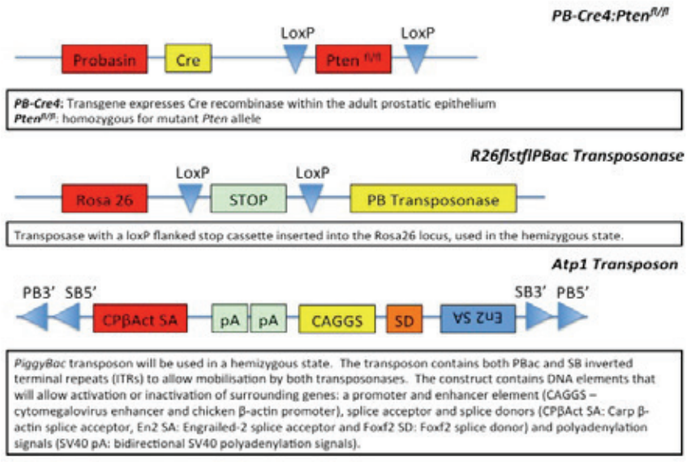


Figure 2
Genetic modifications of the PiggyBac mice.

Figure 3

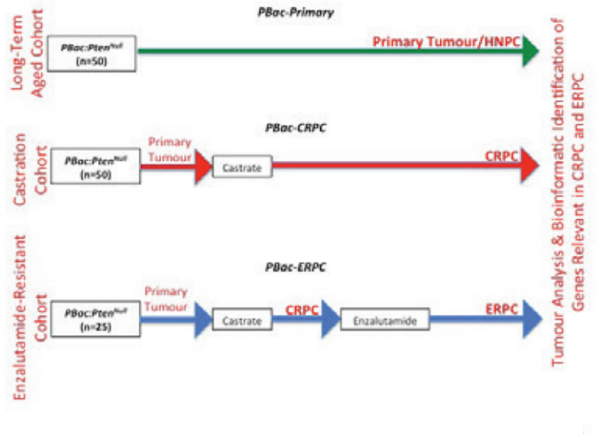


Figure 3
Experimental design for the ageing, castration and enzalutamide-treatment of the PiggyBac (PBac) mice.

Figure 4

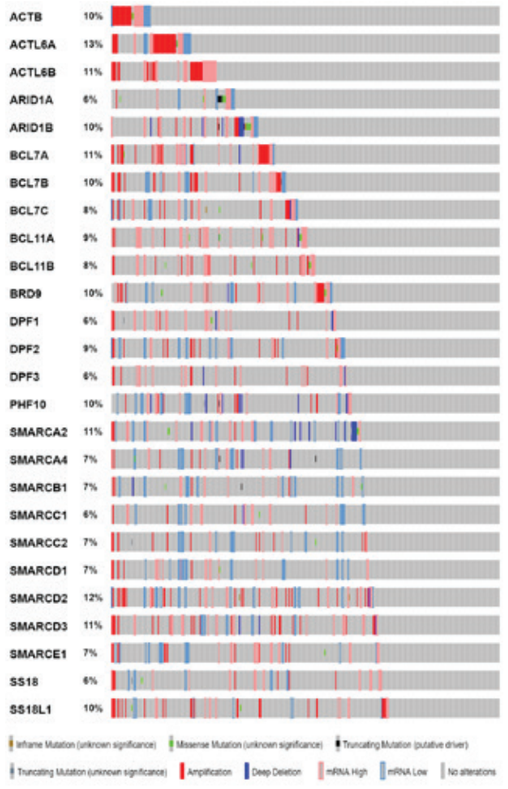


Figure 4
Mutations in the BAF complex in metastatic prostate cancer

Using cross-species oncogenomics, we will overlay identified genes with those from human sequencing projects, allowing better stratification of the human somatic mutational landscape into 'driver' and 'passenger' events. Once validated, candidate genes will provide insight into the biology, as well as offering potential diagnostic, prognostic and therapeutic targets in advanced disease, and offering insight into the mechanisms of CRPC and ERPC.

Role of Arid1a in prostate cancer

ARID1A was also identified as a potential driver in prostate cancer by the Sleeping Beauty screen. ARID1A is part of the BAF complex, and functions as a key regulator controlling DNA accessibility and organisation by chromatin remodelling. The BAF complex itself is highly mutated in metastatic prostate cancer. Including mRNA alterations, the BAF complex is mutated in 60-70% of metastatic prostate cancer cases (Figure 4). The potential for therapeutically targeting the BAF complex in prostate cancer is reviewed in our recent publication. Hartley *et al.*, Expert Opin Drug Discov. 2021; 16:173-181

Publications listed on page 100

LIVER DISEASE AND REGENERATION

Group Leader
Tom G Bird

Honorary Consultant Hepatologist and Wellcome Intermediate Clinical Fellow

Research Scientists
Stephanie May
Miryam Müeller[1]

Scientific Officer
Ee Hong Tan[2,3]

Clinical Research Fellow
Tom Drake[4]

Graduate Students
Anastasia Georgakopoulou[2]
Christos Kiourtis

[1]Wellcome Trust
[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. Our group works at the interface of clinical care and the development of preclinical models to study liver biology. Our focus is to understand dysregulated liver regeneration, particularly during cancer development, with the aim of taking novel therapies from the bench to the clinic to improve patient outcome; both with liver cancer, and those at risk of this devastating disease.

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 currently no therapy is 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 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 we are commencing a clinical trial 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 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 clinical trials, targeting specific therapies to specific tumours for precision medicine in liver cancer.

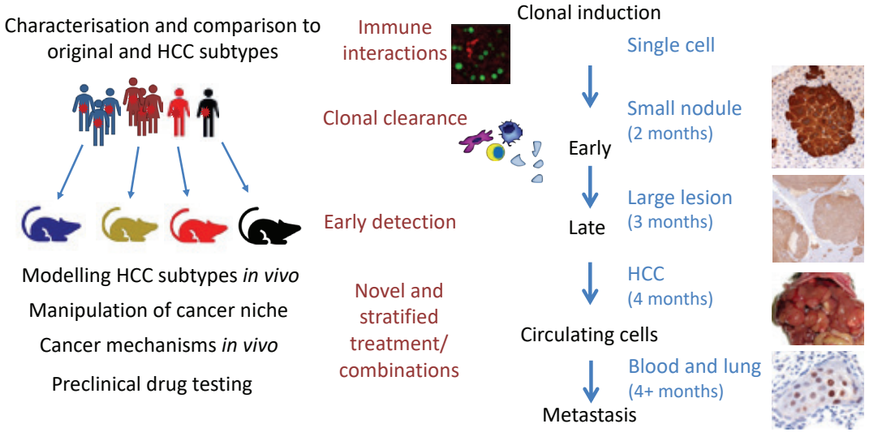


Figure 1
Human HCCs can be grouped into different functional and genetic subclasses.
We are mimicking the genetic alterations in human HCC subclasses using *in vivo* models in the mouse. Our strategy is to induce clonal hepatocytes and then follow the clones as they develop into metastatic HCC. We aim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

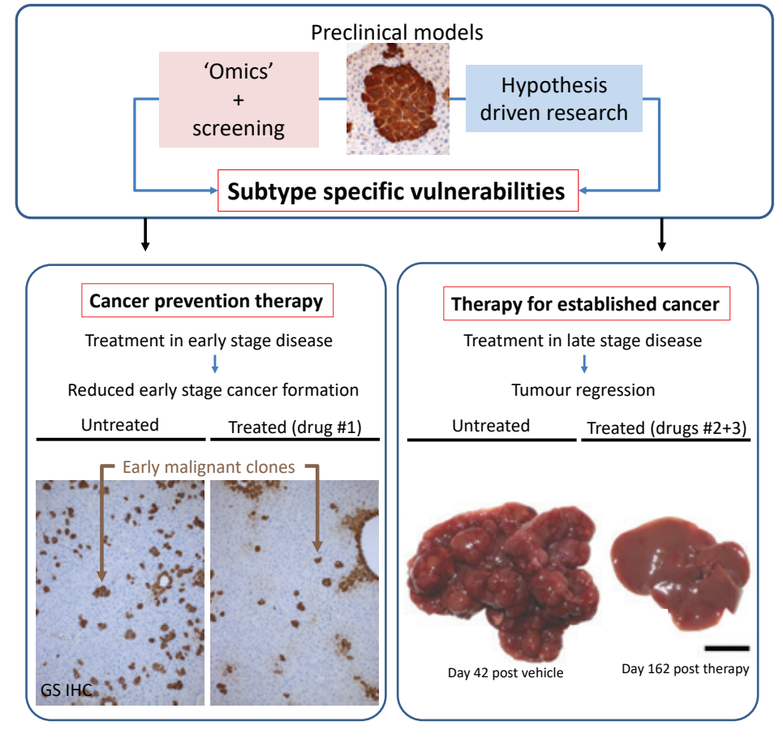


Figure 2
Cancer prevention in preclinical models by targeting early tumour clones.
We are able to explore specific vulnerability of individual liver cancer subtypes. We have identified pathways which are specifically activated in early disease. When we apply therapies to early disease, we are able to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

IN VIVO CANCER BIOLOGY

Group Leader
Karen Blyth

Associate Scientist
Kirsteen Campbell

Research Scientist
Laura Martinez Escardo[1]

Graduate Students
Adiba Khan
Kerri Sweeney[2]
Matthew Winder

[1]Prostate Cancer Research
[2]Breast Cancer Now

Our lab uses *in vivo* models to study cancer processes, interrogating aspects of the disease and cancer-related pathways within a biological context. By validating *in vitro* discoveries in physiologically relevant models we hope to expedite novel therapeutic approaches. The group has expertise in modelling different cancer types but has a specific interest in breast and prostate cancer, and how certain signalling nodes such as the RUNX/CBFβ transcriptional complex and pro-survival factor MCL-1, contribute to tumour progression and metastasis.

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 - often found dysregulated in cancer. Indeed, in around 13% of breast cancer cases, we find genetic aberrations of the *RUNX1* and *CBFβ* genes. Importantly, the nature of these alterations differ between subtypes where mutation and gene loss are associated with oestrogen-receptor positive (ER+) disease; while gain of *RUNX* gene function has been proposed to drive oestrogen-receptor negative (ER-) subtypes of breast cancer. PhD students Kerri Sweeney and Adiba Khan have been exploring the enigmatic role of the RUNX/CBFβ complex using *in vitro* and *in vivo* models of breast cancer.

Adiba has shown that loss of *Cbfβ* in the mammary gland did not overtly alter the normal development of the tissue, but when combined with oncogenic WNT signalling dramatically accelerated onset of mammary tumours in a mouse model of breast cancer, providing *in vivo* evidence that CBFβ has a tumour suppressor role. RNA sequencing analysis of tumours deficient in CBFβ showed enhanced Wnt pathway activation, indicating a role for CBFβ in regulation of Wnt signalling. Interestingly, loss of *Cbfβ* in the *MMTV-PyMT* model did not accentuate tumour development, and indeed acute loss of CBFβ in cell lines derived from these tumours resulted in decreased cell proliferation. CBFβ therefore, plays a context-dependent role in different models of mammary cancer, a conundrum similar to that observed in patients.

In collaboration with Prof Ewan Cameron (University of Glasgow) and funded in part by Breast Cancer Now, Kerri has focused on the role of RUNX1, and in particular to its effects on mammary stemness. Deletion of *Runx1* in an oncogenic *β-catenin* setting accelerated disease onset in an *in vivo* model, and was further accentuated if *Runx2* was also deleted. An increased stem-like transcriptional signature was observed at early stages of tumourigenesis in this model. Similarly, we found that deletion of *Runx1* in 3D mammary cell culture resulted in increased mammosphere and colony forming capabilities and was accompanied by an upregulation of stem cell markers. Further transcriptional profiling of RUNX/CBFβ deleted mammary tumours is underway to unravel the mechanism/vulnerabilities of RUNX pathway alteration in cancer. Notably however, the functional loss of the RUNX/CBFβ complex in mammary tumours evoked changes to the immune composition of tumours that may be seminal in driving tumorigenesis, a hypothesis we are actively exploring.

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. In Campbell *et al*., 2018, *Cell Death Disease*, we have shown that MCL-1 is required for both tumour development and maintenance of established tumours in the breast (Figure 1A). Our preliminary data suggested that MCL-1 also has a role in prostate cancer where it can act as a barrier to tumour cell elimination by prostate cancer therapies. New therapeutic options are urgently required in both cancers as together they account for over 23,000 deaths each year in the UK. Due to its importance in cancers of the blood, inhibitors of MCL-1, so-called "BH3 mimetics", have been developed and are in clinical trials for treatment of haematopoietic malignancies. Whilst high levels of MCL-1 prevent tumour cell death, BH3 mimetics can inhibit MCL-1 and help eliminate cancer cells (Figure 1B). By characterising the role of MCL-1 in breast and prostate cancer we aim to prove MCL-1 a valid target and expedite the use of MCL-1 inhibitors in these cancer types.

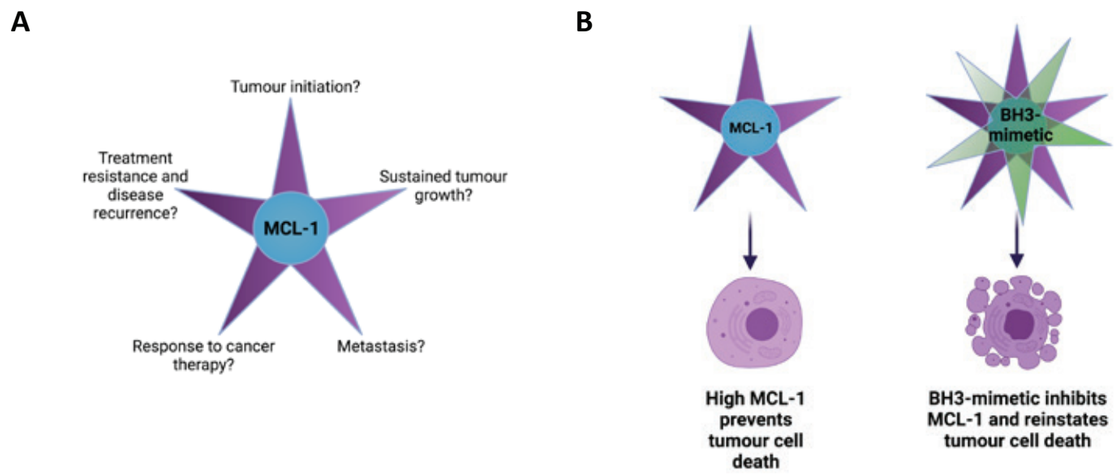


Figure 1
MCL-1 is an important mediator of cancer processes and a credible therapeutic target
A. We have shown that MCL-1 is required for tumour initiation and sustained growth in breast cancer. We are also investigating the role of MCL-1 in metastasis, response to cancer therapy and treatment resistance and recurrence. **B.** High levels of MCL-1 are found in breast and prostate cancer where MCL-1 facilitates tumour cell survival. We are investigating whether drugs that inhibit MCL-1 (BH3-mimetics) can reinstate tumour cell death and improve response to current anti-cancer therapies.

Figures created with BioRender.com

Together with Prof Stephen Tait, we have found that the anti-apoptotic function of MCL-1 is key in breast cancer (Campbell *et al*, 2021, *Cell Death Differentiation*). This is important as the anti-apoptotic function of MCL-1 is inhibited by the BH3 mimetic drugs currently in clinical trials and therefore provides further evidence for the potential anti-cancer effects in breast cancer.

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, with Dr Kirsteen Campbell, aims to unravel the role of MCL-1 at the time of tumour initiation, during the metastatic process, and in mediating cancer therapy resistance and disease recurrence (Figure 1A).

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 in bone metastases. Together with Prof Karen Blyth and Prof Hing Leung, Dr Kirsteen Campbell recently secured funding from Prostate Cancer Research for a 3-year project to investigate whether targeting of MCL-1 can improve response to hormone therapy and/or chemotherapy in advanced prostate cancer. Dr Laura Martinez-Escardo has joined our group this year and also works closely with Prof John Le Quesne and Prof Crispin Miller and their teams to pursue this research.

Publications listed on page 101

EPITHELIAL POLARITY

Group Leader
David Bryant

Research Scientists
Archana Katoch[1]
Álvaro Román-Fernández[2]

Scientific Officer
Emma Sandilands[1]

Graduate Students
Amy Bryson[1,2]
Erin Cumming[3]
Eva Freckmann[4]
Konstantina Nikolatou[5]

[1]UKRI Future Leader Fellowship grant
[2]University of Glasgow
[3]University of Glasgow MVLS Doctoral Training Program
[4]University of Glasgow Industrial PhD Partnership
[5]CRUK Glasgow Centre

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, cells 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.

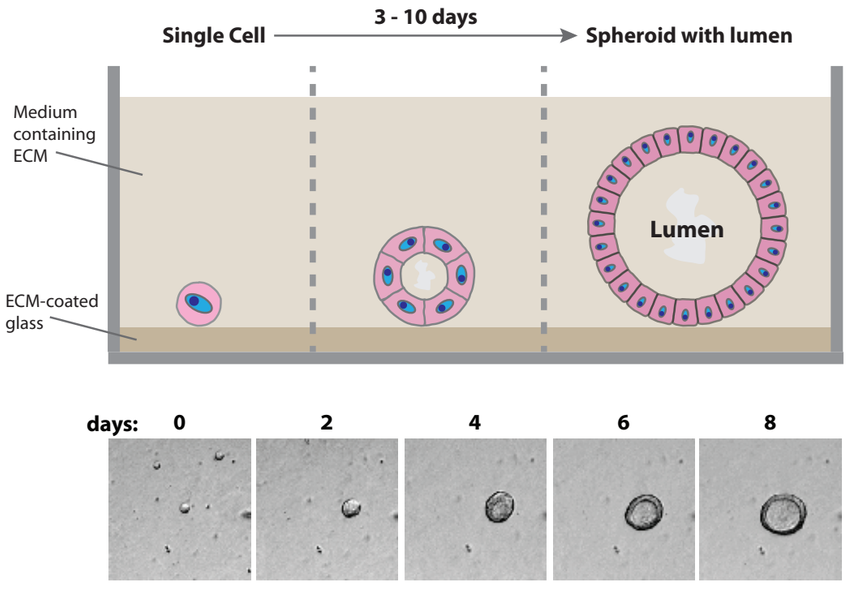


Figure 1
By culturing cells on glass-bottomed chambers coated with extracellular matrix (ECM), we direct the self-assembly of single cells into multicellular spheroid structures with a single, central lumen. This process occurs over 10 days, allowing us to study the dynamics of tissue formation.

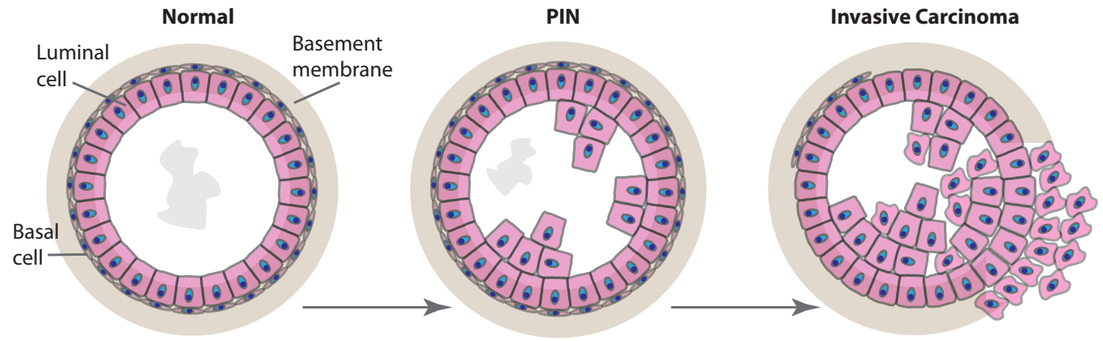


Figure 2
3D cultures of cells to form cysts (also called spheroids or organoids) also allows us to model the loss of normal tissue architecture that occurs in cancer. For example, the progressive disrupted organisation of Normal, to Prostatic Intraepithelial Neoplasia (PIN), to Invasive Carcinoma typifies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

In collaboration with the Blyth, Leung and Zanivan groups, we are interrogating their function in metastasis. We found that many ARFome family members assumed as redundant had highly divergent and sometimes opposing roles in invasion, and showed that there was specificity of signalling between family members. We have identified a key ARF GTPase module based around the IQSEC1 GEF protein that controled metastasis in prostate cancer by controlling phosphoinositide signalling. In collaboration with the Zanivan group, we are studying how ARF GTPases function in PTEN-null tumours, particularly in ovarian cancer. These studies are an important preamble to identify which and how selectively, ARF GTPases may be targets for future therapeutic inhibition studies.

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 are dissecting how podocalyxin controls 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. 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.

Publications listed on page 102

www.beatson.gla.ac.uk/martin_bushell

RNA AND TRANSLATIONAL CONTROL IN CANCER

Group Leader
Martin Bushell

Associate Scientist
Joseph Waldron

Research Scientists
Aditya Chandru
Chiara Giacomelli[2]
Pauline Herviou
Corina Keller[3]
Laura Mincarelli[2]
Ruban Peter–Durairaj[4]
Tobias Schmidt[5]

Scientific Officer
June Munro

Graduate Students
Aldo Bader[1]
Adrianna Dabrowska[4]
James Ettles
Mhairi O'Donnell[4]
Peter Walsh

[1]MRC
[2]CRT
[3]BBSRC
[4]CRUK programme grant
[5]CRUK SMERP

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.

Understanding how eIF4A1 drives tumourigenesis

Recently, we unexpectedly showed how eIF4A1, a rate-limiting helicase of the initial step of mRNA translation, functions to specifically promote oncogenic gene expression.
We found that unwinding of RNA secondary structure requires eIF4A1 to act as a multimer, composed of a loading subunit and one or more unwinding subunits (Figure 1A). The rate of unwinding was dependent on the single stranded RNA sequence bound by the loading subunit, whereby unwinding was highest when the region was composed entirely of purines (Figure 1B). RNA structure profiling showed increased secondary structure ~30-50nt downstream of purines in cells upon eIF4A inhibition (Figure 1C). We performed metabolic pulse-labelling together with quantitative TMT labelling (TMT-pSILAC) to measure protein synthesis rates following eIF4A inhibition (Figure 1D). This showed that mRNAs with an AG10 motif within their 5'UTR were translationally repressed following eIF4A inhibition more significantly than mRNAs with a GC motif (Figure 1E). Our data suggested that eIF4A1 drives oncogenic mRNA translation through unwinding RNA structure during scanning and that this is distinct from its role during ribosome recruitment.

Interestingly, eIF4A1 has a closely related paralogue, eIF4A2, which until recently was believed to function interchangeably with eIF4A1 within the eIF4F complex. However, we have recently shown that eIF4A2 instead acted to repress translation as part of the CCR4-NOT complex, where it bound purine-rich motifs with a slower off rate than eIF4A1, resulting in a block to ribosome scanning and therefore translational repression. Given the opposing associations of these two proteins with translation, the group is currently working to understand the effects of eIF4A inhibitors on eIF4A2 as well as eIF4A1 in cancer models of the small intestine.

Further, as we know that eIF4A2 also acts independently of the eIF4F complex, it is important to address whether either of the two proteins have roles outside of eIF4A which is believed to be restricted to the 5' ends of mRNAs. We are currently analysing both iCLIP and selective TCP-seq datasets to identify the exact RNA binding sites of eIF4A1 and eIF4A2 as well as the footprints of initiating ribosomal complexes associated with each protein. Preliminary data suggested that in addition to binding within the 5'UTRs, both proteins are also enriched for binding within the 3'UTRs (Figure 1F), which may unlock previously unknown mechanisms through which eIF4A exerts its oncogenic functions.

Understanding the role of the two eIF4A helicases in different tumour compartments will be essential in determining vulnerabilities of cell types and tailoring specific strategies to target gene expression programmes within different cancers.

Investigating the role of the CCR4-NOT complex in sensing codon optimality and the downstream consequences

Multiple codons encode the same amino acid. It is now clear that synonymous codon usage affects the expression of proteins through altering the rate of translation elongation and

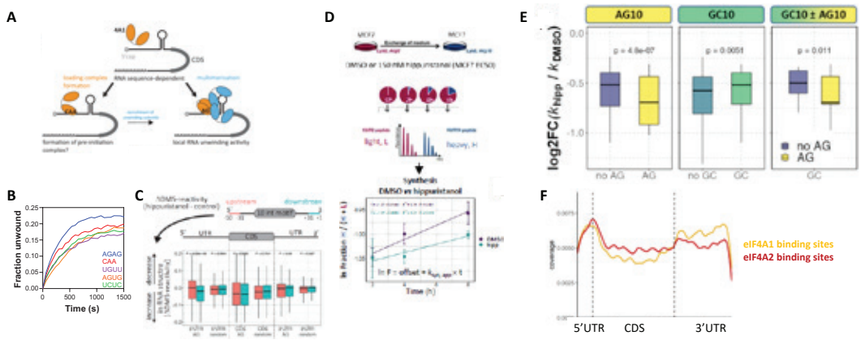


Figure 1
AG motifs stimulate the unwinding activity of eIF4A1 by inducing multimeric formation.
(**A**) eIF4A can form multimer composed of a loading subunit and unwinding subunits. (**B**) Unwinding activity of eIF4A1 helicase is highest with an AG-rich overhang. (**C**) RNA structure profiling upon eIF4A inhibition with hippuristanol in MCF7 cells shows increased structure ~30-50nt downstream of AG rich motifs, but not random motifs from the same 5'UTRs or within the CDS or 3'UTR. (**D**) Protein synthesis rates upon eIF4A inhibition assessed by metabolic pulse-labelling together with quantitative TMT labelling (TMT-pSILAC). (**E**) Protein synthesis rates are decreased in mRNAs with an AG10 motif within their 5'UTRs in contrast to mRNAs with no AG10 motif. The same is not true for mRNAs with an GC10 motif, unless they also possess an AG10 motif. (**F**) Preliminary iCLIP analyses show enriched binding of both eIF4A1 and eIF4A2 in the 5'UTR but also the 3'UTR.

the mRNAs half-life - termed codon optimality. Critically in cancer, it is suggested that mRNAs encoding proteins required for proliferation are stabilised and highly expressed, due to their codon usage and the altered levels of corresponding tRNAs.

The CCR4-NOT complex has been implicated in sensing codon optimality, with Not5p shown to directly bind to the ribosomal E-site, only when the A-site is unoccupied, in yeast. Although its significance is unclear in human cells, several cancer mutations have been identified in NOT3 (the human orthologue of Not5p). As the CCR4-NOT complex has been implicated in most stages of the mRNA's life cycle, we carried out a comprehensive investigation of the post-transcriptional regulation mediated by the CCR4-NOT complex.

We observed that upon CNOT1 knockdown, mRNAs that are enriched for G/C-ending codons were most stabilised in human cells (Figure 2A) - consistent with a role of the CCR4-NOT complex in the preferential degradation of mRNAs based on their codon usage in humans. Interestingly, eIF4A2 and DDX6 have been shown to bind the CCR4-NOT complex and mRNAs that are bound by either of the two helicases have distinct codon optimality compared to mRNAs bound by eIF4A1 (Figure 2B). In fact, although eIF4A1 and eIF4A2 share ~90% amino acid identity, their coding sequences have diverse codon usages (Figure 2C), suggestive of a mechanism which drives their divergent expression.

Interestingly, mRNAs targeted to the ER were shown to require the CCR4-NOT complex for their translation, with these mRNAs possessing reduced ribosome occupancy most notably downstream of the signal sequence (Figure 2D) and were not targeted to the ER following CNOT1 knockdown (Figure 2E). This fits with recent data from the Norman lab, who found reduced extracellular fibronectin, following knockdown of either eIF4A2 or CNOT1 in human fibroblasts. Given the importance of the secretome in providing the right microenvironment for continued tumour growth, targeting the CCR4-NOT complex could therefore be beneficial in slowing tumour growth.

Further work within the group is now investigating the role of the CCR4-NOT complex in protein folding and cellular localisation.

Publications listed on page 102

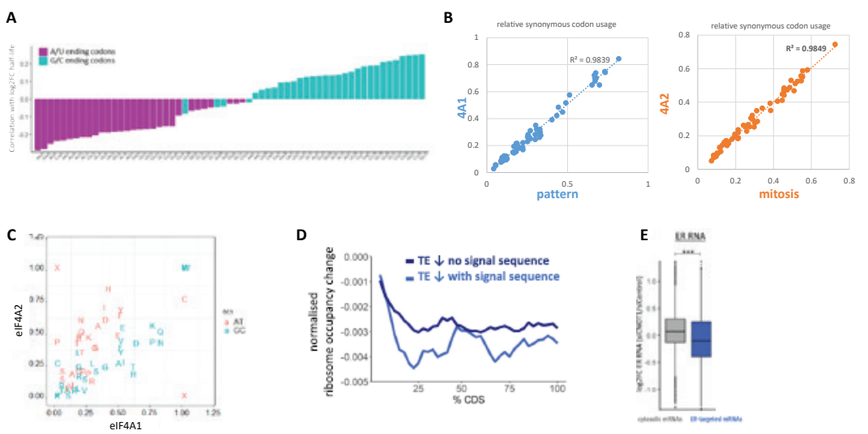


Figure 2
The CCR4-NOT complex senses codon optimality and is required for ER targeting.
(**A**) For each codon the correlation coefficient is plotted between the log2FCs in mRNA half-life following CNOT1 knockdown (siCNOT1 / siControl) and the frequency of that codon within that mRNA, for all mRNAs in HEK293 cells. Codons ending in A/U (magenta) and codons ending in G/C (cyan). (**B**) The codon optimality of mRNAs bound by eIF4A1 and eIF4A2 are distinct, with eIF4A1 bound mRNAs utilising the codons most enriched in genes involved in pattern specification, but eIF4A2 bound mRNAs utilising codons associated with mitosis genes. (**C**) The relative synonymous codon usage of eIF4A1 is distinct from eIF4A2. (**D**) mRNAs with a signal sequence have reduced ribosome occupancy directly downstream of the signal sequence following CNOT1 depletion. (**E**) logFC in ER localised RNA following CNOT1 depletion of RNA normally localised to either the cytosol or ER in control conditions, shows reduced ER targeting following CNOT1 knockdown.

BIOLOGY OF THERAPEUTICS

Group Leader
Ross Cagan

Research Scientists
Hammed Badmos
Bojie Cong
Shree Roychowdhury
Kimmi Stephens

Scientific Officer
Evangelia Stamou

Graduate Students
Sindhura Gopinath
Teena Thakur

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.

My 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 my 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 complex colon cancer fly lines more deeply, we find evidence that 'passengers'—mutations not clearly driving cancer progression—impact a variety of tumour properties including the interactions with normal tissue that regulate tumour expansion such as 'cell competition'. Adding additional mutations can promote a tumour's 'fitness' in the context of the normal organ. We are exploring the relationships between complex mutation profiles, cell competition, and drug response.

Adenoid cystic carcinoma

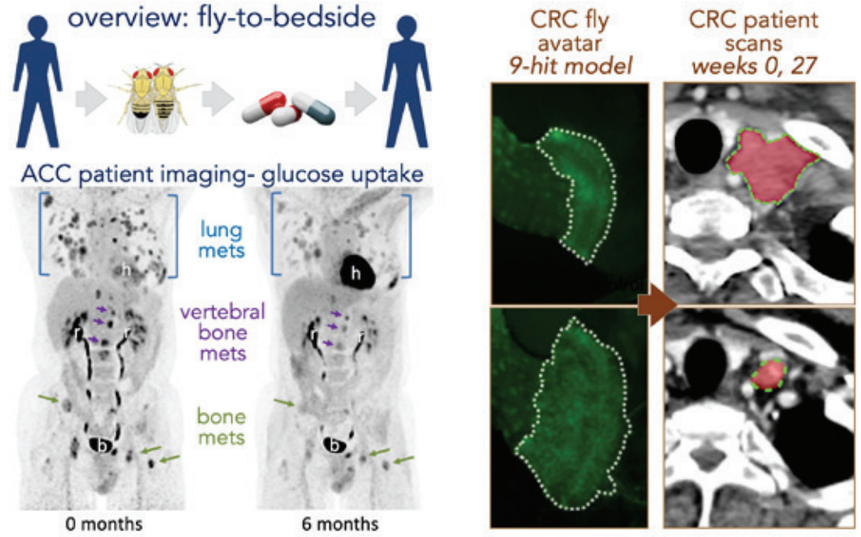
Adenoid Cystic Carcinoma (ACC) is the most common malignant tumour of the minor salivary glands and the second most common of the major salivary glands. Unfortunately, once disseminated there are currently no effective therapies.

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 genomic 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). Similar to our colorectal cancer work, we are now exploring why this drug combination was effective in this patient, and whether it has promise to enter clinical trials for other ACC 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.

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



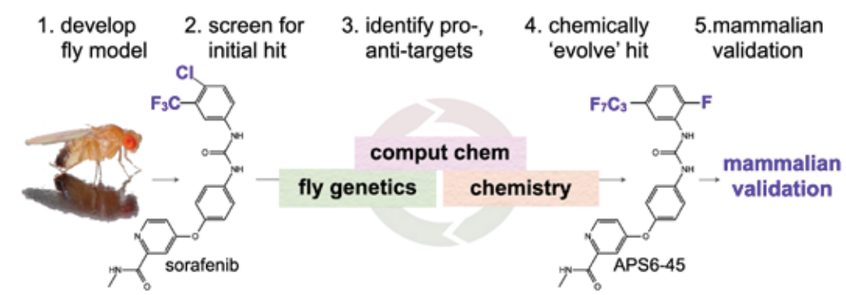
To compare different RASopathy isoforms, we collaborated with Bruce Gelb's laboratory to develop 29 Drosophila models that express human RASopathy isoforms including *PTPN11, KRAS, HRAS, BRAF, RAF1*, and *MEK1*. Different isoforms showed distinct phenotypes as well as different levels of RAS activity as assessed with phosphorylated ERK (pERK), mirroring differences in RASopathy patients. Our models indicate these signalling differences have consequences: while several drugs worked against one or a few fly models, few drugs worked with multiple fly RASopathy models, emphasising the unique whole-body challenge presented by the RASopathies. We are currently working with Maria Kontarides to explore these compounds in mouse RASopathy models, as well as a drug company to help advance our most promising leads towards clinical trials.

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.

Publications listed on page 103

Figure 2
Platform to 'tune' therapeutic leads.



LEUKOCYTE DYNAMICS

Group Leader
Leo Carlin

Fellow of the Royal Microscopical Society (FRMS)

Research Scientists
Frédéric Fercoq[1]
Amanda McFarlane
Ximena L. Raffo Iraolagoitia

Graduate Students
Marco De Donatis
Ryan Devlin[2]
Désirée Zerbst[3]

[1]Breast Cancer Now
[2]TRACC programme; co-supervised with Ed Roberts
[3]Medical Research Scotland; co-supervised with Vignir Helgason

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 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 with pathogens 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. tumour infiltrating lymphocyte (TIL) therapy and immune checkpoint inhibition) highlight the potential for new therapies that can come from a better understanding of how immune cells are (dys) regulated. However, these strategies do not work for all cancers or all patients.

Specialised vasculature and leukocyte dynamics

Our group has a particular interest in the lung and the liver, both as sites of primary tumour development and as targets of metastasis. The extensive capillary network of the lung is unusual in several ways. Alveolar capillaries are of exceptionally small diameter (~5µm) and are in such close proximity to external mucosa that 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. 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 (e.g. Figure 1a, from Mackey *et al.*, 2021, *bioRxiv*), 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 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.

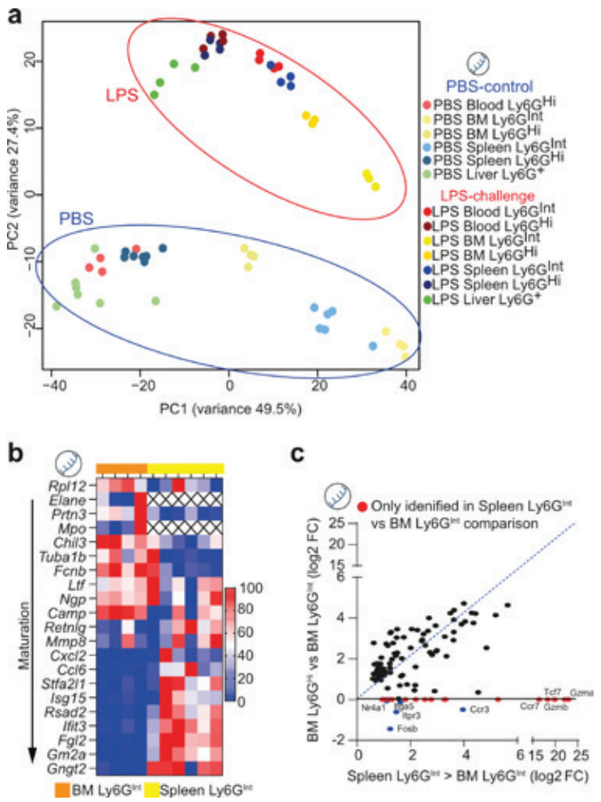


Figure 1
Splenic neutrophil maturation is distinct from the BM.
a PCA plot for Ly6G$^+$, Ly6G^{Int} and Ly6G^{Hi} transcriptomic analysis from the peripheral blood, BM, spleen and liver of PBS-control and LPS-challenged mice. **b** Heatmap showing row scaled expression for genes associated with neutrophil maturation (identified by Xie *et al.*, 2020) for Ly6G^{Int} neutrophils in BM and spleen from naïve mice. Data from transcriptomic analysis. **c** Correlation for genes identified in process networks differing between spleen Ly6G^{Int} and BM Ly6G^{Hi}, shown as log2 fold change (FC) compared to BM Ly6G^{Int}. Data from transcriptomic analysis.

Using a combination of flow cytometry, transcriptional profiling and proteomics, we have extended our understanding of fundamental neutrophil biology by uncovering important differences in neutrophils that originate from the bone marrow and the spleen (Figure1 a-c from Mackey *et al.*, 2021, *bioRxiv*). Production of neutrophils outside of the bone-marrow niche is associated with several pathologies including cancer, so this knowledge will help us to further address how neutrophil regulation is modified by the presence of a tumour and potentially how to target these changes more specifically without affecting the protective roles of neutrophils.

Publications listed on page 103

IMMUNE CELLS AND METASTASIS

Group Leader
Seth Coffelt

Research Scientists
Sarah Edwards[1]
Wilma Hoevenaar[2]
Toshiyasu Suzuki[3]

Scientific Officer
Anna Kilbey[1]

Clinical Research Fellow
Josip Vrancic[1,4]

Graduate Students
Hannah Hayman[5,6]
Mark Lawrence[7,8]
Robert Wiesheu[2]

Intern
Amy Lawlor[9]

[1]CRUK Glasgow Centre
[2]Breast Cancer Now
[3]McNab
[4]co-supervised by Patricia Roxburgh
[5]MRC
[6]co-supervised by Joanne Edwards and Antonia Roseweir
[7]Pancreatic Cancer UK
[8]co-supervised by Jen Morton
[9]University of Glasgow

The contribution of immune cells to cancer progression and metastasis is now well established. Our lab is focused on a particular type of immune cell, called a gamma delta (γδ) T cell. We are exploring the involvement of γδ T cells in breast, colon and pancreatic cancers. γδ T cell is actually an umbrella term that encompasses 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.

In 2021, our lab contributed to three scientific papers. Two lab members (Wilma and Rob) gave oral presentations at the 9th International γδ T Cell Conference, which was held virtually from Beijing, China. Toshi won best flash talk at the annual Beatson Institute Conference, as well as best poster prize (out of 600 posters) at the British Society for Immunology Congress in Edinburgh. Anna was recognised for her exceptional contribution to research during the pandemic. We were also happy to receive funding from the Cancer Research Institute (New York) and Worldwide Cancer Research.

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 has yielded a number of new targets for pro-metastatic γδ T cells, including co-inhibitory and co-stimulatory molecules expressed on the surface of these cells. We have found that a subset of lung γδ T cells expressed constitutively levels of PD-1 and ICOS. Manipulation of these molecules on lung γδ T cells *in vitro* and *in vivo* has shown that PD-1 regulates IL-17A expression, while the function of ICOS remains unknown. Excitingly, mammary tumours regulated expression of another co-inhibitory molecule on lung γδ T cells, which was the receptor TIM-3. Blocking TIM-3 on lung γδ T cells increased IL-17A expression. These data have broader implications for cancer patients on immunotherapy drugs targeting PD-1 and TIM-3, suggesting that increased IL-17A after anti-PD-1 or anti-TIM-3 treatment may contribute to resistance mechanisms. We are currently testing the impact of these drugs on metastasis progression and resistance to immunotherapy.

Another project in the lab is investigating the anti-tumour functions of γδ T cells. We have identified a subset of cells with cancer-killing functions (Figure 1A). We are testing different methods to increase their killing ability to use in adoptive cell transfer experiments to target primary and secondary tumours.

Colorectal cancer

We have continued our collaboration with Owen Sansom and Adrian Hayday (Francis Crick Institute) to investigate the role γδ T cells in mouse models of bowel cancer. We are particularly interested in the gut-resident γδ T cell population that express the Vγ7 chain T cell receptor chain and their role in cancer progression. We have found that these cells counteract intestinal adenoma formation and kill transformed enterocytes in mice. When tumours develop, however, these cells are largely excluded from the tumour microenvironment. We have found that Butyrophilin-like 1 (BTNL1), a molecule expressed on gut epithelial cells required for survival of Vγ7 cells, is absent from tumours in the bowel. This observation has led to an examination into the mechanism of BTNL1 loss. We have found that deletion of the tumour suppressor *Apc* induces the down-regulation of *Btnl1* mRNA using organoids derived from our mouse models. This down-regulation of *Btnl1* is accompanied by decreased expression by gut-specific transcription factors (Figure 1B). We are now using the organoid system *in vitro* to study the molecular link between *Apc* deletion and γδ T cell exclusion in tumours.

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 uncovering the mechanism by which γδ T cells promote metastasis. During lockdown, we discovered that macrophages and fibroblasts are reduced in pancreatic tumours from γδ T cell-deficient mice, indicating that γδ T cells regulate these cells in some way to support metastasis. Currently, we are investigating the mechanisms by which this occurs.

Publications listed on page 104

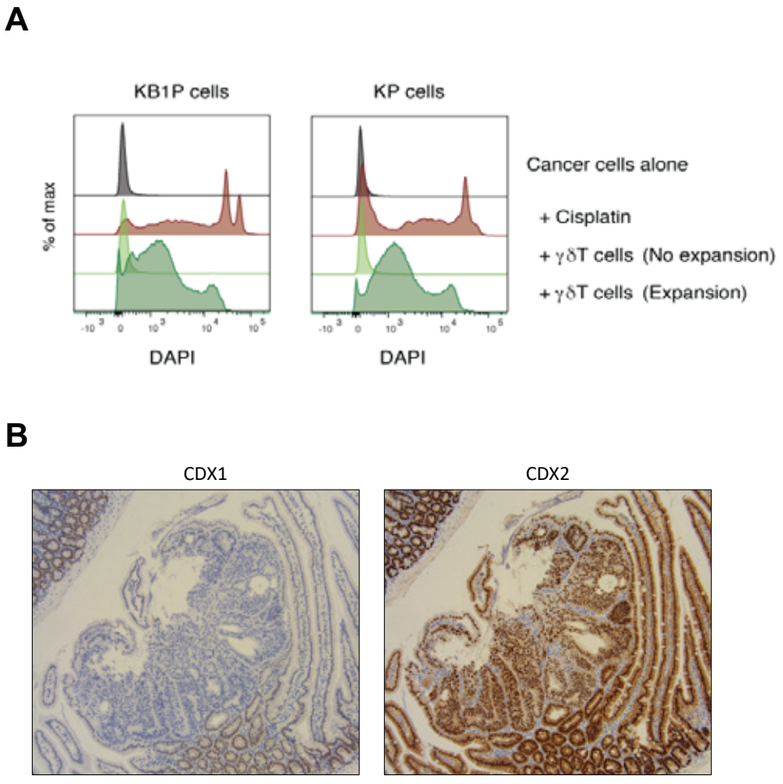


Figure 1
γδ T cells in mammary, pancreatic and colon cancers. (**A**) KB1P and KP mouse mammary cancer cell lines were co-cultured with CD27+ γδ T cells before and after expansion at a ratio of 1 T cell to 10 cancer cells. Cell death was measured by flow cytometry using DAPI. Cisplatin was used as positive control. Representative histograms of DAPI uptake by KB1P or KP mammary cancer cells for four different culture conditions shown. (**B**) Expression of CDX1 and CDX2 transcription factors in tumours from *Villin-Cre*^{ERT2};*Apc*$^{F/+}$;*Kras*^{G12D} mice, detected by immunohistochemistry.

www.beatson.gla.ac.uk/julia_cordero

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

Group Leader
Julia Cordero

Research Scientists
Karen Bellec[1]
André Carvalho[2]
Jessica Perochon[2]
Sofía Polcowñuk[3]

Scientific Officer
Yachuan Yu

Graduate Students
André Medina
Jade Phillips
Yuanliangzi Tian[4]

[1]University of Glasgow
[2]Wellcome Trust/Royal Society
[3]CRUK Pioneer Award
[4]China Scholarship Council

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.

Publications listed on page 104

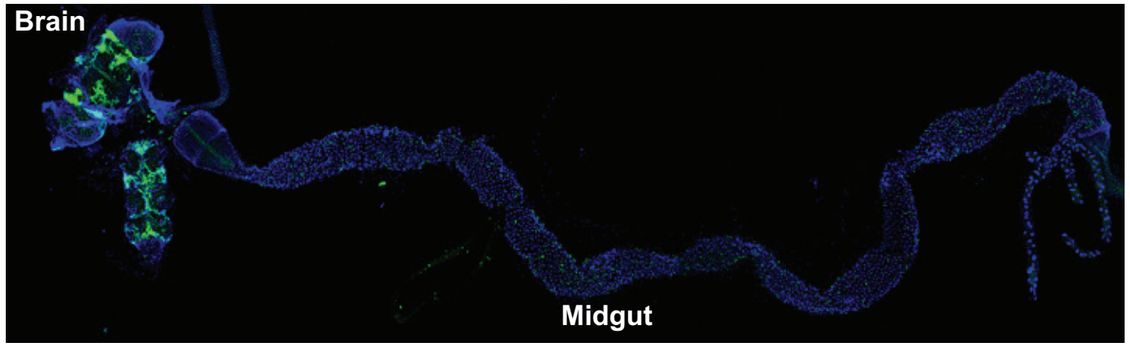


Figure 1
Image of the adult Drosophila brain midgut. Hormone secretory cells (green), all cell nuclei (blue).

Image by Andre Medina

PANCREATIC CANCER EVOLUTION AND THERAPEUTIC DEVELOPMENT

Group Leader
Fieke Froeling

Clinical Senior Lecturer
Honorary Consultant
Medical Oncologist

Research Scientist
Selma Rebus[1]

[1]CRUK Precision-Panc

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 an average survival of less than a year after diagnosis, pancreatic cancer (PC) 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 genomic medicine, one of the challenges for therapeutic development for pancreatic cancer is its heterogeneity and large cellular plasticity. Research within the field has however 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 PC exist as a continuum, with a classical tumour that has more indolent biology on one end, a highly aggressive squamous/basal-like tumour on the other, and a range of cellular states in between (Fig. 1B).

The cell-to-cell differences that drive this cellular plasticity are determined by a complex interplay of multiple genetic and non-genetic factors (Fig. 1B). Our research aims to better understand the dynamics and evolution of PC progression with the overall goal to develop novel, biomarker directed therapies. To do so, we use 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.

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, we are starting the PRIMUS-004 platform trial with the aim to offer a range of signal seeking second-line studies with extensive molecular profiling and evaluation of candidate selection biomarkers. This not only provides a clear pathway for translation of preclinical discoveries into scientifically driven clinical trials, it also allows reverse translation of clinical observations into the laboratory to keep advancing our knowledge and refine therapeutic approaches.

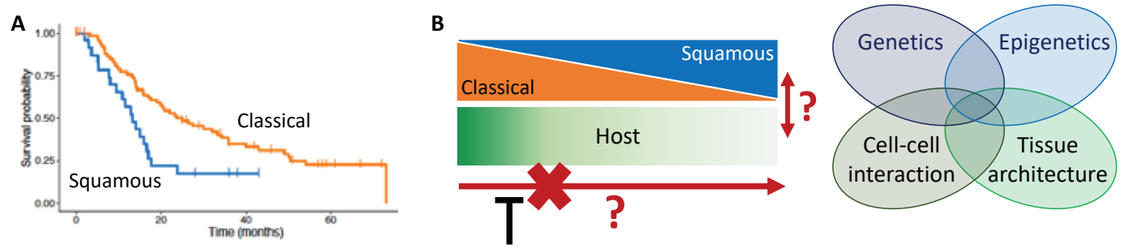


Figure 1
A) Two consensus subgroups, or lineages, of pancreatic cancer (PC) are a "classical" and a poorer prognostic "squamous/basal-like" subtype. Overall survival by subtype is shown.
B) Recent research is indicating subtypes of PC exist as a continuum, with co-evolution of tumour and host cells driving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

With thanks to the patients, translational research samples will be taken at baseline, at 2 weeks, at 10 weeks and at progression. Additional blood samples for pharmacokinetic and pharmacodynamic analysis will also be collected. The molecularly characterised, clinically well-annotated, data collected as part of PRIMUS-004 and the Precision-Panc Master Protocol, enable us to perform further in-depth studies to understand pancreatic cancer progression and treatment response.

Publications listed on page 105

www.beatson.gla.ac.uk/payam_gammage

MITOCHONDRIAL ONCOGENETICS

Group Leader
Payam Gammage

Research Scientists
Andrew Shaw
Elisabetta Tolla

Scientific Officer
Jacqueline Tait–Mulder

Graduate Student
Mahnoor Mahmood

Masters Student
Morgan McIntosh

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 are among the most common pan-cancer mutational events, comprising 25 of the 30 most mutated genes in all cancer (Figure 1a), ii) that mtDNA mutational status is unaffected by nuclear DNA mutation burden or MSS/MSI state (Figure 1b,c), iii) that recurrent hotspots define the patterning of severe mtDNA mutations (Figure 1d) and iv) that mtDNA mutation state offers major prognostic benefit in colorectal cancer (Figure 1e) (Gorelick *et al.*, 2021, *Nature Metabolism*). These findings illustrate some of the major impacts of mitochondrial genetics in cancer for the first time, shining a light on a whole additional genetic system of potential therapeutic targets that have been 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.

Publications listed on page 106

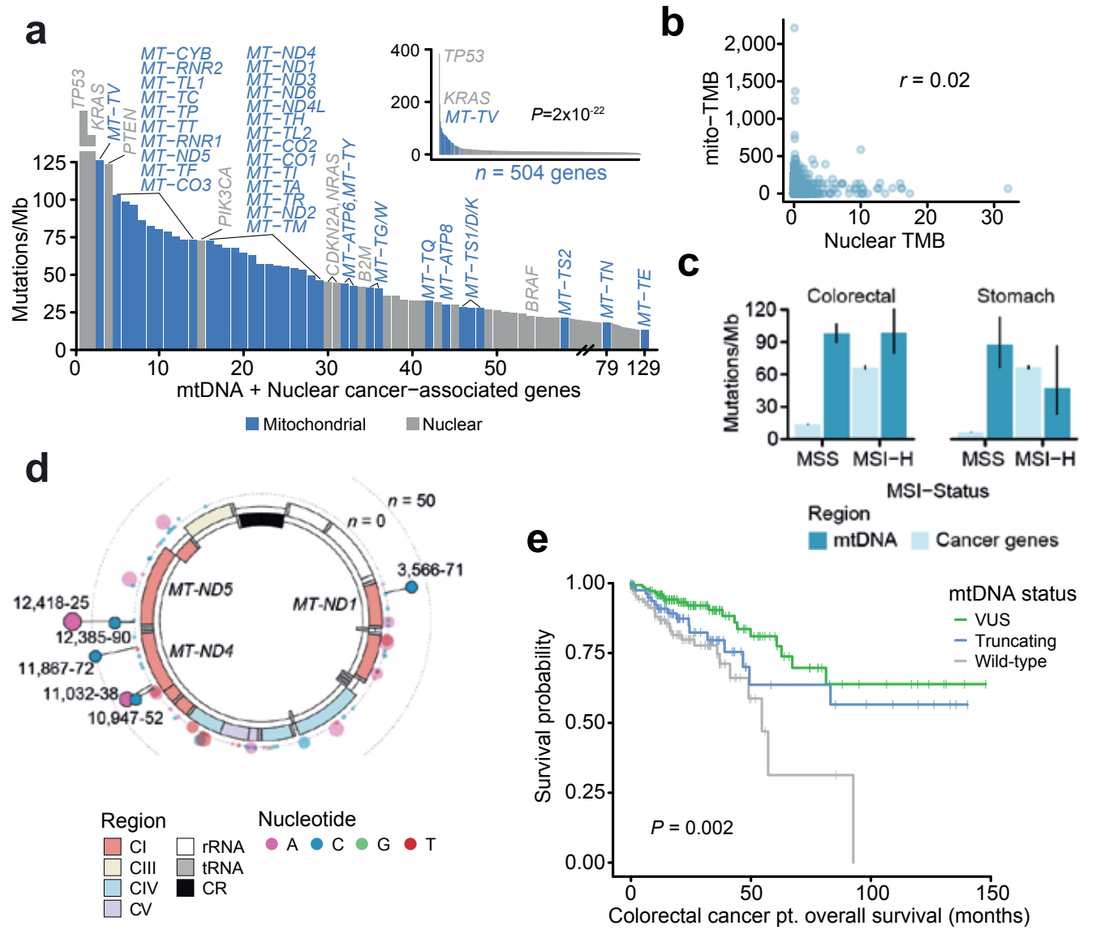


Figure 1

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).

www.beatson.gla.ac.uk/danny_huang

UBIQUITIN SIGNALLING

Group Leader
Danny Huang

Associate Scientist
Lori Buetow

Research Scientists
Karolina Majorek
Mark Nakasone
Feroj Syed

Scientific Officer
Michael McIlwraith

Graduate Students
Chatrin Chatrin
Alice Wicks[1]

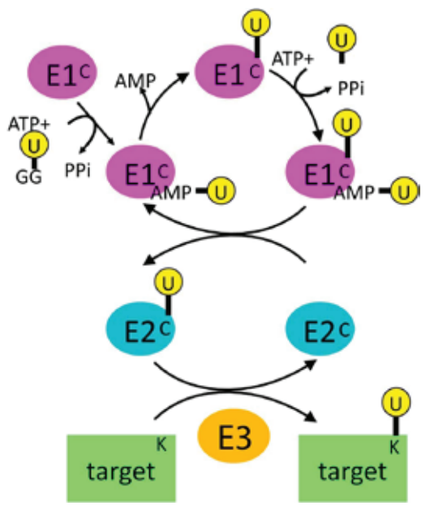
[1]BBSRC CTP

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

Ubiquitin conjugation cascade

Covalent attachment of Ub involves three key enzymes, namely E1, E2 and E3 (Figure 1). E1 adenylates Ub's C-terminus in the presence of Mg2+ and ATP, followed by formation of a covalent thioester intermediate with Ub. E1 then recruits an E2 and transfers the thioesterified Ub to the E2's catalytic cysteine, forming an E2~Ub thioester intermediate (~ indicates the thioester bond). E3 generally consists of an E2-binding module (HECT, RING, RBR or U-box domain) and a protein-protein interaction domain that can recruit the substrate directly or indirectly.

Figure 1
Enzymatic cascade for Ub modifications

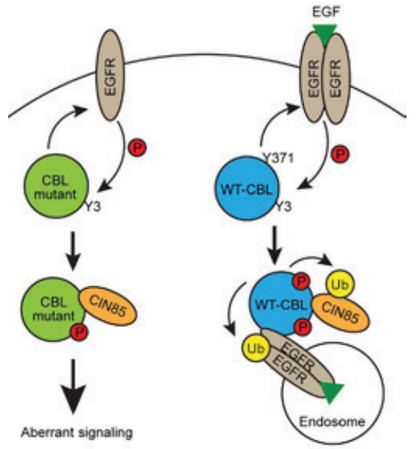


With this configuration, E3 recruits E2~Ub and the substrate to promote Ub transfer from the E2 to a lysine side chain on the substrate. In humans, there are ~600 RING E3s, and we are interested in uncovering their regulation and function, and exploring the Ub system for cancer therapeutics.

Deregulation in CBL ubiquitin ligase

CBL proteins (CBLs) are RING E3s that negatively regulate RTKs, 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 inactivate 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 up-regulated PI3K-AKT signalling cascade to drive oncogenesis (Ahmed *et al.*, 2021, Oncogene; Figure 2). Our on-going work is aiming to develop therapeutics targetting CBL mutant-EGFR interaction and thereby reducing the oncogenic property of CBL mutant.

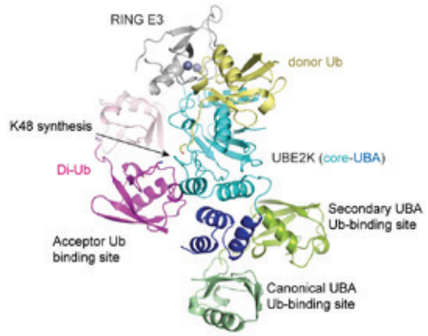
Figure 2
Oncogenic mechanism of CBL mutant



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 often due to amplification or high expression of MDM2. 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 explored whether targeting the RING domain is a suitable strategy. We showed that MDM2 E3-inactive mutant could not ubiquitinate and degrade p53. However, these mutants retained the ability to bind p53, thereby limiting p53's transcription activity in cells. Upon stresses, cells expressing E3-inactive MDM2 showed rapid p53 activation (Nomura *et al.*, 2017, Nature Structural and Molecular Biology). In collaboration with Prof. Karen Vousden's group at the Francis Crick Institute, we showed that the expression of MDM2 E3-inactive mutant was tolerated in adult mice. Despite high levels of p53, MDM2 mutant was able to restrain p53 activity sufficiently for normal growth. Upon high dose of γ-irradiation, p53 activity was rapidly activated in various tissues, but most tissues were able to dampen p53 activity and regained homeostasis (Humpton *et al.*, 2021, Genes & Development). These studies support the view that inhibitors that target MDM2 E3 activity could activate p53 in tumours with reduced on-target toxicity. We have described a strategy for preparation of the MDM2 RING domain for structural analyses to enable rapid development of MDM2 RING inhibitors (Magnussen & Huang, 2021, Journal of Molecular Biology). On-going work focuses on developing molecules that target MDM2 E3 activity.

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).

Publications listed on page 106

GROWTH FACTOR SIGNALLING AND SQUAMOUS CANCERS

Group Leader
Gareth Inman

Research Scientist
Jasbani Dayal[1]

Scientific Officers
Craig Nourse
Michela Raponi
Christina Schoenherr

Graduate Students
Max Bone[2]
Irati Ricón-Santoyo
Mairi Treanor-Taylor[3]

[1]DEBRA
[2]British Skin Foundation
[3]CRUK TRACC programme

The transforming growth factor beta (TGFβ) superfamily comprises approximately forty related cytokines, including the bone morphogenetic proteins, the growth and differentiation factors, activin, nodal and the TGFβs (TGFβ1, TGFβ2, TGFβ3). As well as playing important physiological roles during development and adult tissue homeostasis TGFβ family members 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, Head and Neck SCC and other squamous tumour subtypes 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 are inactivated in 30% of sporadic cSCC and that TGFβ signalling can have potent tumour suppressive effects in the face of other mutational events *in vivo*. 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 display 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 show overexpression of TGFβ1 and many tumours upregulate TGFBR1 expression relative to normal tissue. Taken together, these observations indicate that TGFβ signalling may also act to promote tumour progression in both cSCC and HNSCC and we are focusing our initial efforts into understanding the potential tumour promoting effects of TGFβ signalling in cSCC and HNSCC in a panel of patient derived cell lines (PDCLs).

cSCC is a life-threatening complication for patients who suffer from recessive dystrophic epidermolysis bullosa (RDEB), a skin blistering disease caused by germline mutations in collagen VII, an anchoring fibril component in the skin. Unlike in sporadic cSCC, RDEB SCC tumours do not contain inactivating mutations in TGFβ

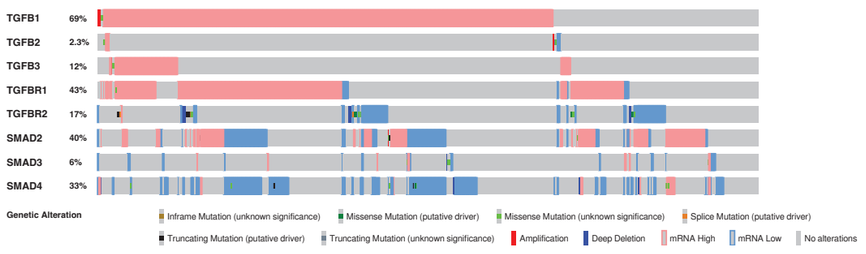


Figure 1
Oncoprint analysis of TGFβ canonical signalling components in HNSCC.
Cbioportal (Cerami et al., Cancer Discov. 2012, and Gao et al., Sci. Signal. 2013) analysis of HNSCC (TCGA, Pancancer Atlas) reveals frequent mutational alteration and downregulation of mRNA expression of TGFBR2 and SMAD4 but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

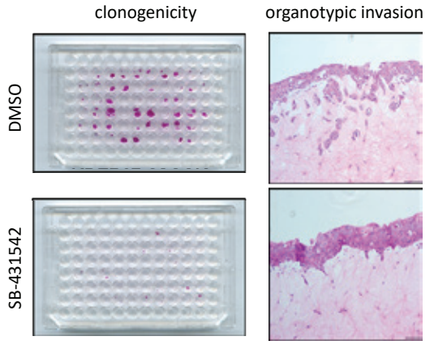


Figure 2
Inhibition of endogenous TGFβ signalling blocks clonogenicity and invasion of RDEB cSCC tumour cells.
Treatment of RDEB cSCC PDCLs with the TGFBR1 kinase inhibitor SB-431542 blocks clonogenic potential (left panels) and invasion in 3D organotypic assays using RDEB cancer associated fibroblasts embedded in type 1 Collagen-Matrigel gels (right panels) compared to DMSO treated controls.

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 may have therapeutic benefit for patients with these tumours but in some it maintains tumour suppressive activity. Our efforts are focusing on developing biomarkers for TGFβ tumour promotion and in understanding the molecular processes by which TGFβ signalling acts to drive proliferation, migration and invasion in these tumours.

The molecular landscape of cSCC

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 (Figure 3). In contrast to most other epithelial malignancies, more than a third of patients develop multiple primary cSCC. Metastasis occurs in ~5% of cases, and there are few effective treatments for advanced cSCC, with five-year 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 (Figure 4) 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.

Publications listed on page 106

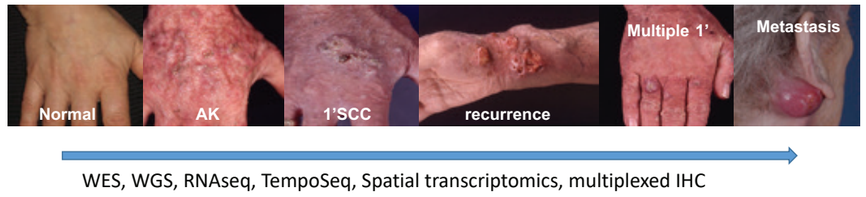


Figure 3
Disease progression of human cSCC.
Images illustrating disease progression

(Courtesy of Prof Charlotte Proby, University of Dundee). We are performing molecular profiling of human disease progression using NGS approaches, Immunohistochemistry and spatial transcriptomics.

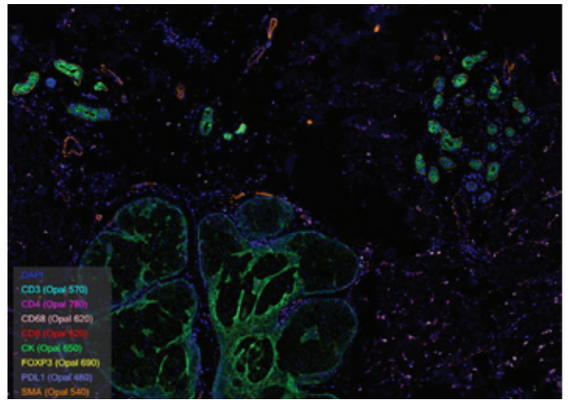


Figure 4
Multiplexed immunohistochemistry of human cSCC.
Image of a human cSCC primary tumour (courtesy of Leah Officer-Jones and John Le Quesne) stained with the indicated antibodies.

www.beatson.gla.ac.uk/robert_insall

CELL MIGRATION AND CHEMOTAXIS

Group Leader

Robert Insall FRSE

Research Scientists
Simona Buracco
Peggy Paschke
Shashi Singh[1]

Scientific Officers
Peter Thomason
Luke Tweedy

Graduate Students
Sophie Claydon
Adam Dowdell
Elena Mandrou[1]
Chris Walsh[2]

[1]EPSRC Physics of Life grant
[2]CRUK Glasgow Centre

The cell migration and chemotaxis lab studies how & why cells move, using a wide range of multidisciplinary tools, including cell biology, computer modelling and machine 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: Pseudopods and 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. Pseudopods – the structures that actually move cells – are not made following a decision, but constantly generated in random directions. Steering and migration occur when pseudopods that point in the best directions are selected and maintained. We have shown that this is true in multiple different cultured melanoma lines, and constructed computer models that reproduce individual migrating cells. We also 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. 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.

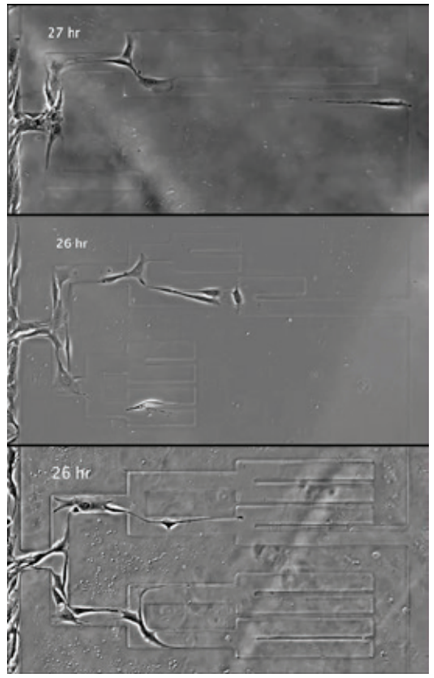


Figure 1
Pancreatic cancer cells steering themselves through a microfluidic maze using a self-generated gradient of lysophophatidic acid. This reproduces many of the features of metastasis

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 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 in view of the size of the complex and its dynamic behaviour.

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.

Publications listed on page 107

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

STEM CELL AGEING & CANCER

Group Leader
Kristina Kirschner

Scientific Officers
Ya-Ching Hsieh[1]
Craig Nourse

Graduate Students
Shafi Mohammed Alotaibi[2]
Maria Terradas-Terradas[3]
Fei Yan

Masters Student
Alison Purcell

[1]CRUK Glasgow Centre
[2]Saudi Government Scholarship
[3]MRC DTP studentship

The aim of our research is to understand how ageing influences stem cell behaviour and cancer outgrowth. We aim to use this knowledge for early detection of cancers before the cancer occurs and to identify and test new clinical therapies to prevent or treat cancer at an early stage. We also consider the influence of the ageing tumour microenvironment and effects of senescence on the tumour niche. We then examine cross talk of stem cells with their surroundings. In the majority of our studies, we use mouse models and primary patient samples.

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 cell (HSC) 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. Myeloid cancers often originate from defects in the haematopoietic stem and progenitor cell (HSPC) compartment in which a single mutation can often account for disease. For instance, the JAK2V617F mutation is sufficient for the development of myeloproliferative disease (Baxter et al., 2016). Therefore, studying HSC ageing is essential for gaining insights into mechanisms underlying the transformation of aged HSPCs into cancer stem cells.

Upon the accumulation of 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 (juxtacrine) (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).

Single cell approaches to investigate primary and secondary senescence

The role of secondary senescence *in vitro* and *in vivo* remains elusive since its discovery in 2012. Thus far, it was assumed that primary and secondary senescence phenotypes are identical. A recent publication from the lab showed, for the first time, that both senescence types differ transcriptomically. We found that Notch blunted the SASP in secondary senescence with a simultaneous accumulation of collagens. The lab aims to understand the roles different senescence phenotypes play in cancer. We combine single cell omic approaches with advanced mouse models to assess consequences of secondary senescence in genetic model systems.

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.

Longitudinal profiling of clonal haemopoiesis mutations

Clonal haemopoiesis of indeterminate potential (CHIP) is defined as the clonal expansion of HSPCs in healthy aged individuals with a myeloid bias. 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. We have previously shown an association between an increase in biological age acceleration and the presence of CHIP, as well as finding transcriptional differences between young and old HSCs carrying the *Jak2V617F* mutation (Robertson *et al.,* 2019, Kirschner *et al.,* 2017).

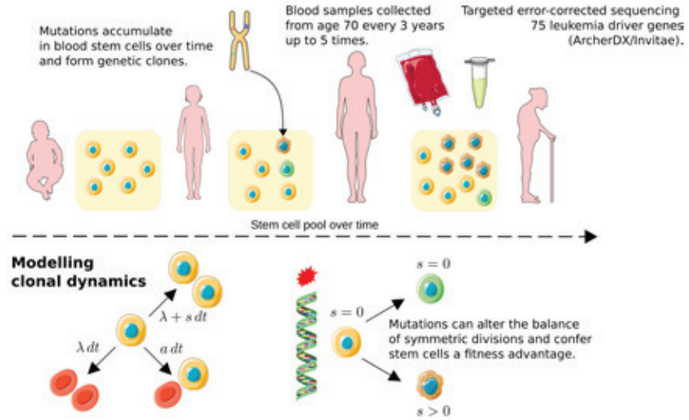


Figure 1
Mathematical modelling to interrogate stem cell fitness in clonal hemopoiesis
Mathematical models are used to estimate stem cell fitness alterations caused by mutations associated with clonal hemopoiesis. This allows us to predict outgrowth of fit clones early and progression toward leukaemia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

CHIP is characterised by mutations in leukaemia driver genes in healthy aged individuals. Several groups reported that CHIP is driven by somatic mutations in *DNMT3A*, *TET2*, and *JAK2* genes, mutations previously described as drivers of myeloid malignancies. Such mutations can increase stem cell fitness, leading to growth advantages over neighbouring cells and eventually disease. We hypothesise that identifying the distinct stem cell fitness conferred by individual CHIP mutations will enable us to predict clonal dynamics, and hence estimate risk of progression towards age-related disease.

Several studies have predicted fitness effects of CHIP in cross-sectional cohorts. As these use single-time point data, inferring fitness had to rely on assumptions about when in life mutations occurred. This introduced uncertainty and made mutation-specific estimates challenging. Current estimates showed a wide uncertainty in mutational fitness and strong dependence on an accurate, yet difficult, estimated time of mutation. Longitudinal data offer a direct and accurate way to infer fitness effects of individual variants.

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 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.

Publications listed on page 107

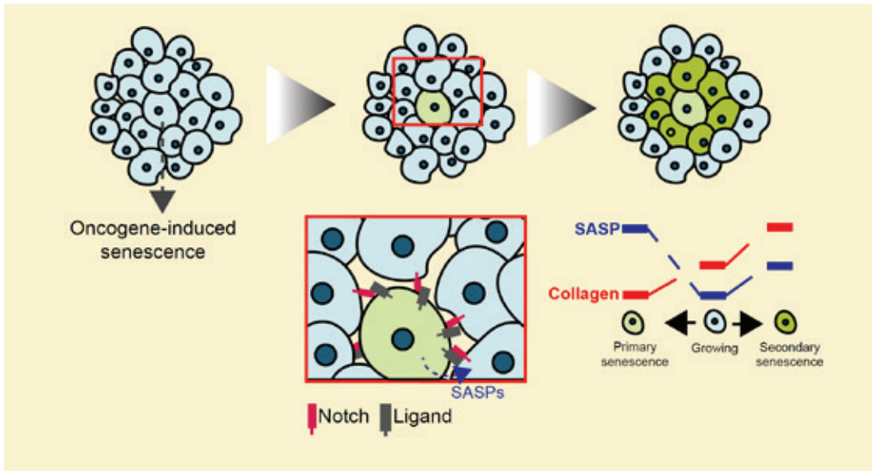


Figure 2
Model of Notch-mediated secondary senescence.
Secondary senescence *in vitro* and *in vivo* requires Notch, rather than SASP alone as previously thought, with primary and secondary senescence being distinct molecular endpoints. A blunted secretory phenotype and the induction of fibrillar collagens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

www.beatson.gla.ac.uk/john_lequesne

DEEP PHENOTYPING OF SOLID TUMOURS

Group Leader
John Le Quesne FRCPath

Research Scientists
Zhangyi He[1]
Ian Powley[2]
Hannah Williams

Scientific Officers
Fiona Ballantyne
Silvia Cusumano[2]
Rachel Pennie

Histopathology Technology Manager
Leah Officer[1]

Graduate Student
Bethany Woodvine[3]

[1]CRT translational alliance
[2]Mazumdar-Shaw Molecular Pathology Chair
[3]Midlands Integrative Biosciences Training Partnership

Solid tumours are complex assemblages of malignant cells, inflammatory cells, fibroblasts, blood vessels and other tissue types, and are perhaps 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 a potentially extremely hostile habitat, we must understand this 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 have been establishing our phenotyping technologies in the Institute since autumn 2020. We have developed numerous highly multiplexed IF/ISH staining assays using Ventana autostainer platforms and we routinely collect multidimensional images from human and mouse tumour tissues using Akoya Mantra and Polaris imaging platforms, as well as the CODEX 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?

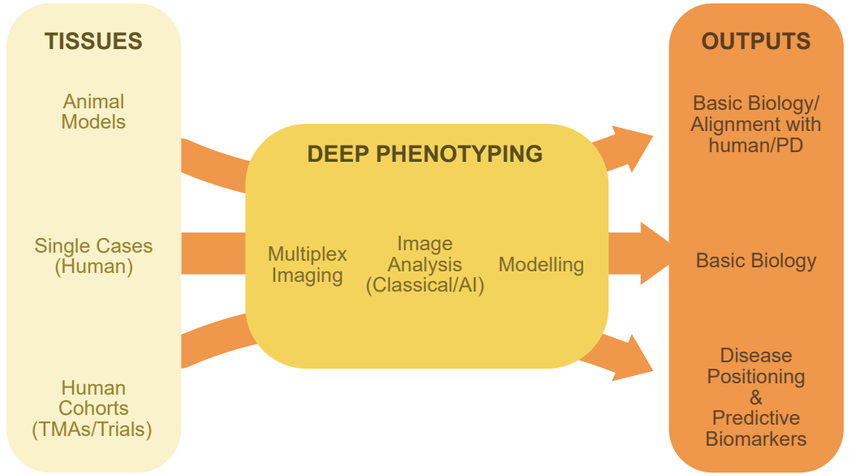


Figure 1
Workflow schematic of deep phenotyping methods. The basic pipeline (centre) is applied to a range of tissue types to achieve answers to diverse scientific questions.

To achieve this, we are applying highly multiplexed panels of markers to identify tumour and immune cell phenotypes, for instance using our CODEX 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.

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 of tumours 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

As a group 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.

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.

Publications listed on page 107

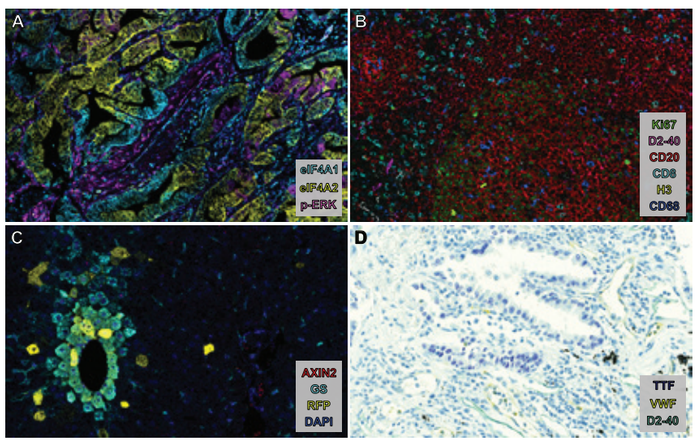


Figure 2
Example multiplex images. **A**) Spectrally unmixed multiplex staining of eIF4A1, eIF4A2 and P-ERK in archival human lung adenocarcinoma tissue . **B**) CODEX image of indicated protein markers on human tonsil tissue sections; only a small subset of the stained markers are shown **C**) Spectrally 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.

www.beatson.gla.ac.uk/hing_leung

PROSTATE CANCER BIOLOGY

Group Leader
Hing Leung

Clinical Lecturer
Mark Salji[1]

Scientific Officers
Ernest Mui[2]
Linda Rushworth[2]

Visitors
Mario Cangiano[3]
Maha AlRasheedi

[1]NHS Educating Scotland
[2]Prostate Cancer Foundation; Horizon 2020
[3]Horizon 2020 ITN, in conjunction with GenomeScan

University of Glasgow

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. Our recent research applied a range of preclinical models to investigate molecular drivers of treatment resistance, aimed at the discovery of new therapeutic strategies.

In this report, we describe our recent advances in multi-omics and network-based analyses to probe the molecular basis of advanced prostate cancer including castration resistant disease. Our findings support the idea that Prostate Specific Membrane Antigen (PSMA) may have a potentially functional role in promoting treatment resistance in prostate cancer in addition to its role as an imaging marker.

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 castration resistant prostate cancer (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). In these early studies, we have adopted a relatively focussed platform (for instance, the proteome) as a starting point for investigating the molecular basis driving CRPC, which will inevitably result in a relatively 'narrow' perspective of the underlying biology. To provide an added dimension of our knowledge in CRPC, we have now carried out a multi-omic analysis of our orthograft models of hormone naïve and castration resistant prostate cancer as well as network-based regulon analysis of CRPC.

Multi-omics analysis identifies potential roles for tumour N-acetyl aspartate accumulation in Castration Resistant Prostate Cancer

Untargeted RNA sequencing, proteomics, and metabolomics analyses were performed on xenografts derived from three independent sets of hormone naïve and matched CRPC human cell line models grown as murine orthografts. We tested the feasibility of multi-omic analyses on models of CRPC in revealing pathways of interest for future validation investigation (Figure 1). Untargeted metabolomics revealed N-acetylaspartate (NAA) and N-acetylaspartylglutamate (NAAG) commonly accumulating in CRPC across three independent models, while 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). Based on pathway analysis integrating multiple omic levels, we hypothesise that increased NAA in CRPC may be due to upregulation of NAAG hydrolysis via NAALADLases providing a pool of Acetyl Co-A for upregulated sphingolipid metabolism and a pool of glutamate and aspartate for nucleotide synthesis during tumour growth.

Our findings are highly relevant to clinical prostate cancer. PSMA is an important imaging marker of prostate cancer. PSMA based PET imaging is rapidly transforming the detection of low burden prostate cancer metastatic lesions as well as cancer recurrence (both hormone naïve and castration resistant disease)(Figure 2). In addition, PSMA based therapy is rapidly gaining momentum as part of an effective treatment for advanced metastatic disease. Here, our data supports the idea that PSMA may functionally contribute to disease progression and represents a valid target for therapy.

Figure 1

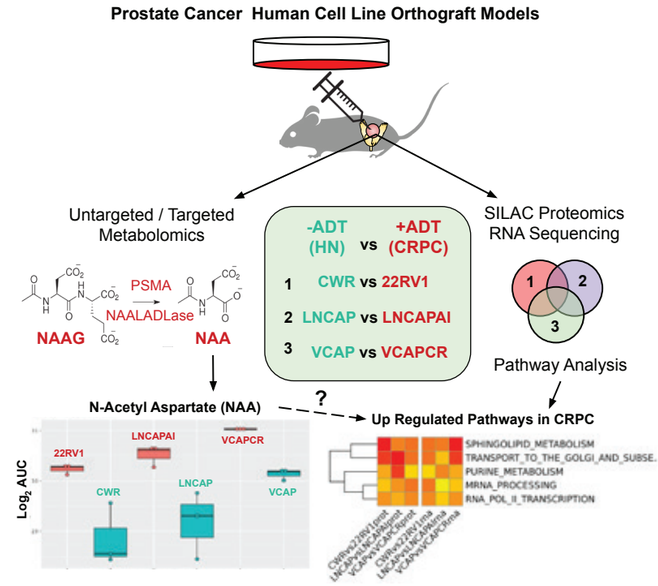


Figure 2

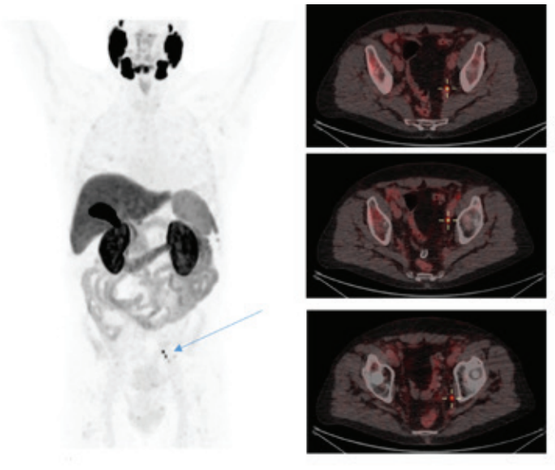
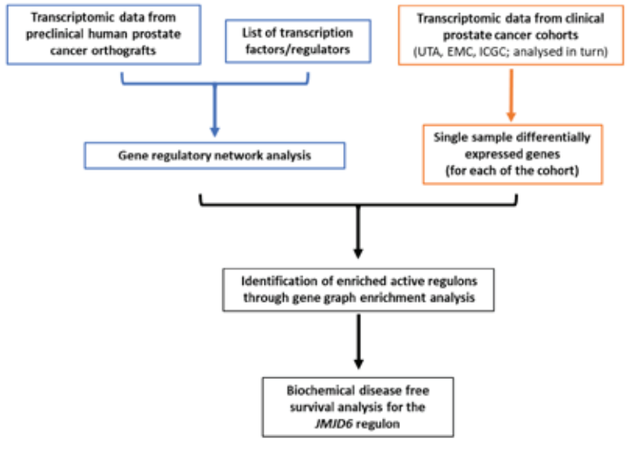


Figure 3



Transcriptomic gene regulation network analysis of human prostate orthografts

We exploited a graph-based enrichment score to integrate transcriptomic data from gene regulation network identified in our prostate orthografts and differentially expressed genes in clinical resected prostate tumours (Figure 3). We tested whether a network of genes similarly regulated by transcription factors (gene products that control the expression of target genes) are associated with patient outcome. We identified regulons (networks of genes similarly regulated) from our preclinical prostate cancer models and further evaluated the top ranked JMJD6 gene related regulated network in three independent clinical patient cohorts.

JMJD6 belongs to the Jumonji C (JMJC) domain-containing family of proteins. JMJD6 is thought to function mainly as a lysyl 5-hydroxylase. Its ability to regulate the transcriptional activity of p53 through hydroxylation of a lysine in the p53 C-terminus is highly relevant in cancer biology. Upregulated JMJD6 expression has been implicated in tumour growth, tumour metastasis and high tumour pathological grades. Our transcriptomic network analysis highlighted the value of future studies on JMJD6 mediated function in prostate cancer biology.

Concluding comment

The use of a multi-omics approach and the application of a network-based analysis have potential in revealing important insight into CRPC.

Publications listed on page 108

Figure 1
Schematic representation of multi-omics analysis on data from three sets of isogenic hormones naïve and castration resistant prostate cancer orthograft models.

Figure 2
An example of ^{18}F-PSMA PET/CT scan on patient with biochemical recurrence following radical prostatectomy. (Images provided by Dr David Colville, Department of Nuclear Medicine, NHS Greater Glasgow & Clyde)
Left panel shows Maximum Intensity Projection image to represent three-dimensional visualization of signals from PSMA binding tracer. Background signals (strong – salivary glands, liver and kidney; weak – small bowel) can be seen, along with signals in left pelvis (highlighted by blue arrow), in keeping in metastatic deposits in the pelvic lymph nodes, confirming the presence of recurrent prostate cancer.
Right panel represents fused axial CT images confirming focal uptake within matching sub-centimetre pelvic lymph nodes with the largest node measuring 5 mm in diameter.

Figure 3
The workflow of the gene regulatory network analysis. (UTA, EMC and ICGC signify three independent patient cohorts).

MOLECULAR IMAGING

Group Leader
David Lewis

Research Scientists
Robert Bielik
Algernon Bloom[1]
Piotr Dzien[2]

Graduate Students
Irene Caldera Quevedo
Emer Curley[2]

[1]CRUK RadNet
[2]CRUK Glasgow Centre

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 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 was characterised by high [^{11}C]acetate uptake. Evident on dual-isotope autoradiographs, these tumour sub-regions appear 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 shows distinct transcriptional, proteomic and metabolic signatures. Regions with higher glucose consumption are more proliferative with activation of cell cycle genes, Myc targets and the unfolded protein response. While regions of high acetate uptake have signatures for 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 utilise glucose for synthesis of serine and glycine and use acetate to replenish the TCA cycle intermediates. In contrast, acetate-avid tumours use 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.

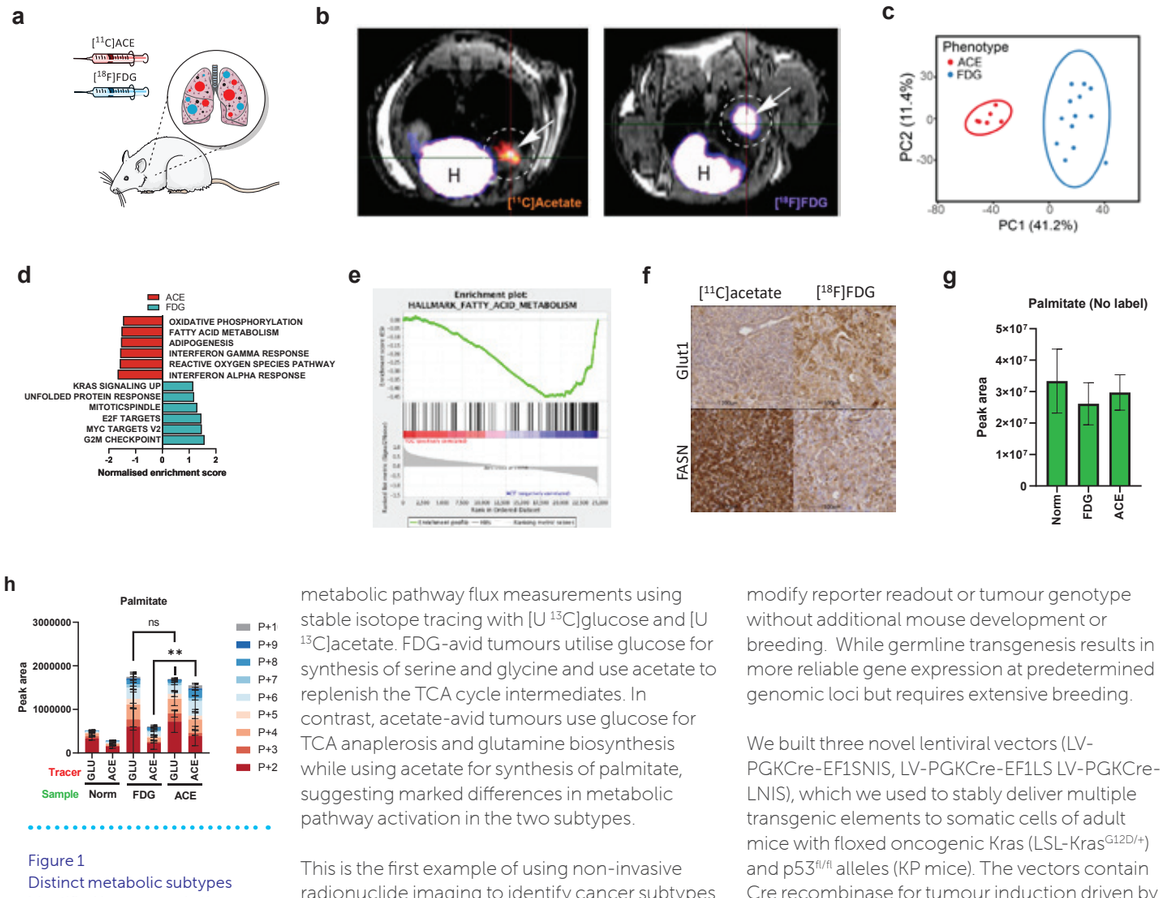


Figure 1
Distinct metabolic subtypes identified in oncogenic Kras driven lung cancer.
(**a**) and (**b**) dual radioisotope PET imaging with sequential [^{11}C] acetate and [^{18}F]FDG injections indicating respective tumour regions with elevated fatty acid synthesis and glucose uptake. (**c**) Principle component analysis from transcriptomic analysis of metabolic phenotypes, showing distinct separation. (**d**) Gene-set enrichment analysis highlighting elevated fatty acid metabolism as a key differentially expressed pathway (**e**). (**f**) Immunohistochemistry of Glut-1 and FASN in [^{11}C]acetate and [^{18}F]FDG-avid tumours. (**g**) Metabolomics of steady-state palmitate and (**h**) isotopologues following [U ^{13}C]glucose and [U ^{13}C]acetate infusion tracing into palmitate.

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.

Tomographic imaging of spontaneous tumorigenesis and treatment response

Genetically engineered mouse models (GEMMs) of cancer recapitulate the genetic and microenvironmental heterogeneity characteristic of clinical tumours. There is evidence that GEMMs are more predictive of patient response than subcutaneous tumours. However, visualising tumour formation and treatment response is difficult as tumours develop with varying latency and at autochthonous sites.

We are therefore further developing radionuclide imaging of NIS as a method for visualising oncogenesis and drug response in transgenic mouse models. NIS imaging is an improvement on current optical methods, as it does not suffer from the same photon scatter and absorbance as light as, for example, bioluminescence. We have taken two approaches to the gene delivery of NIS: somatic induction and germline transgenesis. Somatic induction has the advantage that the vector can be readily customised at the bench to modify reporter readout or tumour genotype without additional mouse development or breeding. While germline transgenesis results in more reliable gene expression at predetermined genomic loci but requires extensive breeding.

We built three novel lentiviral vectors (LV-PGKCre-EF1SNIS, LV-PGKCre-EF1LS LV-PGKCre-LNIS), which we used to stably deliver multiple transgenic elements to somatic cells of adult mice with floxed oncogenic Kras (LSL-Kras$^{G12D/+}$) and p53$^{fl/fl}$ alleles (KP mice). The vectors contain Cre recombinase for tumour induction driven by a constitutively active PGK promoter and reporter genes (luciferase/ mStrawberry, sodium iodide symporter (mNIS)/ mStrawberry and luciferase/ sodium iodide symporter (mNIS) driven by the high activity promoter EF1.

Mice were longitudinally imaged using ([^{18}F]TFB PET and $^{99m}TcO_4$– SPECT) and lesions down to tens of nanolitres could be repeatedly imaged. Imaging following drug therapy identified single initiating lesions with significantly reduced $^{99m}TcO_4$–uptake and elevated cleaved caspase 3 (CC3) staining, indicating that imaging identified true responders 24 hours after treatment. This is the first example of a radionuclide reporter gene used to monitor spontaneous tumour development in a live mouse. This is important, as unlike bioluminescent approaches, it enables sensitive three-dimensional imaging of tumour development *in vivo* that is unaffected by overlying tissue-depth or tissue pigmentation. We are exploiting the tomography of radionuclide imaging to track single lesions at nanolitre resolution during cancer therapy and identifying inter- and intratumoural heterogeneity in drug response.

Publications listed on page 109

www.beatson.gla.ac.uk/laura_machesky

MIGRATION, INVASION AND METASTASIS

Group Leader

Laura Machesky FMEDSci, FRSE

Associate Scientist
Amelie Juin

Research Scientists
James Drew
Esther Garcia[1]
Jamie Whitelaw[1]

Scientific Officer
Heather Spence

Graduate Students
Hakem Albilasi[2]
Anh Le
Elaine Ma[3]
Juda Milvidaite[3]
Savvas Nikolaou
Sonia Rolo

[1]EPSRC/UKRI Physics of Life
[2]Saudi Government Scholarship
[3]EPSRC Physics of Life, Centre for Doctoral Training Bioengineering

We are particularly interested in the mechanisms of cell migration and invasion and how the tumour microenvironment governs cell behaviour, depending on mechanical stiffness, nutrient availability and signalling. We recently discovered that the actin and RAC1 regulatory protein CYRI can control the cellular balance between migration and macropinocytosis. This finding suggests an important role for CYRI, RAC1 and the Scar/WAVE complex in regulating the plasticity of tumour cells to survive starvation and escape harsh conditions by migrating. Pancreatic tumours are especially fibrotic and nutrient-starved, adding to their aggressiveness. Our goal is to find new ways to manipulate tumour cell plasticity and expose vulnerabilities that could be targeted against metastasis.

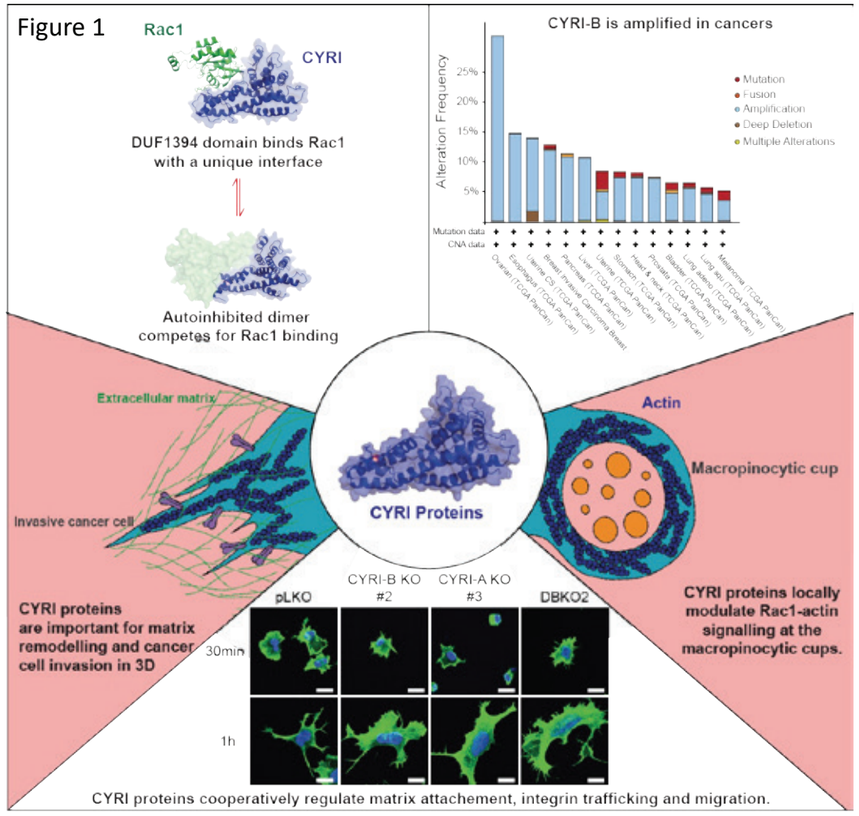
One of the ways that tumour cells survive in the hostile tumour microenvironment is by repurposing their actin cytoskeletal migration machinery to take in large gulps of the surrounding liquid by macropinocytosis. Migration and macropinocytosis use the same basic actin machinery, so can compete with each other, but the mechanisms controlling this competition are not well understood. PhD student Anh Le discovered an important role for CYRI-A in regulating the balance between macropinocytosis and invasive cell migration (Le *et al.*, 2021, *Journal of Cell Biology*) (Figure 1). 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 are unable to perform macropinocytosis, but show enhanced invasive migration, suggesting a competition between these processes (Le *et al.*, 2021, *Journal of Cell Biology*).

Working with Tamas Yelland and Shehab Ismail, Anh and Savvas went on to solve the crystal structure of CYRI-B in a complex with RAC1 (Yelland, Le *et al.*, 2021, *Structure*). This structure and their biochemical work revealed a mechanism for how CYRI and RAC1 binding could be regulated by dimerisation of CYRI in a way that opposes RAC1 binding. We also learned that the interface between CYRI and RAC1 is unique among RAC1 interacting proteins and is likely shared with CYFIP proteins. CYFIPs are members of the Scar/WAVE complex, which is activated by binding to active RAC1. Together, our study suggests a mechanism by which the Scar/WAVE complex is regulated negatively by CYRI proteins, opposing branched actin formation that drives lamellipodia and macropinocytic cup formation.

We also have ongoing work to study the interaction between pancreatic cancer cells and the liver metastatic niche (Figure 2). Postdoctoral fellow James Drew, with student Elaine Wing See Ma, has developed a co-culture system for pancreatic cancer cells and normal liver spheres. The liver sphere system was developed in Prof. David Hay's lab in Edinburgh and is used for study of normal liver function. We adapted this for 3D co-culture and are using confocal imaging, proteomics (with Kelly Hodge and Sara Zanivan) and RNA-sequencing to identify the conversation between the tumour cells and their mini-liver niche.

Taken together with our recent study connecting metabolic energy flux with mechanosensing in pancreatic cancer cells (Papalazarou *et al.*, 2020 *Nature Metabolism*), we are building up a picture of how pancreatic cancer cells respond to the microenvironment. Stiff fibrotic matrix induces a highly migratory state and the challenges of breaching matrix barriers require cells to enhance their ATP production and recycling to meet demand. The tumour environment also can be depleted of nutrients, which induces macropinocytosis, as cells switch to alternative nutrient sources such as proteins.

Figure 1



Macropinocytosis and migration use the same actin machinery, so can compete for cellular resources. Essentially, tumour cells have to make decisions about whether to walk or to eat and CYRI, RAC1 and Scar/WAVE provide this flexibility for cells, but with high energy demand. Our goal is to understand how cells meet the energy demands of navigating the tumour microenvironment and to look for vulnerabilities that we can exploit against metastasis.

Publications listed on page 109

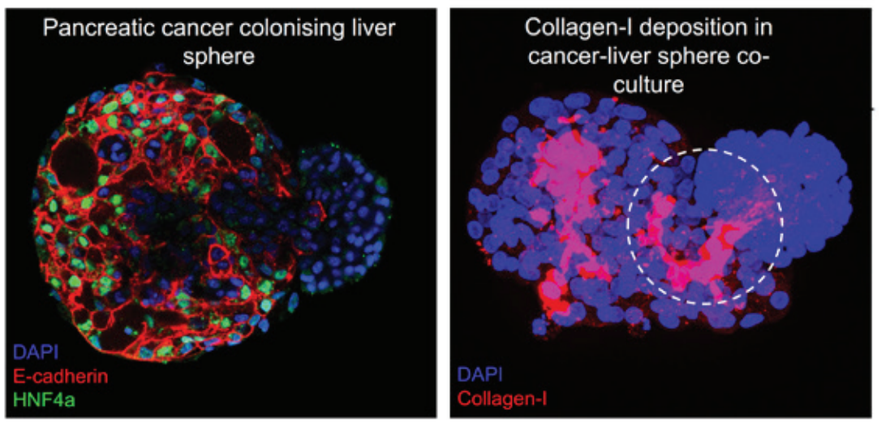


Figure 2
Pancreatic cancer cells colonising liver spheres
Left picture shows staining for DNA- DAPI, blue, Epithelial junctions- E-cadherin, red; Hepatocyte identity- HNF4a, green. Right picture shows staining for DNA- DAPI; Collagen-I, red.

COMPUTATIONAL BIOLOGY

Group Leader
Crispin Miller

Research Scientists
Holly Hall
Tamara Luck

Graduate Student
Boyu Yu

The computational biology group is interested in how the processes that control gene expression are altered in tumour cells, how these changes occur, and how they drive oncogenic transformation and tumour progression. We are studying these systems by using classical- and deep- machine learning approaches to study multiomics datasets arising from clinical and *in vitro* studies.

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?

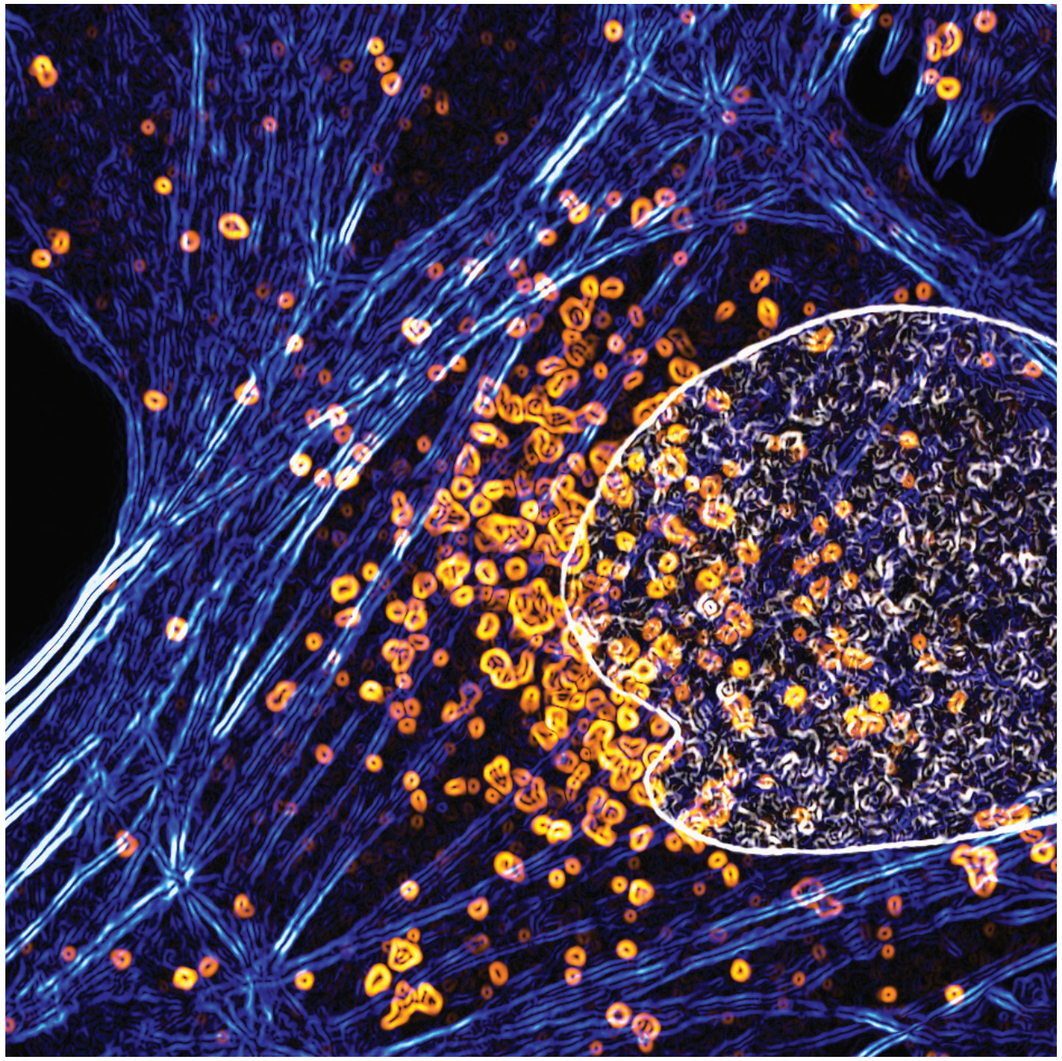
Our work exploits the availability of a large and diverse cohort of well annotated genome sequences from different species. This allows comparative genomics to be used to pursue regulatory patterns from an evolutionary perspective. In parallel, the availability of large cohorts of DNA- and RNA-sequenced 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.

Tamara Luck, a postdoc in the group is interested in regulatory sequences embedded within coding sequences, and how mutations in and around these regulatory sites can impact on protein levels. Boyu Yu, a new 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 recently joined the lab as a postdoc to develop computational models of mesothelioma 'omics data (Tsim et al. 2021).

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 the Information Services team to expand the High-Performance Computing infrastructure that will underpin our data science efforts across the Institute.

Publications listed on page 110



An osteosarcoma cell (U2OS) was stained with markers for the cell recycling and degradation centre, the lysosome (LAMP-2, yellow), the actin cytoskeleton (Blue, Phalloidin) and the nucleus (Grey, DAPI/DNA). The cell was imaged on the Zeiss 880 super-resolution microscope at the Beatson Institute and processed using FIJI (ImageJ) software.

Image by David McEwan

www.beatson.gla.ac.uk/jennifer_morton

PRECISION-PANC PRECLINICAL LAB

Group Leader

Jennifer Morton

Research Scientists
Karen Pickering[1]
Mathias Tesson[1]

Scientific Officers
Saadia Karim
Kay Kong[1]
Loveena Rishi Delori[2]

Graduate Students
Laura Lapienyte[2]
Curtis Rink[1]
Dale Watt[3]

[1]CRUK PRECISION-Panc
[2]Pancreatic Cancer UK
[3]CRUK Glasgow Centre

The focus of research in our lab is to better understand how pancreatic cancer develops and progresses and use this knowledge to identify and test new clinically relevant therapies and combinations. To do this, we use mouse models of pancreatic cancer that recapitulate human tumours in terms of both the genetic aberrations and the dense fibrotic and immuno-suppressive stroma. These models, therefore provide a clinically relevant platform, in which to test and validate or de-validate novel tumour cell and microenvironment targeted therapies.

Pancreatic cancer, or pancreatic ductal adenocarcinoma (PDAC), kills over 430,000 people every year. It is one of the deadliest epithelial malignancies, and both incidence and mortality are rising. In the UK alone, there are around 10,500 new cases every year, equivalent to about 30 new cases every day. Less than 8% of those patients will survive their disease for five years, and only 1% are likely to survive beyond ten years. Indeed, despite improvements in surgical management and significant investment in clinical trials, cure rates have only minimally increased over the last 50 years.

Many years of research have improved our understanding of disease 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 patient may confer sensitivity to targeted therapies. For that reason, part of our work involves modelling mutations in the genes that are mutated in smaller subsets of human pancreatic cancer with a view to understanding the biological consequences of 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 in the human disease. 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, able to influence tumour cell proliferation, survival, metabolism, migration, immune surveillance, and response to chemotherapy. Therefore, it is essential to investigate pancreatic tumour biology *in vivo*, in spontaneous tumours with a physiological microenvironment and immune response.

Modelling genetic subsets of patients

With regard to the recurring mutations in patients that may be actionable, we have developed several models to mimic these patients and identify therapeutic targets. Our suite of models covers the majority of 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 (10-15%). Using KPC mice as a backbone (*Pdx1-Cre; Kras*$^{G12D/+}$; *Trp53*$^{R172H/+}$), we have now developed a genetically engineered mouse (GEM) 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. RNF43 inhibits Wnt/β-catenin signalling by reducing membrane Frizzled. Thus, to test whether this subset of patients might be uniquely sensitive to pathway inhibition we are testing a clinically-relevant porcupine inhibitor in these mice.

Mutations in DNA damage repair genes have also been reported in ~15% of pancreatic cancers. We developed models of these patients, by deleting *Atm* or *Brca1* in the KPC mouse model and found differential sensitivities to DNA damaging agents. We are now extending these studies to include radiotherapy, as we predict that these mutations will render tumours more sensitive to radiation. Using our small animal radiotherapy research platform (SARRP) we have developed a protocol for tumour-targeted radiotherapy in GEM models of pancreatic cancer (Figure 1), which we are applying to these models. The use of radiotherapy in pancreatic cancer treatment has been limited thus far, however, this may be due to a lack of understanding of the effect of radiation on the pancreatic TME. Irradiation results in tumour cell death and release of tumour-associated antigens that can elicit a cytotoxic T cell response against the tumour. However, this is impeded by the release of inflammatory cytokines and chemokines which can result in altered fibroblast secretory output, ECM remodelling, macrophage polarisation and an even more immunosuppressive microenvironment. Thus, we are using our models to investigate responses in individual cells in the TME to determine the mechanisms controlling pro-tumourigenic immune and fibrotic responses with the aim of identifying rationale therapeutic combinations to promote anti-tumourigenic immune responses while inhibiting pro-tumourigenic immune and fibrotic responses.

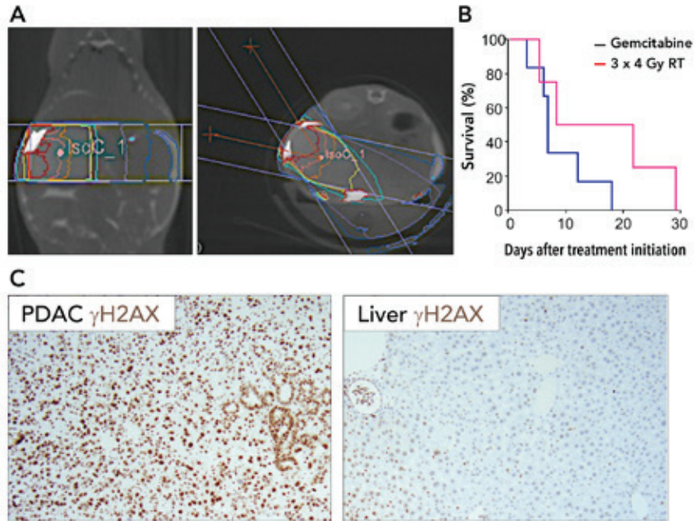


Figure 1
A) Example of CT imaging guided arc radiotherapy plan in a KPC mouse. **B)** Kaplan-Meier survival analysis of KPC mice treated as indicated and aged until clinical endpoint. **C)** Immunohistochemical staining for γH2AX in PDAC and liver from irradiated KPC mice, showing DNA damage in the tumour, but not in the surrounding tissue.

Tumour heterogeneity

Microdissection and single cell sequencing studies in human pancreatic cancer have recently revealed that both the tumour and stromal compartments display significant heterogeneity in terms of gene expression and function. For example, antibody-based single cell analysis (CyTOF) has highlighted two stable populations of cancer-associated fibroblasts with distinct expression profiles and immune cell interactions and defined by differential expression of CD105. CD105+ fibroblasts are tumour-permissive, whilst CD105- fibroblasts exhibit tumour restrictive behaviour which is dependent on the adaptive immune system.

The level of heterogeneity in mouse models has been the subject of some debate, both in terms of inter- and intra-tumour heterogeneity. We have now shown that these models do exhibit significant transcriptional heterogeneity, particularly between animals, despite identical initiating mutations (Figure 2). We now want to investigate the spatially resolved transcriptional landscape of tumours in these models, to monitor and understand this multi-level heterogeneity during tumour progression and in response to therapeutic intervention. This understanding is vital for the development of novel therapeutic strategies to improve the dismal statistics associated with this disease.

Publications listed on page 110

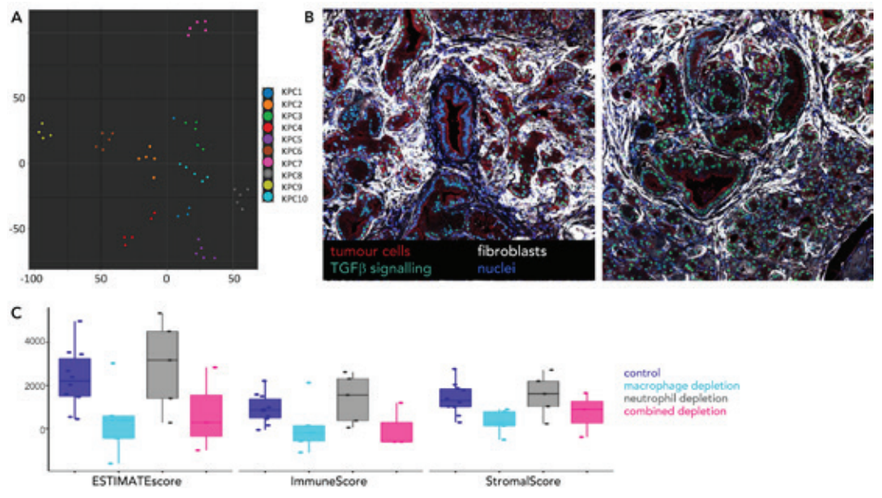


Figure 2
A) t-SNE plot and unsupervised clustering of biopsies taken from KPC autochthonous tumours (each colour represents 1 mouse; each dot represents 1 of 5 biopsies). **B)** co-IF for the markers indicated highlighting significant heterogeneity in the pancreatic cancer microenvironment. **C)** ESTIMATE (Estimation of STromal and Immune cells in MAlignant Tumors using Expression data) evaluation, using gene expression data, of the abundance of immune and stromal components in tumours from KPC mice treated as indicated.

www.beatson.gla.ac.uk/daniel_murphy

MYC-INDUCED VULNERABILITIES/THORACIC CANCER RESEARCH

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.

Group Leader
Daniel J. Murphy

Research Scientists
Jennifer Crowe[1]
Pooyeh Farahmand[2]
Sarah Laing[3]
George Skalka[4]

Scientific Officer
Jennifer Doig[5]

Clinical Fellow
Claire Rooney[6]

Graduate Student
Declan Whyte[7]

[1]Puma Biotechnologies
[2]Mick Knighton Mesothelioma Research Fund/British Lung Foundation
[3]CRUK Early Detection
[4]Merck
[5]CRUK Accelerator Award PREDICT-Meso
[6]Chief Scientist Office
[7]Pancreatic Cancer UK

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 huge number of human cancers arising from almost every tissue type. In many *in vivo* settings, MYC overexpression is sufficient to initiate or exacerbate tumourigenesis and MYC is moreover typically required to sustain the cancerous phenotype. A successful therapeutic strategy that exploits MYC overexpression is likely to 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 scientific community.

MYC and KRAS drive immune evasion

Evasion of the immune system defines the underlying principal behind the therapeutic success of immunotherapy across many cancer types. MYC is well known to induce expression of PD-L1, which inactivates cytotoxic T cells upon binding to PD1, but new data from multiple labs, including ours, indicates that PD-L1 expression is not the sole immune evasion strategy deployed by MYC. In 2020, we showed that MYC and KRAS combine to potently 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.

These transcriptional changes occur immediately upon activation of KRAS or modest overexpression of MYC in culture, and importantly, they persist throughout tumour progression *in vivo*. Mechanistically, we identified repressive transcriptional complexes, comprising MYC and MIZ1, binding directly to key regulators of Type I Interferons in pancreatic ductal adenocarcinoma, and enabling tumours to elicit CXCL13 production in nearby macrophages and thereby recruit anti-tumour immune cells to limit tumour progression, resulting in extended survival. Since publication, this provocative finding of active suppression of the Type I Interferon cascade by the MYC/KRAS pathway has been reproduced in multiple cancers, 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 widespread benefit as a 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, prominently including 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, allowing cells to balance the rate of ATP consumption with that of 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 some 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 a 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 in order to sustain the tumour phenotype. As there are presently no clinically proven inhibitors of KRAS, our observation raises the exciting possibility that simultaneously inhibiting signalling components upstream and downstream of KRAS with existing therapeutic agents may benefit lung cancer patients whose disease is driven by mutant KRAS.

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 goal of improving patient outcomes for this dreadful disease. We have developed a new mouse model that will enable us to investigate the interplay between asbestos-driven chronic inflammation and the major 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 mutations. This 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 2021

Although COVID-19 continued to limit benchwork throughout 2021, my lab met with considerable success in securing competitive funding from multiple sources. I led a successful team bid for programme funding from the CRUK Early Detection and Diagnosis Committee, securing over £2.1M in funding for IAMMED-Meso (Integrated Analysis in Mouse and Man for Early Detection of Mesothelioma), bolstered by an additional £300K from Asthma UK/British Lung Foundation for DEBIT-Meso (Differential gene Expression in Bystander Immune Transcriptomes). This funding will enable a deep-dive characterisation of the cellular and molecular content of patient and mouse model pleural effusions to identify biomarkers that may either predict progression to mesothelioma from benign disease or more accurately diagnose occult malignancy from seemingly benign disease. This work is fully integrated within the PREDICT-Meso umbrella group led by Kevin Blyth maximising the potential for rapid translation of our research into the clinic. In a separate development, our collaboration with CRUK Translational Discovery Labs and Merck Pharmaceuticals was extended for an additional year. Additionally, I contributed as a named co-Investigator and co-author on successful team bids for the CRUK Scotland Centre and the MRC National Mouse Genetics Network Cancer Cluster.

Our development of mouse models of Mesothelioma started to yield dividends with our first major publication in this field. In collaboration with the Willis, MacFarlane, Le Quesne labs of MRC Toxicology, we demonstrated a potential therapeutic benefit of targeted suppression of mTORC and PI3K in epithelioid mesothelioma, published in Nature Communications. Our growing visibility in mesothelioma research additionally spawned several new collaborations with teams in Turin, Imperial College London and Queens Uni Belfast.

PhD student and PCUK Future Leader in pancreatic cancer research Declan Whyte completed his project showing a new role of NUAK1 in regulating centrosome number and submitted his thesis for examination.

The lab sadly saw the early departure of European postdocs, Björn Kruspig and Katarina Gyuraszova, citing a combination of Brexit and COVID in their decision to depart the lab for Germany. We wish Björn, Katarina, and their new son Eric the very best on their new adventure.

Publications listed on page 112

INTEGRIN CELL BIOLOGY

Group Leader
Jim Norman

Associate Scientist
Cassie Clarke

Research Scientists
Madeleine Moore
Sergi Marco-Martin
Luis Pardo

Associate Clinical Scientist
Iain Macpherson

Scientific Officer
Louise Mitchell

Clinical Research Fellow
Dominik Kössinger

Graduate Students
Michalis Gounis
Jasmine Peters

One of the main challenges faced in treating cancer is the likelihood that, at the time of diagnosis, malignant cells have already left the primary tumour and spread to other organs. Thus, even following complete removal of the primary tumour, these disseminated cells can reside within 'primed metastatic niches' only to reappear later as metastasis. Primary tumours re-wire their metabolism in order to initiate and establish themselves, and we have found that these metabolic alterations can influence metastatic niche priming. Our research programme is dedicated to furthering our understanding of how metabolic re-wiring in the primary tumour leads to the release of factors, such as tumour metabolites and extracellular vesicles, which influence the microenvironment of other organs to prime them for metastasis. Armed with this information, we aim to target the therapeutic vulnerabilities of the metastatic microenvironment and develop strategies to oppose metastasis.

Metabolic rewiring drives release of extracellular vesicles to promote metastasis in mammary carcinoma

(i) Release of glutamate from mammary cancer cells drives invasion and metastasis by promoting release of extracellular vesicles containing mitochondrial DNA.

The CRUK Beatson Institute has an ongoing programme to map cancer-associated metabolomic landscapes. This has indicated that alterations to the serum metabolome presage metastatic onset. In mammary cancers, elevated circulating glutamate is a prominent feature of the metastasis-associated metabolome, and this is owing to upregulated expression of the glutamate-cystine antiporter, xCT (SLC7A11) in metabolically-stressed cancer cells. Extracellular glutamate then activates a metabotropic glutamate receptor, mGluR3 to drive invasive behaviour by enlisting membrane trafficking events dependent on the Rab27 GTPase. Consistently, Rab27a knockout mice bearing autochthonous MMTV-PyMT mammary tumours display reduced metastasis to the lungs. Through exploring the cellular mechanisms responsible for this, we have found that Rab27a participates in a membrane trafficking process in which mitochondrial material (including mitochondrial DNA (mtDNA)) is packaged into extracellular vesicles (EVs). Moreover, a detailed investigation into the cellular mechanisms controlling glutamate-driven EV release indicates that the mitochondrial metabolic sensing kinase, PINK1 is responsible for the packaging of mtDNA into the EV lumen. mtDNA-containing EVs are then released from cancer cells to evoke invasive behaviour in neighbouring cells by activating a toll-like receptor, TLR9. Thus, this work has led us to discover a pathway through which metabolic rewiring in cancers can drive invasion and metastasis by releasing mtDNA-containing exosomes to influence the behaviour of other cells in the tumour microenvironment and beyond. We are currently investigating how mtDNA-containing EVs can drive metastasis by activating TLR9 in elements of the innate immune system, such as neutrophils, which are recruited to primed metastatic niches.

(ii) Cells from lung micrometastases have altered glutathione levels which promote sphingomyelinase-2-dependent extracellular vesicle release

Primary tumours re-wire their metabolism to establish themselves and grow and, as we have seen above, this leads to altered invasive behaviour. We proposed that when invasive cells emanating from primary tumours arrive in metastatic target organs (such as the lung), they need to further re-wire their metabolism to adapt to the different environmental challenges posed by these locales before they can initiate metastatic outgrowth. In collaboration with Karen Blyth's laboratory, we have isolated cells from early lung micro-metastases and compared their metabolism with that of cells from their 'parent' primary tumours in the mammary gland. This yielded the surprising finding that cells from lung micro-metastases have consistently reduced levels of glutathione (reduced and oxidized) by comparison with cells from the primary mammary tumour and that this was associated with a marked increase in EV release. Furthermore, EV release from micro-metastatic cells is strongly dependent on the expression of sphingomyelinase-2, whereas EVs are released from primary tumour cells in a sphingomyelinase-2 independent manner. These findings indicate that alterations to redox metabolism made by cells as they establish lung metastases drives sphingomyelinase-2 dependent EV release. We are currently investigating how production of these EVs may assist in maintaining redox balance in micro-metastatic cells, and how they communicate with the immune system to help prime the metastatic niche.

Publications listed on page 112

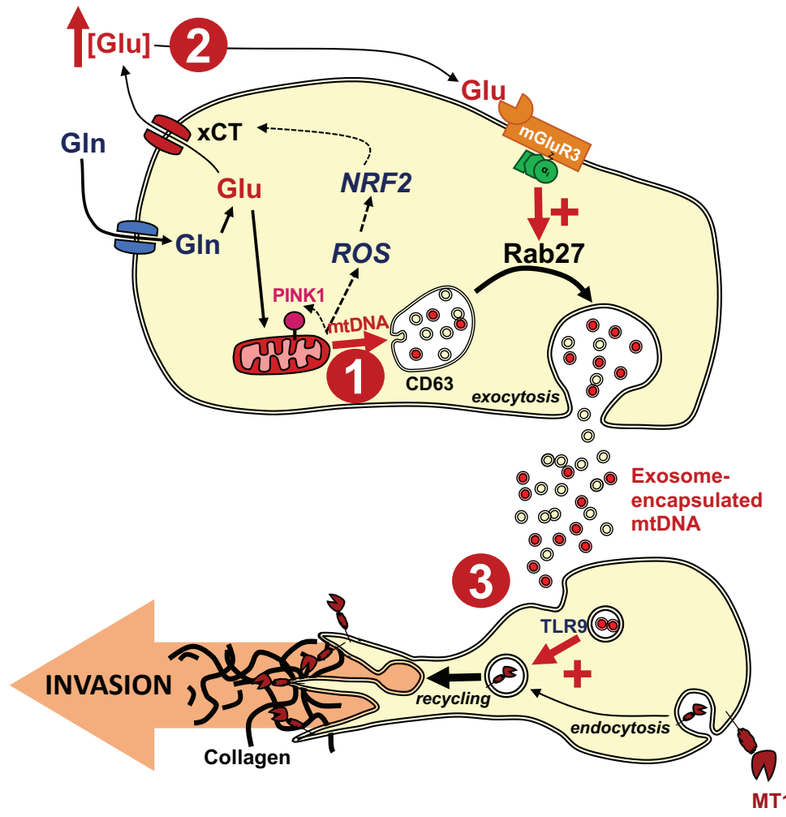


Figure 1
Re-wiring of metabolism in cancer cells drives invasion by promoting release of mitochondrial DNA-containing extracellular vesicles.
Three sequential events contribute to mechanisms through which metabolic alterations may evoke intercellular communication: [1] Mitochondrial damage/depolarisation increases levels of PINK1 to promote physical interaction of late endosomes with mitochondria. This leads to transfer of the mitochondrial chromosome into the lumen of intralumenal vesicles of late endosomes; [2] A combination of glutaminolysis and upregulation of xCT (SLC7A11) leads to increased secretion of glutamate to drive Rab27-dependent exocytosis of EVs loaded with mitochondrial DNA (mtDNA) and [3] mtDNA transported within these EVs activates a TLR9-dependent mechanism to promote pro-invasive endosomal trafficking of MT1-MMP in other cells.

IMMUNE PRIMING AND THE TUMOUR MICROENVIRONMENT

Group Leader
Ed Roberts

Research Scientists
Sarwah Al-Khalidi
Chiara Pirillo

Scientific Officer
Alberto Bravo Blas

Graduate Students
Ryan Devlin[1]
Youhani Samarakoon
Amy Shergold

[1]CRUK TRACC programme

In recent years tumour immunotherapy has led to dramatic patient benefit in a variety of cancers previously refractory to treatment. Despite these successes, only a minority of patients currently benefit from immunotherapy and more work is needed to expand their applicability. Using cutting-edge microscopy and flow cytometry, we are studying the dysfunctional initiation of anti-tumour immune responses in the lymph node. This understanding will inform future work seeking to augment anti-tumour immune responses and to increase the number of patients who can benefit from existing immunotherapies.

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 suboptimally 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 DC 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 DC 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 DC at the tumour site, how DC 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 DC 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 DC 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.

Publications listed on page 113

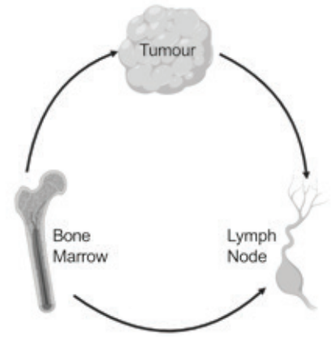


Figure 1
The DC lifecycle
DC precursors develop in the bone marrow and migrate to the tumour and the lymph node. Once within the tumour they sample proteins from the microenvironment and then mature and migrate to the lymph node. There the DC which migrated straight to the lymph node and those which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

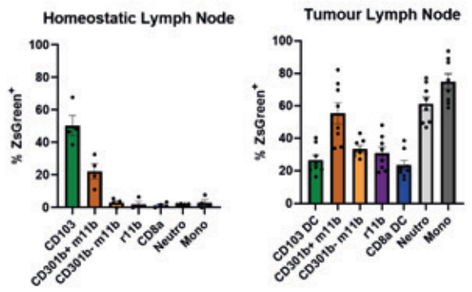


Figure 2
Tumour antigen is handled uniquely
ZsGreen expressed within the lung is carried to the lymph node by migratory DC, but the protein remains restricted to the migratory DC. When the same protein is expressed in a tumour, the protein is carried to the lymph node by migratory DC in a similar fashion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

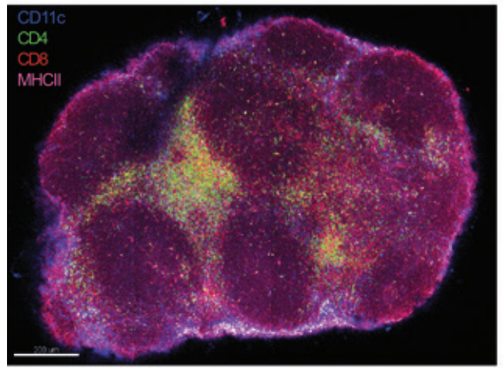


Figure 3
Lymph node organisation
A whole cleared lymph node stained for T cell, B cell and DC markers shows the organisation of a lung tumour-draining lymph node.

TUMOUR CELL DEATH AND AUTOPHAGY

Group Leader
Kevin Ryan FRSE

Associate Scientist
David McEwan

Research Scientists
Chelsea Gerada
Elzbieta Kania[1]
Marcus Ladds[2]
Victoria Wang[3]

Scientific Officers
Valentin Barthet
Jaclyn Long

Graduate Students
Martina Brucoli
Jia Li
Hannah Mearns[2]
Christoph Nössing

[1]Kay Kendall Leukaemia Fund
[2]Cancer Research UK Early Detection
[3]Henry Wellcome Fellowship

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

Autophagy in cancer

Autophagy (literally, 'self-eating') is a 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 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 - the autophagosomes. Their formation 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).

Autophagy is controlled by an expansive array of cues that can rapidly alter the rate of autophagy, allowing the cell to quickly adapt to stimuli. The cell can utilise autophagy to bring about the selective degradation of cellular components including mitochondria, protein aggregates and intracellular pathogens.

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 serves to promote 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 in different tumour types how and when autophagy ceases to be tumour suppressive and switches to support tumour growth and preservation.

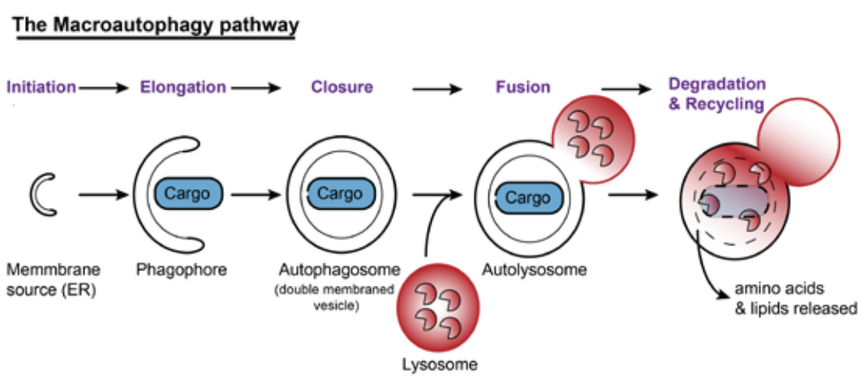
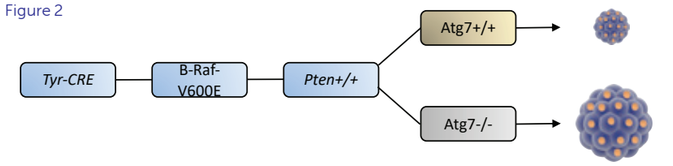


Figure 1
The autophagy pathway
The process of macroautophagy occurs in the cytoplasm of the cell and proceeds through various stages to encapsulate cargoes destined for degradation. Ultimately fusion occurs with a lysosome that provides hydrolases required for cargo degradation. The breakdown products are the recycled or further catabolised.



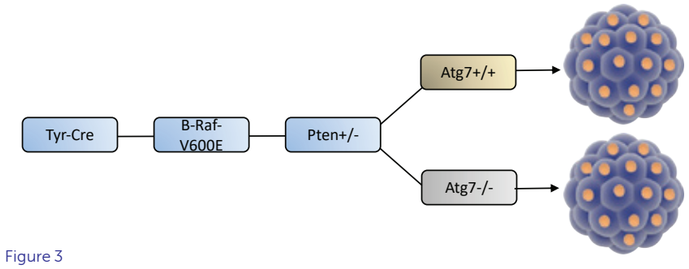


Figure 2
Pten status determines the impact of autophagy loss in the progression of melanoma. In a mouse model of melanoma driven by a mutant of B-Raf (V600E), deletion of the essential autophagy gene *Atg7* accelerates tumour development. However, in mice also hemizygous for *Pten*, the loss of autophagy has no further impact on melanoma progression.

Figure 3
Autophagy controls the ductular reaction – a key stage in hepatocellular carcinoma. Loss of *Atg5* or *Atg7* in the liver leads to tissue destruction and inflammation. As a result, hepatocytes dedifferentiate into liver progenitor cells which expand into the ductular reaction. These liver progenitor cells then differentiate back into hepatocytes to resolve/repair the damage. If this cycle persists, this leads to fibrosis followed by cirrhosis and finally, hepatocellular carcinoma.

The complex role of autophagy in cancer development

We previously showed that p53 tumour suppressor status can determine how autophagy affects the development of pancreatic ductal adenocarcinoma (PDAC). We found that in animals expressing mutant Ras and wild-type p53 in their pancreata, the deletion of the essential autophagy genes *Atg7* or *Atg5* blocked the formation/establishment of PDAC and induced excessive accumulation of pre-cancerous lesions. In contrast, in animals expressing mutant Ras, but lacking p53, inhibition of autophagy no longer blocked PDAC formation and even enhanced disease progression, indicating a tumour suppressive role for autophagy in this context.

In the context of the tumour suppressor PTEN, deletion of *Atg7* revealed that autophagy was again tumour suppressive in mice hemizygous for *Pten*, but this effect was lost in animals that were Pten null in their pancreata.

Switching to a different model of cancer development, a mouse model of melanoma driven by a mutant allele of B-Raf (V600E), we again showed that loss of Atg7 was tumour promoting in animals wild-type for *Pten*, but that this difference was lost in mice hemizygous for this allele (Figure 2).

Collectively, these studies across multiple cancer models provide compelling evidence that autophagy has a role in tumour suppression.

Autophagy in Hepatocellular Carcinoma progression

This previous work led us to question the underlying mechanisms behind the tumour suppressive/promoting role of autophagy as this knowledge is critical for the safe application of autophagy-directed therapeutics.

Through collaboration with Dr. Tom Bird, we examined the formation of liver tumours in mice expressing the liver specific albumin-Cre and floxed alleles for the essential autophagy genes *Atg5* and *Atg7*. These were combined with *Pten* floxed alleles, as *Pten* is known to be involved in the development of hepatocellular carcinoma (HCC) and its status affects how the autophagy pathway impacts tumour development.

During the progression of HCC, the liver can undergo a change called the ductular reaction in which there is an expansion of duct-like structures (Figure 3). Ductular reaction is caused by chronic liver injury and is particularly associated with the transition from fibrosis to cirrhosis, an event which significantly increases the risk of HCC development.

Interestingly, ablation of autophagy in the liver by deletion of either *Atg5* or *Atg7* and hemizygous *Pten* deletion resulted in tissue damage and a ductular reaction (Figure 3). Using this experimental system, we performed lineage tracing to show that the ductular reaction arose through the dedifferentiation of hepatocytes. Moreover, we found that the ductular reaction was associated with deregulation of transcription factors YAP and TAZ, where that co-deletion of these genes reversed the dedifferentiation and the development of HCC (Figure 3). Together, these findings not only provided insight into the role of autophagy in tumour development, but they also constituted a significant step forward in understanding of the origins of HCC that was previously controversial.

Publications listed on page 113

COLORECTAL CANCER AND WNT SIGNALLING

Group Leader
Owen Sansom FRSE, FMEDSci FRCPS(Glasg)

Associate Scientist
Andrew Campbell[1]

Research Scientists
Constantinos Alexandrou[2]
Dustin Flanagan[3]
Nuray Gunduz[1]
Georgios Kanellos[2]
John Knight
Tamsin Lannagan[4]
John Mackey
Noha Mohamed[5]
Arafath Najumudeen[6]
Alexander Raven[7]
Johan Vande Voorde[1]
Nikola Vlahov[8]

Scientific Officers
Catriona Ford[1]
Kathryn Gilroy[7]
Tam Jamieson
Rachel Ridgway

Clinical Research Fellows
Michael Gillespie
Mark White

Graduate Students
Sigrid Fey[10]
Ella Gilchrist
Jasmine Ho
Joshua Leach[11]
Laura Millett[12]
Rachael Smith[10]
Teena Thakur
Lucas Zeiger[1]

Scientific Writer
Nathalie Sphyris

[1]CRUK Grand Challenge "Rosetta"
[2]Cancer Research Technology
[3]University of Glasgow/MRC
[4]University of Glasgow/CRUK Accelerator Award
[5]AstraZeneca
[6]Novartis
[7]CRUK Grand Challenge "SpecifiCancer"
[8]University of Leicester/ Wellcome Trust
[9]McNab
[10]Pancreatic Cancer UK
[11]MRC Clinical Research Training Fellowship
[12]CRUK Glasgow Centre

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 development. Our overarching goals are to identify early-stage diagnostic biomarkers and develop stage- and subtype-specific targeted therapies.

Colorectal tumours that arise left or right to the splenic flexure differ profoundly in their epidemiology, histopathogenesis, and molecular landscapes. Left-sided CRCs 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 oncogenes and tumour suppressors, such as *KRAS*, *TP53*, and *SMAD4*, as well as the acquisition of chromosomal instability. Right-sided CRCs arise through an alternative serrated neoplasia pathway, so-called because the precursor lesions harbour a distinctive saw-tooth crypt morphology. Right-sided CRCs carry a worse prognosis than left-sided tumours and are frequently characterised by oncogenic mutation of *BRAF*, microsatellite instability (MSI), and aberrations in TGFβ signalling. In this past year, we have pursued a multi-pronged approach to develop tractable subtype-specific models of CRC ranging from early-stage adenomas through to treatment-refractory, *KRAS*-mutant left-sided CRCs, and oncogenic BRAF-driven right-sided 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 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 disrupts 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 identify 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.

Oncogenic KRAS-driven metabolic reprogramming unveils novel therapeutic vulnerabilities

To delineate how oncogenic KRAS alters the molecular landscape of APC-deficient cells and identify actionable therapeutic vulnerabilities, we performed transcriptomic and metabolomic profiling of VilCre^{ER}*Apc*$^{fl/fl}$*Kras*$^{G12D/+}$ intestinal tissues, compared with VilCre^{ER}*Apc*$^{fl/fl}$*Kras*$^{+/+}$. We found a significant enrichment of pathways associated with mRNA translation and metabolism in VilCre^{ER}*Apc*$^{fl/fl}$*Kras*$^{G12D/+}$, manifesting as elevated rates of cell proliferation and protein synthesis, and extensive metabolic reprogramming. Concomitant deletion of *Apc* and oncogenic activation of KRAS in the mouse intestinal epithelium increased glutamine consumption through a pronounced upregulation of genes associated with glutamine transport and metabolism (Najumudeen *et al.*, 2021 *Nature Genetics*). Using mass spectrometric imaging to map the spatial distribution of glutamine in intestinal tissues *in situ*, we detected paradoxically reduced levels of intracellular glutamine in VilCre^{ER}*Apc*$^{fl/fl}$*Kras*$^{G12D/+}$, relative to VilCre^{ER}*Apc*$^{fl/fl}$*Kras*$^{+/+}$ counterparts, and decreased channelling of glutamine derivatives through the tricarboxylic acid cycle. These findings suggested a metabolic fate other than glutaminolysis for glutamine in this molecular setting. Indeed, we found selective upregulation of the glutamine antiporter SLC7A5/LAT1, which exchanges intracellular glutamine for essential amino acids, (such as leucine, isoleucine, histidine, and lysine), that stimulate mTOR signalling and fuel protein synthesis.

Targeted deletion of *Slc7a5* in the intestinal epithelium of VilCre^{ER}*Apc*$^{fl/fl}$*Kras*$^{G12D/+}$ mice restored intracellular glutamine levels and decreased the translocation of essential amino acids (Figure 2A), suppressing mTOR signalling, protein synthesis, and the hyperproliferative crypt-progenitor phenotype. Consequently, *Slc7a5* deletion attenuated polyp formation and sensitised tumours to mTOR inhibition, prolonging the survival of VilCre^{ER}*Apc*$^{fl/fl}$*Kras*$^{G12D/+}$ mice (Figure 2B). Deletion of *Slc7a5* also compromised tumour formation and metastasis (Figure 2C and 2D) in the aggressive, metastasis-prone, KRAS-driven "KPN" model of CRC (VilCre^{ER}*Kras*$^{G12D/+}$*Trp53*$^{fl/fl}$*Rosa26*$^{N1icd/+}$). These

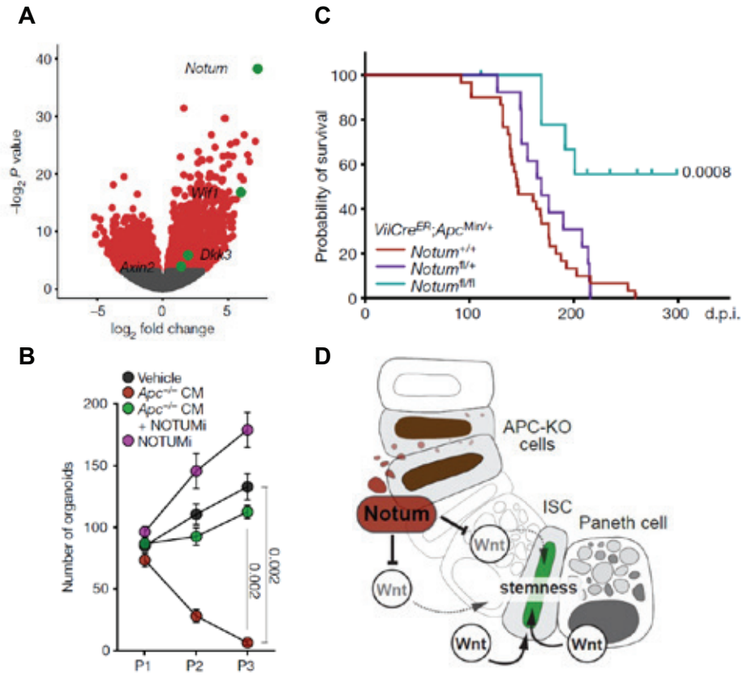


Figure 1
NOTUM is a prospective target for APC-deficient adenomas.
(**A**) Volcano plot showing genes differentially expressed between VilCre^{ER}*Apc*$^{fl/+}$ tumour tissue (n=5) and wild-type small intestine (n=3). Red, significantly altered genes; Green, Wnt-antagonists. (**B**) Number of organoids 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.

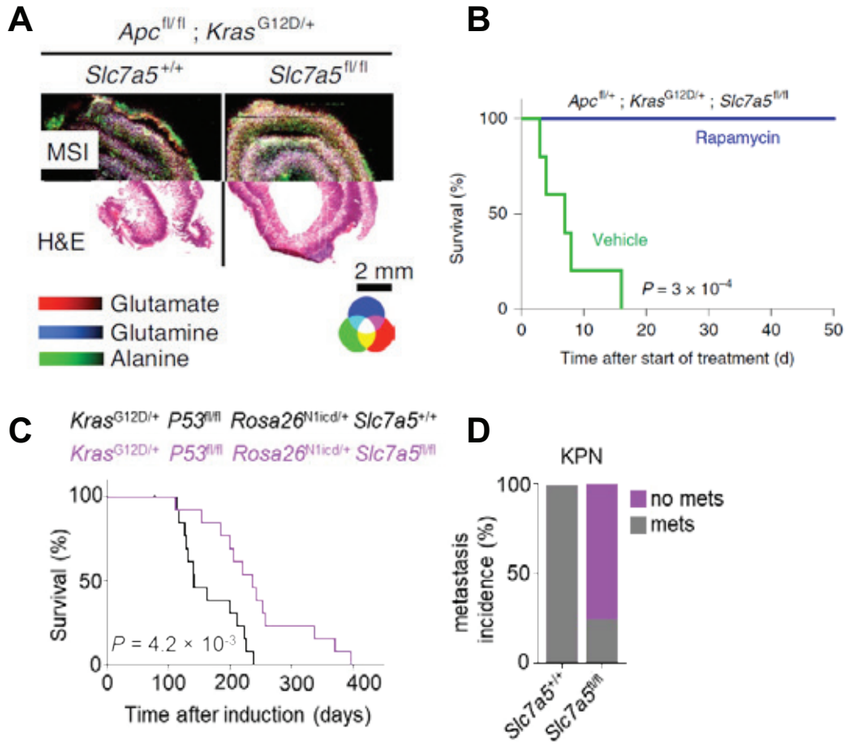


Figure 2
Targeting the glutamine antiporter SLC7A5 alters amino acid dynamics and extends survival of tumour-prone mice.
(**A**) Haematoxylin and eosin (H&E) staining (bottom) and ion intensity distribution of glutamine, glutamate, and alanine (top) in intestinal tissues from VilCre^{ER} *Apc*$^{fl/fl}$ *Kras*$^{G12D/+}$ *Slc7a5*$^{+/+}$ and VilCre^{ER} *Apc*$^{fl/fl}$ *Kras*$^{G12D/+}$ *Slc7a5*$^{fl/fl}$ mice. Ion intensities, determined by mass spectrometric imaging (MSI), are shown in the red-green-blue scale. (**B**) Survival curves for vehicle- and rapamycin-treated VilCre^{ER} *Apc*$^{fl/+}$ *Kras*$^{G12D/+}$ *Slc7a5*$^{fl/fl}$ mice. Mice were aged until they developed symptoms of intestinal disease and, subsequently, treated with vehicle or rapamycin until endpoint. (**C**) Survival curves for VilCre^{ER} *Kras*$^{G12D/+}$ *Trp53*$^{fl/fl}$ *Rosa26*$^{N1icd/+}$ *Slc7a5*$^{+/+}$ and VilCre^{ER} *Kras*$^{G12D/+}$ *Trp53*$^{fl/fl}$ *Rosa26*$^{N1icd/+}$ *Slc7a5*$^{fl/fl}$ mice aged until clinical endpoint. (**D**) Incidence of metastasis (mets) in VilCre^{ER} *Kras*$^{G12D/+}$ *Trp53*$^{fl/fl}$ *Rosa26*$^{N1icd/+}$ *Slc7a5*$^{+/+}$ (KPN *Slc7a5*$^{+/+}$) and VilCre^{ER} *Kras*$^{G12D/+}$ *Trp53*$^{fl/fl}$ *Rosa26*$^{N1icd/+}$ *Slc7a5*$^{fl/fl}$ (KPN *Slc7a5*$^{fl/fl}$) mice aged until clinical endpoint.

findings advocate the development of combinatorial therapeutic strategies targeting SLC7A5 and mTOR, and hold promise particularly for CRCs of the metabolic CMS3 subtype that are enriched for *KRAS* mutations, but also for the highly aggressive CMS4 class.

Modelling and targeting right-sided colon cancers

We developed a new mouse model of right-sided colon cancer, driven by oncogenic BRAF (BRAF^{V600E}) and loss of the epithelial TGFβ-receptor ALK5/TβRI (rather than a Wnt-activating *Apc* mutation), which faithfully recapitulated the topography, molecular landscape, and metastatic tropism of human *BRAF*-mutant right-sided colon cancer (Leach *et al.*, 2021 *Nature Communications*). Unlike intestinal tissues from APC-deficient (VilCre^{ER} *Apc*$^{fl/+}$) or VilCre^{ER} *Braf*$^{V600E/+}$ (B) mice, the right-sided colonic tumours that developed in VilCre^{ER} *Braf*$^{V600E/+}$ *Alk5*$^{fl/fl}$ (BA) mice did not show nuclear β-catenin accumulation (Figure 3A) and lacked expression of the adult ISC-marker *Lgr5* and its associated ISC signature (Figure 3B), indicating that these tumours lacked Wnt-pathway activation. Instead, BA right-sided tumours exhibited a foetal-like progenitor (*Ly6a/Sca1*)$^{+}$ phenotype (Figure 3B), which also correlated with *BRAF*-mutant status, right-sidedness (Figure 3C), MSI-high status, CMS1 class - the signature of pre-neoplastic sessile serrated adenomas in humans - and shorter survival post relapse (Figure 3D). In addition, BA tumours tended to metastasise to the peritoneum, similarly to human right-sided disease. Importantly, thus far existing mouse mutant BRAF-driven models developed lesions primarily in the small intestine, rather than the colon, and exhibited deregulated Wnt signalling, unlike patient-derived right-sided serrated precursor lesions which rarely harbour mutations in *APC* or *CTNNB1* (encoding β-catenin) and display membranous, not nuclear, β-catenin localisation.

We further implicated the transcriptional coactivator YAP as the driver of the Wnt-low, foetal-like signature, and found that microbial-driven inflammation supported the initiation and progression of these tumours, consistent with their preference for the microbe-rich right colon and their responsiveness to antibiotic treatment. Going forward, we will use this model to evaluate how the different environmental contexts of right- and left-sided tumours influence tumour evolution and response to therapy, and in particular interrogate the impact of microbiota and bacterial biofilms on the stem cell subpopulations of right-sided tumours. Building on the findings from the BEACON clinical trial, whereby doublet and triplet combinations targeting the EGFR/MAPK pathway showed modest efficacy in *BRAF*-mutant CRC, we will use our right-sided colon cancer model to evaluate novel targeted therapies.

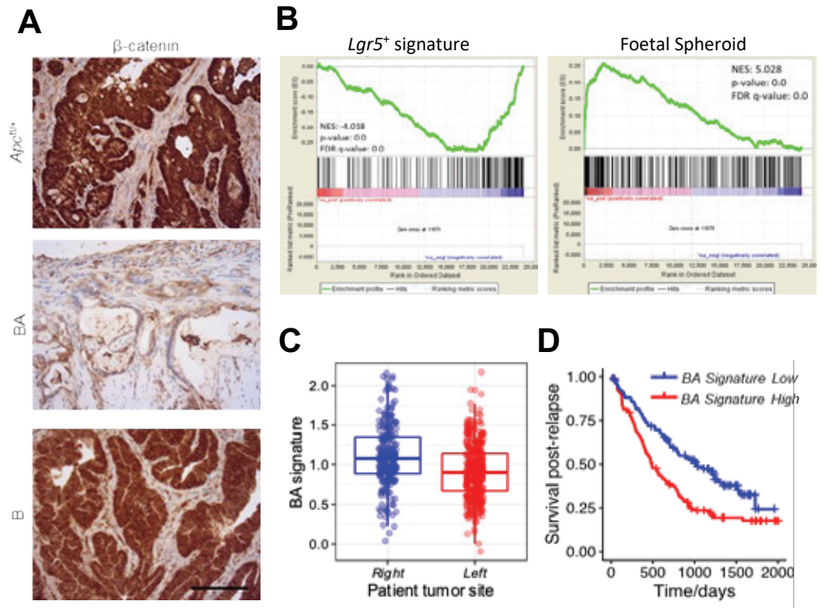


Figure 3
Combined mutation of *Braf* and *Alk5* drives patient-relevant right-sided colon cancer in mice.
(**A**) Immunohistochemistry for β-catenin in intestinal tissues from VilCre^{ER} *Apc*$^{fl/+}$, VilCre^{ER} *Braf*$^{V600E/+}$ *Alk5*$^{fl/fl}$ (BA), and VilCre^{ER} *Braf*$^{V600E/+}$ (B) mice. Scale bar, 100 μm. (**B**) Gene set enrichment analysis showed suppression of the *Lgr5*$^{+}$ ISC signature (left panel) and positive enrichment of the foetal spheroid signature (right panel) in BA right-sided colonic tissues relative to WT control tissues. (**C**) Expression of the BA signature in right- vs left-sided tumours from CRC patients, showing that the BA tumour model aligned closely to human right-sided disease. (**D**) Expression of the BA signature was associated with a shorter survival after relapse.

Overall, these approaches will further inform our understanding of CRC pathogenesis, and provide a platform for the development of novel stage- and subtype-specific therapies.

Publications listed on page 113

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

ADVANCED COLORECTAL CANCER

Group Leader
Colin Steele

Scientific Officer
John Falconer

Clinical Research Fellows
Laura Gould
Alistair McLaren[1]
Ross McMahon
Colin Wood

Graduate Students
Lydia Melissourgou-Syka
Natalie Peckett[1,3]

[1] TRACC programme
[2] Chief Scientist Office
[3] University of Edinburgh

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

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

Assessing the heterogeneity of colorectal liver metastases

Assessment of human colorectal liver metastases (CRLM) suggests that different subtypes exist. These can be detected histologically and separated into 'immune', 'stromal' and 'canonical' using transcriptomic

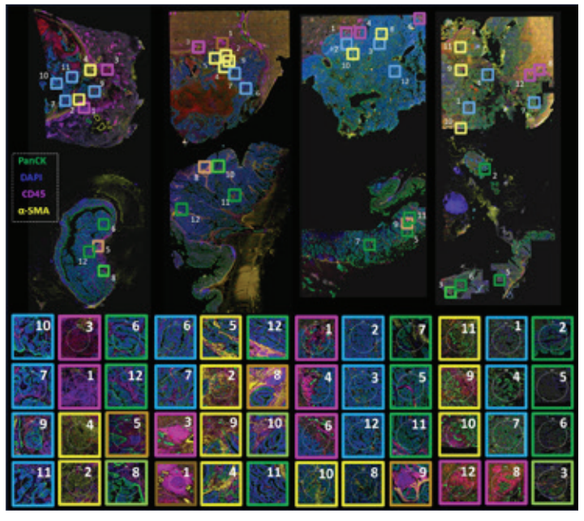


Figure 1
Transcriptomic assessment of heterogeneity, primary and metastatic sites in 4 patients.

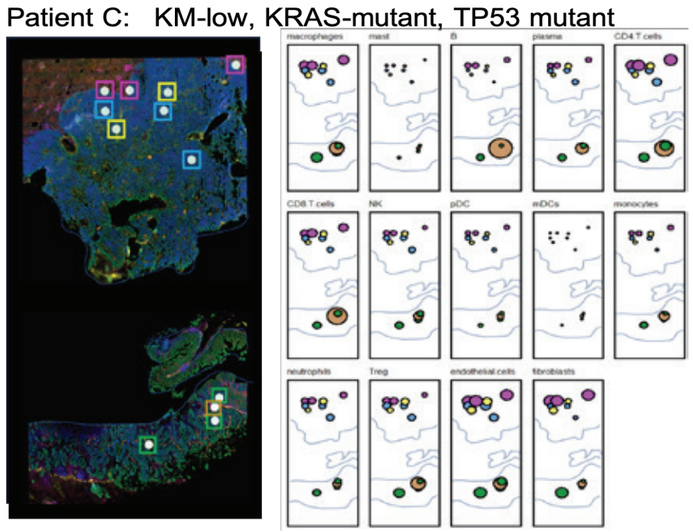
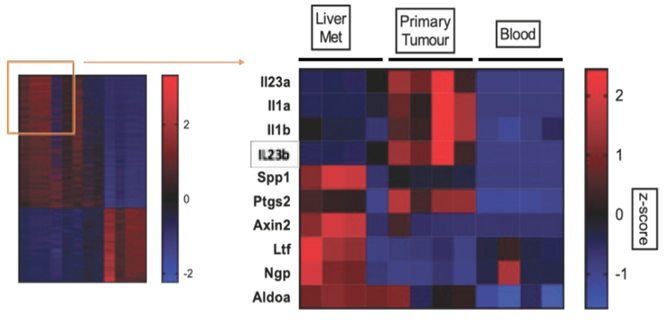


Figure 2
Immunofluorescence. Pink CD45, Blue pan-CK, Yellow alpha-SMA. Immune cell deconvolution representing cellular component derived from transcriptome of each area.

analysis (Pitroda *et al.,* 2018, *Nature Comms*). Patients from the immune subgroup do very well following surgical resection and can be cured of their disease. It is likely these patients may also respond to commonly used immunotherapies, however, this is as yet still to be clearly elucidated. We are making efforts to accurately subtype the disease in our patients (Figure 1) and we have partnered with Nanostring to assess the heterogeneity of these subtyped tumours.

We have identified that CRLM in certain patients are profoundly immunosuppressed with very few activated T cells evident within the microenvironment of these tumours (Figure 1, patient C), while others have significant upregulation of adaptive immune responses particularly at the edges of metastases (Patient B). We observe 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 have contrasting survival based on their immune response, with patients able to obtain long term survival following surgery for liver metastases if they display a strong adaptive immune response. This area 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.

Figure 3
Site specific differential gene expression within neutrophils



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.

Publications listed on page 117

MITOCHONDRIA AND CANCER CELL DEATH

Group Leader
Stephen Tait

Research Scientists
Asma Ahmed[1]
Florian Bock[2,3]
Rosalie Heilig[4]
Alfredo Montes-Gomez[5]
Joel Riley[2]

Scientific Officer
Cat Cloix[2]

Clinical Research Fellow
Anna Kössinger[4]

Graduate Students
Esmée Vringer[4]

Masters Student
Jordan Lee[2]

[1]Prostate Cancer UK
[2]CRUK programme
[3]University of Glasgow
[4]SNF Fellowship
[5]CRUK small molecule award

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.

A non-cell autonomous mechanism of drug persistence

Non-genetic means of drug tolerance or persistence are a major barrier to effective killing of cancer cells. Using BCL-2 targeting BH3-mimetics as prototypic cancer killing drugs, we aimed to understand mechanism(s) of drug persistence. Selection of drug resistant cells following BH3-mimetic (venetoclax) treatment revealed – perhaps not too surprisingly – that resistant cells expressed higher levels of anti-apoptotic BCL-2 and MCL-1. Importantly, venetoclax was found to upregulate BCL-2 and MCL-1 independent of apoptotic resistance, since treatment also led to BCL-2 and MCL-1 upregulation in death resistant BAX/BAK deleted cells. We initially assumed that resistance was cell intrinsic, however, quickly found that upon venetoclax (or other therapies), cells release a factor that promotes BCL-2 and MCL-1 expression in neighbouring cells enabling therapeutic resistance. Consistent with the mechanism being a transient state of drug persistence (as opposed to a genetic mechanism of resistance), removal of apoptotic stress reverted resistant cells to a sensitive state.

Apoptotic stress triggers cells to release pro-survival FGF-2

We next aimed to understand how apoptotic stress could promote cell survival. We found that FGF-2 (pro-survival growth factor) could be released by cells upon apoptotic stress. Following binding its cognate receptors, FGF-2 activates MAPK causing transcriptional upregulation of MCL-1 and BCL-2. Accordingly, neutralisation of FGF signalling or inhibition of MAPK reduced the emergence of drug persistence. Suggesting a potential relevance in human cancer, we found a correlation between FGF-activation, anti-apoptotic BCL-2 expression and worse prognosis in thymoma. In summary, we propose a model whereby apoptotic stress promotes survival (negatively impacting therapeutic efficacy) through an FGF-2 mediated, non-cell autonomous mechanism (Figure 1). Ongoing work aims to understand the occurrence of this mechanism *in vivo*, its impact on chemotherapeutic efficacy and mechanistic basis – *how does BCL-2 inhibition lead to release of FGF-2*?

Wound-healing as a physiological setting of apoptosis induced survival

Cancer processes are invariably subverted forms of physiological functions. Thus, we aimed to understand the "day job" of FGF2 driven survival signalling. Specifically, we investigated wound-healing, in collaboration with Yaron Fuchs (Technion). Interestingly, MCL-1 was found to be highly upregulated upon wound-healing in an FGF and MAPK dependent manner (Figure 2). Moreover, inhibition of MCL-1 upregulation – either through MAPK or FGF inhibition, reduced the kinetics of wound-healing. Our data suggests that wound-healing may represent a physiological setting of apoptotic stress induced survival. Cancer is often referred to as a wound that doesn't heal, while speculative, constant cycles of wounding and repair – in our opinion – provide an ideal setting to promote transformation or therapeutic resistance, by engaging apoptotic stress induced survival.

Finally, we say a sad goodbye to long-time lab stalwarts, Joel Riley and Florian Bock. Both start their own labs in Maastricht (Flo) and Innsbruck (Joel). Can't wait to see the exciting data coming out their new groups in due course, knock em dead guys! Huge welcome to Rosalie Heilig , who joins us as an SNF supported postdoc following her PhD in Lausanne.

Publications listed on page 117

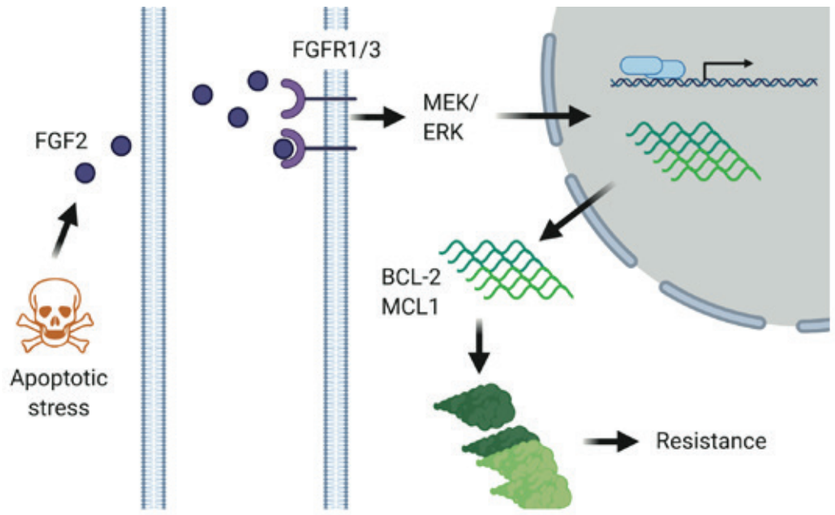


Figure 1
Apoptotic stress triggers an FGF-2 mediated non-cell autonomous mechanism of cell survival
Summary model: Upon an apoptotic stress, BCL-2 inhibition can lead to increased release of FGF-2. FGF-2 has non-cell autonomous survival effects, upregulating MCL-1 and BCL-2 via MAPK dependent transcriptional mechanisms.

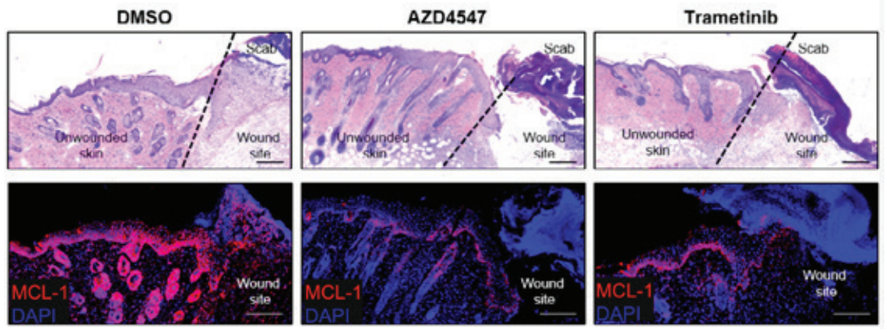


Figure 2
Upon wound-healing anti-apoptotic MCL-1 is upregulated in an FGF receptor and MAPK dependent manner
Sub-cutaneous wounds were inflicted under control (DMSO), inhibition of FGFR (AZD4547) or MAPK (trametinib). Wounding leads to an upregulation of anti-apoptotic MCL-1 that is inhibited either by FGFR or MAPK inhibition.

ONCOMETABOLISM

Group Leader
Saverio Tardito

Research Scientists
Tobias Ackermann
Victor Villar Cortes

Scientific Officer
Ruhi Deshmukh

Graduate Student
Maria Francesa Allega

At the foundation of cellular and tissue growth stands the transfer of chemical energy from nutrients into macromolecules. Tumours are no exception to this principle, and unavoidably seek metabolic states that support 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 and glutamate metabolism in brain and liver cancer

Glutamine and glutamate are instrumental to physiological processes, such as neurotransmission in the brain and ammonia homeostasis in the liver. At the same time, they are obligate substrates for anabolism of tumours originating in these organs, such as glioma and hepatocellular carcinoma. Glutamine synthetase (GS) catalyses the ligation of glutamate and ammonia and is the only known enzyme able to synthesise glutamine in mammalian cells (Figure 1, bottom right). We previously showed that GS-derived glutamine provides the nitrogen required for nucleotide biosynthesis in glutamine-restricted glioblastoma, the most aggressive type of glioma. Currently, we are assessing the effects of GS interference on the metabolism and growth of human primary glioblastoma cells and xenografts.

The liver is an ammonia-detoxifying organ and maintains homeostatic levels of circulating ammonia and glutamine. The functional unit of the liver constitutes an elegant example of metabolic zonation. In fact, the periportal zone, where hepatocytes express the urea cycle enzymes, converts the majority of ammonia into urea. The ammonia escaping this metabolic zone is captured by the hepatocytes surrounding the central vein, which express GS. This enzyme has a high affinity for ammonia, and incorporates it into the non-toxic glutamine, that can be returned to blood circulation. In liver tumours this metabolic zonation is disrupted. Liver tumours with an overactive WNT/β-catenin signalling pathway show a widespread GS expression.

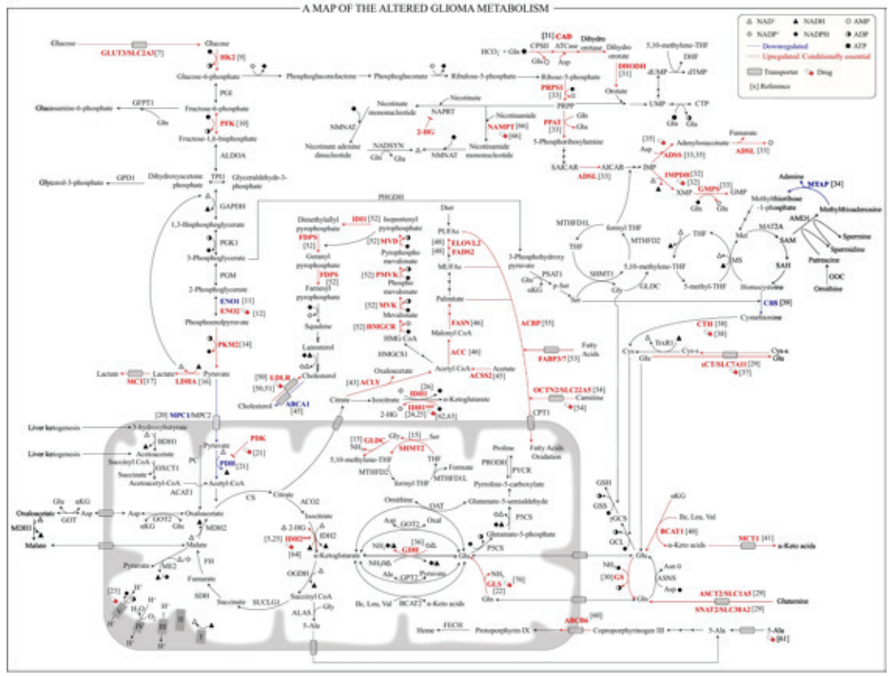
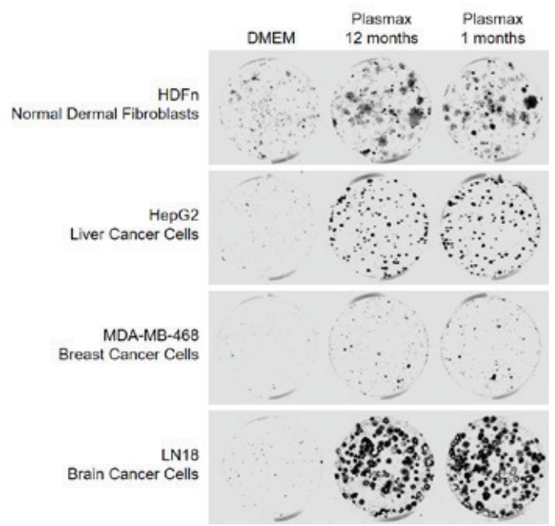


Figure 1
A map of the altered glioma metabolism.
The manually curated metabolic map highlights the altered reactions reported in gliomas. Deshmukh, Allega & Tardito, 2021, *Trends Mol Med.* 27:1045-1059.

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. It is available for biomedical research at Ximbio.com.

Figure 3
Colony formation in DMEM and Plasmax™.
(**A**) Normal Human Dermal Fibroblasts (HDFn), HepG2, LN18 and MD-MB-468 cells were seeded in DMEM in 24 wells plates, at 1x104, 2x104, 1x104, 2x104 cells/well respectively. The day after seeding (day 0) medium was replaced with 2ml/well of DMEM or Plasmax™, both supplemented with 2.5% dialysed FBS. Different Plasmax™ media were used for the experiment: either prepared on day0 from frozen stock components (Plasmax week 0) or left at 4°C for up to 20 weeks (Plasmax week 2–20). To prevent nutrient exhaustion, all media were replaced at days 4 and 5. Cells were trypsinysed and counted with a CASY cell counter. Values are mean ± SD obtained from 4 wells. Cell numbers were interpolated with a logistic growth curve (Graph Pad Prism 8.3). (**B**) Representative images of cells cultured in DMEM or PlasmaxTM at day 4 of the experiment described above. Cells were stained with Calcein AM 10 μM) for 20 minutes, and images aquired with a Zeiss Axiovert 25 microscope, objective magnification 2.5x.



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 peritumoural 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 these bases, glucocorticoids constitute excellent candidates to design novel metabolic combination therapies for the treatment of glioma.

More physiological cell culture media to obtain results more relevant to human tumour 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, a standard culture medium known as DMEM is distant from the nutrient levels found in normal human blood and it profoundly skews the metabolism of cancer cells in culture (Vande Voorde *et al.*,2019, Sci Adv.; Ackermann *et al.*, 2019 Trends Cancer). For example, glucose in DMEM is at five-fold the normal glycaemia. A similar ratio applies to glutamine, the most abundant amino acid in circulation. Conversely, non-essential proteinogenic amino acids normally circulating in blood are missing from DMEM.

On this basis, we developed Plasmax™ (Figure 2) a cell culture medium with nutrients and metabolites at the concentration normally found in human blood. The newly formulated medium allows the culture of mammalian cells with reduced supplementation of foetal bovine serum (Figure 3). We are currently testing Plasmax™ in a variety of cell culture systems, including murine normal, stem and cancer cells, as well as in primary human bone marrow derived mesenchymal stromal cells.

In 2020, Plasmax™ became the first physiological medium to be commercially available (Ximbio.com). In 2021, we tested the stability of Plasmax demonstrating that the medium stored at 4°C for up to 12 months, supported cell growth and colony formation comparably to freshly produced medium and better than DMEM (Figure 3).

The availability of a physiologically relevant cell culture medium will further reduce the inconsistencies between *in vitro* and *in vivo* results, thus favouring more translational biomedical research.

Publications listed on page 117

TUMOUR MICROENVIRONMENT AND PROTEOMICS

Group Leader
Sara Zanivan

Research Scientists
Sam Atkinson
Emily Kay[1]
Alice Santi
Britt Sterken[2]

Scientific Officers
Maja Bailey
Lisa Neilson

Clinical Research Fellow
Doug Cartwright

Graduate Students
Teresa Glauner
Grigorios Koulouras
Ilaria Puoti

[1]CRUK Therapeutic Discovery Laboratories
[2]Breast Cancer Now

High grade serous ovarian cancer (HGSOC) and triple negative breast cancer (TNBC) have limited treatment options, as only few targeted therapies effectively kill cancerous cells and patients frequently develop resistance to standard therapies. The tumour microenvironment actively supports cancer pathology and is populated by a variety of cell types that also offer alternative routes for therapy. Our research focuses on cancer-associated fibroblasts (CAFs), as we and 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 pro-tumorigenic 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 (Figure 1) that actively support cancer progression. CAFs were also shown to secrete EVs whose cargos could aid tumour progression by supporting cancer cell growth and invasion (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 functions. 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*; Hernandez-Fernaud, Ruengeler *et al.*, 2017 *Nat Commun*; 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.

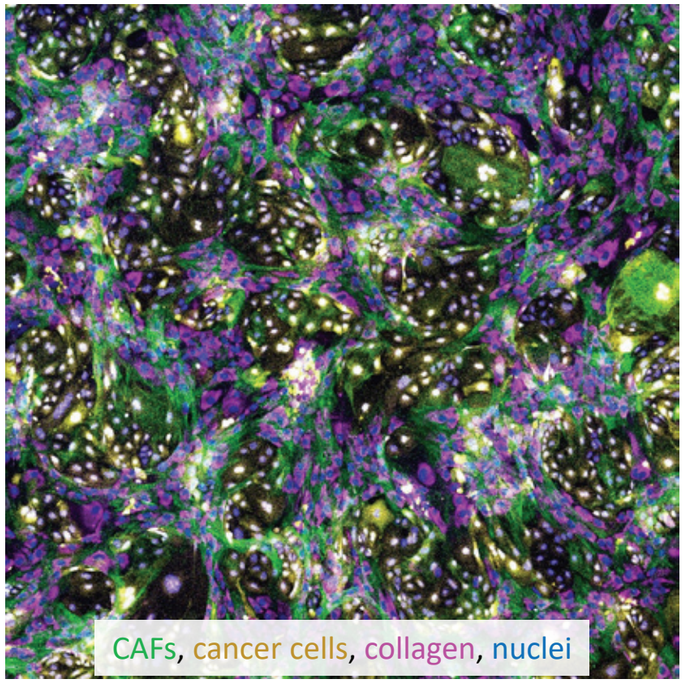


Figure 1
CAFs-cancer cells co-culture
Immunofluorescence image of high grade serous ovarian cancer (HGSOC) cancer cells and patient-derived CAFs, showing that CAFs produce high amounts of collagen. Purple = collagen, Yellow = HGSOC cells, Green = CAFs, Blue = Nuclei.

Image by Teresa Glauner.

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 influence 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 secreted by CAFs play 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 can 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.

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 acetylations and methylations, 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-metastatic ECM (Kay *et al.*, 2020, *bioRxiv*). We are now further investigating the potential of targeting acetyl-CoA production in CAFs in cancer.

News

This year, Teresa Glauner presented her work on linking collagen-producing cancer-associated fibroblasts and immune cells at the British Society for Immunology Congress 2021. Moreover, we have been awarded a CRUK Early Detection and Diagnosis Project Grant to work in collaboration with the Proteomics team, the Liver Disease and Regeneration team (Tom Bird), the Computational Biology team (Crispin Miller) as well as University of Oxford and University of Nottingham to identify biomarkers for early detection of liver cancer.

Publications listed on page 118

DRUG DISCOVERY

Cholangiocarcinoma mouse model expressing KrasG12D/+ and Pten-/- under Ck19 promoter (Jen Morton and Curtis Rink), stained with DAPI (nuclei), and antibodies specific for EphA2 (red) and MRTF (green), a transcription factor which translocates into the nucleus when active.

Image by Sergi Marco Martin

DRUG DISCOVERY UNIT

Co-Leads

Justin Bower

Heather McKinnon

Chemistry
Alan Bilsland
Marta Bon
Kenneth Davies
Alana Hopkins
Stuart Francis
Duncan McArthur
Kirsten McAulay
Ridvan Nepravishta
Charles Parry
Paul Shaw
Mairi Sime
John Taylor
Christopher West
Ryan West

Biology
Anna Estevan Barber
Amy Bryson
Jonathan Clark
Daniel Croft
Sebastian Greenhough
Luke Hutchnison
Nicholas Johnson
Craig Mackay
Laura McDonald
Mokdad Mezna
Francesca Pellicano
Nicholas Rooney
Dominika Rudzka

Structural Sciences
Kenneth Cameron
Nichole Cerutti
Jodie Ford
Jennifer Konczal
Camila Pasquali
Katie Pollock
Alexander Schuettelkopf
Steven Vance

Informatics
Richard Papworth

KRAS Project Co-Ordinator
Katharina Schraut

Lab Support
Michael Kilday

The Drug Discovery Unit bridges the gap between bench science and clinical development, providing a mechanism to deliver urgently needed treatments for cancer patients where there is a clear unmet medical need. Through our focus on exciting science coupled with a collaborative mindset, we have worked with our Beatson colleagues to develop an exciting portfolio of targets that has gained considerable attention from the drug discovery community. Our flagship project seeks to generate reversible inhibitors of KRAS^{G12D}, a highly challenging target with profound importance in cancer biology. In 2019, we partnered with Novartis to take forward this innovative and exciting KRAS project.

Identifying new therapeutic targets from basic research fuels the pipeline for future medicines. In 2020, we implemented our Working Group strategy to bring together like-minded researchers with a focus on therapeutic discovery. With drug discovery staff working side by side with Institute researchers, we can foster a closer framework for collaborative research and more effectively progress novel therapeutic opportunities into clinical development.

KRAS

KRAS is one of the most heavily pursued targets in cancer therapeutics, but despite decades of research, little progress has been made. Missense gain-of-function mutations of the three RAS isoforms are found in 27% of all human cancers, with KRAS being the most mutated isoform: 85% of all RAS mutations are in KRAS, 11% in NRAS and 4% in HRAS. There are clear cancer-type specific mutational profiles for RAS. KRAS mutations dominate over NRAS and HRAS in PDAC (100%), colorectal (86%) and lung adenocarcinoma (LuAd) (96%), whereas NRAS is highest in melanoma (94%) and HRAS in head and neck squamous cell carcinoma (86%).

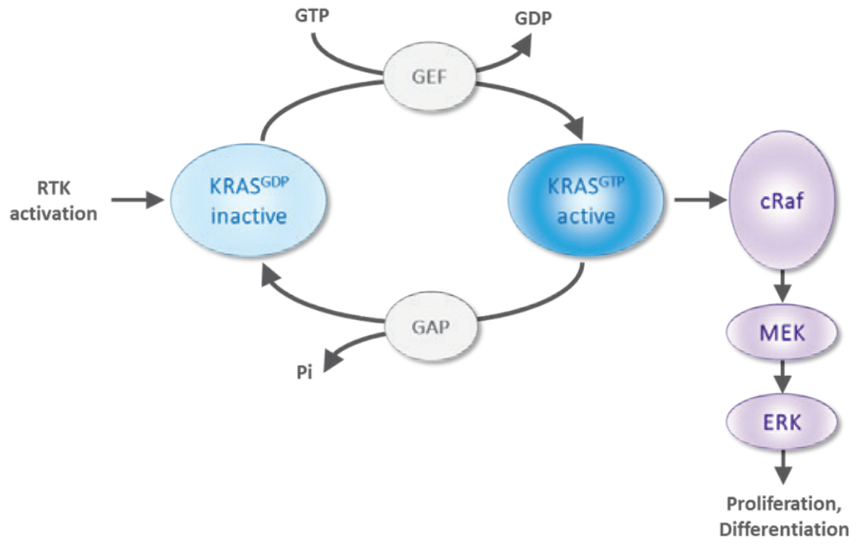


Figure 1
KRAS acts as a molecular switch, rapidly converting to an active state upon receptor tyrosine kinase (RTK) stimulation, by exchanging GDP for GTP via a guanine nucleotide exchange factor (GEF). Active KRAS binds to effectors such as cRaf and activates downstream signalling through the MEK/ERK pathway, driving proliferation and differentiation. KRAS is rapidly de-activated by a GTPase activating protein (GAP), switching back to the inactive GDP form. However, mutant KRAS is resistant to GAP-mediated hydrolysis, resulting in prolonged activation of downstream pathways.

Whilst there are >130 missense mutations of RAS in cancer, hotspot mutations at G12, G13 and Q61 account for 98% of these. G12 mutations are by far the most prolific of all KRAS mutations (83%), and of these G12D dominates (41%). Interestingly, substitutions are cancer-type specific with G12D followed by G12V being more frequent in PDAC whilst G12C is the highest substitution in lung adenocarcinoma (LuAd). The most tractable approach to directly inhibit KRAS has been via covalent binding to KRAS^{G12C} mutant protein, and there has recently been great progress in this area with Amgen (AMG 510/Sotorasib) and Mirati (MRTX849) now in clinical trials. However, this approach is clearly limited to KRAS^{G12C} mutant cancers and is pre-disposed to resistance mechanisms. We have taken a more challenging approach but one that is likely to have a more profound impact on patient treatment if successful, by targeting KRAS^{G12D} with non-covalent inhibitors. We initiated our KRAS project in 2010 and from the initial fragment screen we have made significant progress, putting us in a very competitive position within the RAS inhibitor field.

Key to this success is the Structure-Based Drug Design capability within the Drug Discovery Unit. Using state-of-the-art biophysical techniques such as Nuclear Magnetic Resonance (NMR) and Surface Plasmon Resonance (SPR), alongside an in-house crystallography platform and substantial expertise in computational chemistry and modelling, we have successfully evolved small fragment molecules into high-affinity cell active compounds.

Establishing strong and productive collaborations is a key approach that the Unit adopt for all projects, to ensure the best chance of success in delivering new treatments for patients, in the shortest possible timeframe. Within the Institute, we work closely with Owen Sansom's group, who have a research focus on mutant KRAS–driven models of colorectal cancer. We have also collaborated with the National Cancer Institute's RAS Initiative programme in the USA thanks to funding from Sixth Element capital via the CRT Pioneer Fund, and in 2019 we signed a collaboration deal with Novartis to further optimise our KRAS inhibitors and potentially identify a clinical development candidate.

Translational science

Translating breakthroughs in cancer biology into new therapeutics is the foundation of drug discovery research. Our location at the heart of the Institute enables us to work closely with researchers to identify the most promising opportunities as they emerge, and importantly, to work together to take these forward into drug discovery projects. This year we have initiated our Working Group strategy, building on a model of collaboration to put in place a framework for closer integration between research groups and drug discovery staff. This approach not only fosters a culture of translational research, including training of early-stage researchers, but also creates a portfolio of therapeutic assets and capability, as a foundation for building alliances with pharmaceutical partners.

Publications listed on page 119

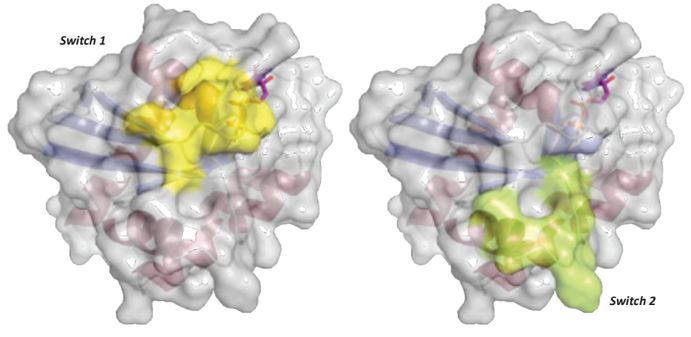


Figure 2
KRAS protein structure showing Switch 1 and Switch 2 which undergo conformational change upon activation to enable binding of effectors such as Raf1.

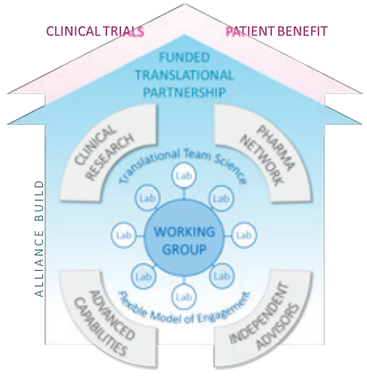


Figure 3
Working group model as a foundation for building translational partnerships that drive novel therapies into clinical trials for patient benefit.

ADVANCED TECHNOLOGIES

Intestinal Forest - lymphocytes (red in optically cleared small intestine. Maximum intensity projection of 100um z-stack. Green: epithelial cells; Blue: leucocytes; Yellow: alpha-SMA – Winner of 2021 'Images of Research' Competition by the Scottish Microscopy Society.

Image by Frédéric Fercoq

BEATSON ADVANCED IMAGING RESOURCE

Head
Leo Carlin

Fellow of the Royal Microscopical Society (FRMS)

Scientific Officers
Tom Gilbey
Lynn McGarry
Ewan McGhee
Nikki Paul
David Strachan
Peter Thomason

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

The Beatson Advanced Imaging Resource (BAIR) team works closely with the Institute's researchers to uncover and interrogate important molecular pathways in cancer. The BAIR is thus involved at some stage in nearly every study from researchers at the Institute that contains a light micrograph, or a flow cytometry plot or uses sorted cells for downstream analysis using one of the other advanced technologies.

All of the beautiful fluorescence light microscopy images you see in this report were captured in BAIR.
We are keen and able to assist from experimental design right through to the finished figures. We train scientists in all stages of modern cytometric and microscopical research, from advice on 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

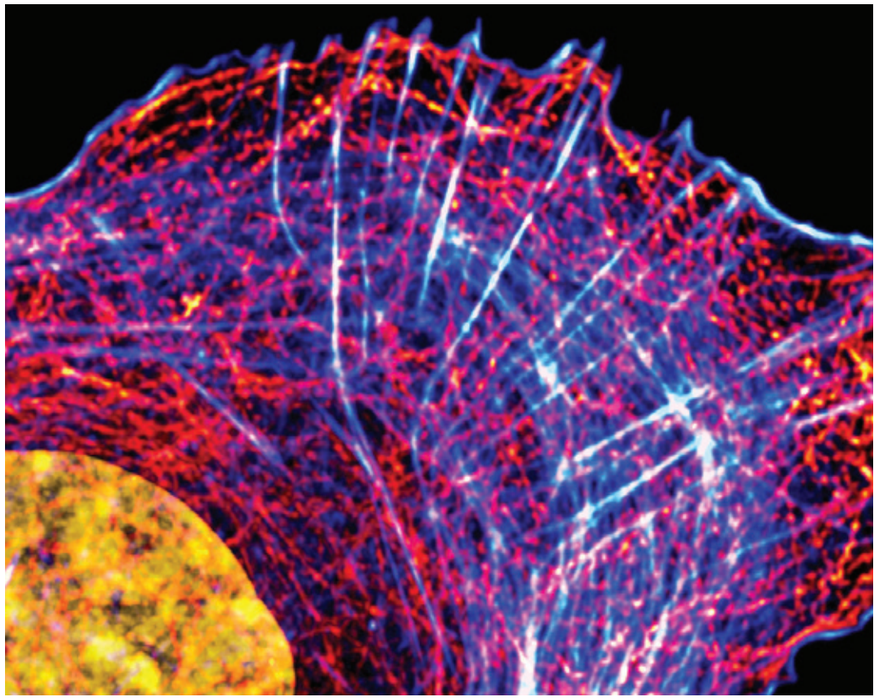
We have the expertise and instruments to:

- 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.

We have welcomed the ability to increase capacity and activity in the BAIR over the year. The team have continued to innovate in the way they support our scientists by providing a hybrid of virtual and face-to-face training and support, and taking on experimental work on behalf of our researchers at times when training wasn't possible. Our Zeiss Elyra 7 Lattice Structured Illumination Microscope (SIM) has become part of a key capability in superresolution microscopy in addition to our two Airyscan systems. Building on technology recognised by the 2014 Nobel Prize in Chemistry, it allows very rapid SIM imaging at sub 100 nanometre resolution and single molecule localisation microscopy down to 10s of nanometres. This means that researchers at the Institute can pinpoint important cancer mechanisms in living cells and tissues down to a sub-organelle level. As explained above, this microscope sits within a suite of technologies and expertise being deployed across the strategic aims of the Institute and in support of the major networks that we lead.

Publications listed on page 120



An osteosarcoma cell (U2OS) was stained with markers for structural elements of the cell. In red/Orange indicates microtubules (Beta-Tubulin), Actin cytoskeleton is seen in blue (Phalloidin) and the nucleus is in yellow (DAPI stain). The cell was imaged on the Zeiss 880 super-resolution microscope at the Beatson Institute.

Image by David McEwan

BIOINFORMATICS AND COMPUTATIONAL BIOLOGY

Head
Crispin Miller

Bioinformaticians
Ann Hedley
Ryan Kwan
Matthew Neilson
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. Our remit also includes mathematical modelling, and a growing focus has been on the application of these techniques to imaging data.

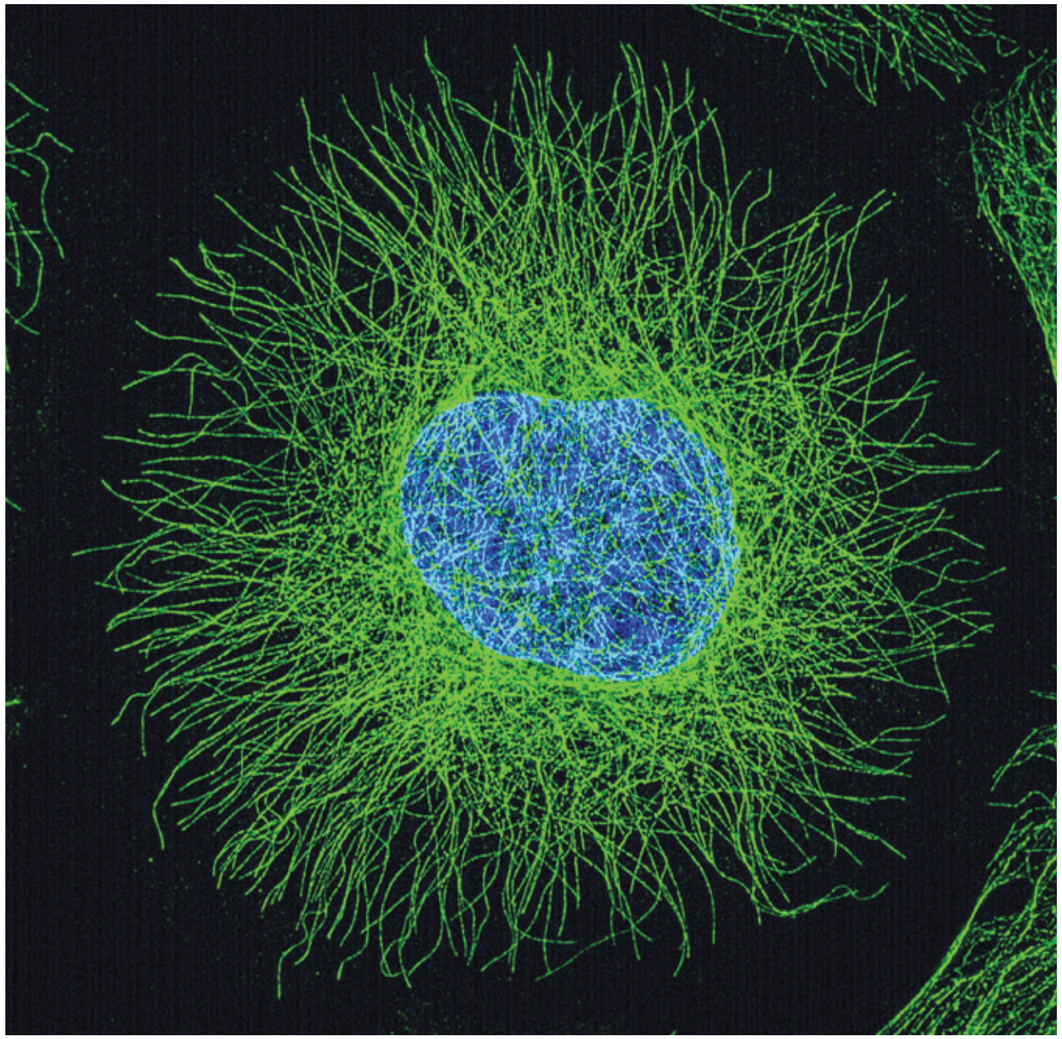
Our team focuses on exploratory data analysis, and our ultimate goal is to provide insights that enhance our understanding of cancer biology. The need for DNA and RNA sequencing analyses has continued to grow, and this has been accompanied by continued interest in using computational and machine learning 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. We are particularly interested in establishing standardised workflows for initial pre-processing and up-stream analysis of single cell data sets in order to automate the more routine parts of the analysis.

Data analysis and modelling is performed using a variety of open-source software environments, programming languages and scripting tools, including R, Bioconductor, KNIME, Fortran, 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 including Oncomine Research Premium Edition, Ingenuity Pathway Analysis and GeneGo MetaCore.

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 postgraduate Cancer Sciences MSc programme at the University of Glasgow.

Publications listed on page 121



Microtubules ovarian cancer cell fixed and stained for microtubules and imaged using Structured Illumination Microscopy (SIM) with a Zeiss Elyra 7 microscope.

Image by Nikki R. Paul

METABOLOMICS

Head
David Sumpton

Scientific Officer
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 of cells, tissues, and biological fluids. This platform is complemented by a Thermo Scientific Altis triple quad that broadens the sensitivity and specificity of the detection for specific metabolites of interest. In addition, an Agilent gas-chromatography mass spectrometry (GC-MS) triple quad instrument provides complementary coverage to our LC-MS systems.

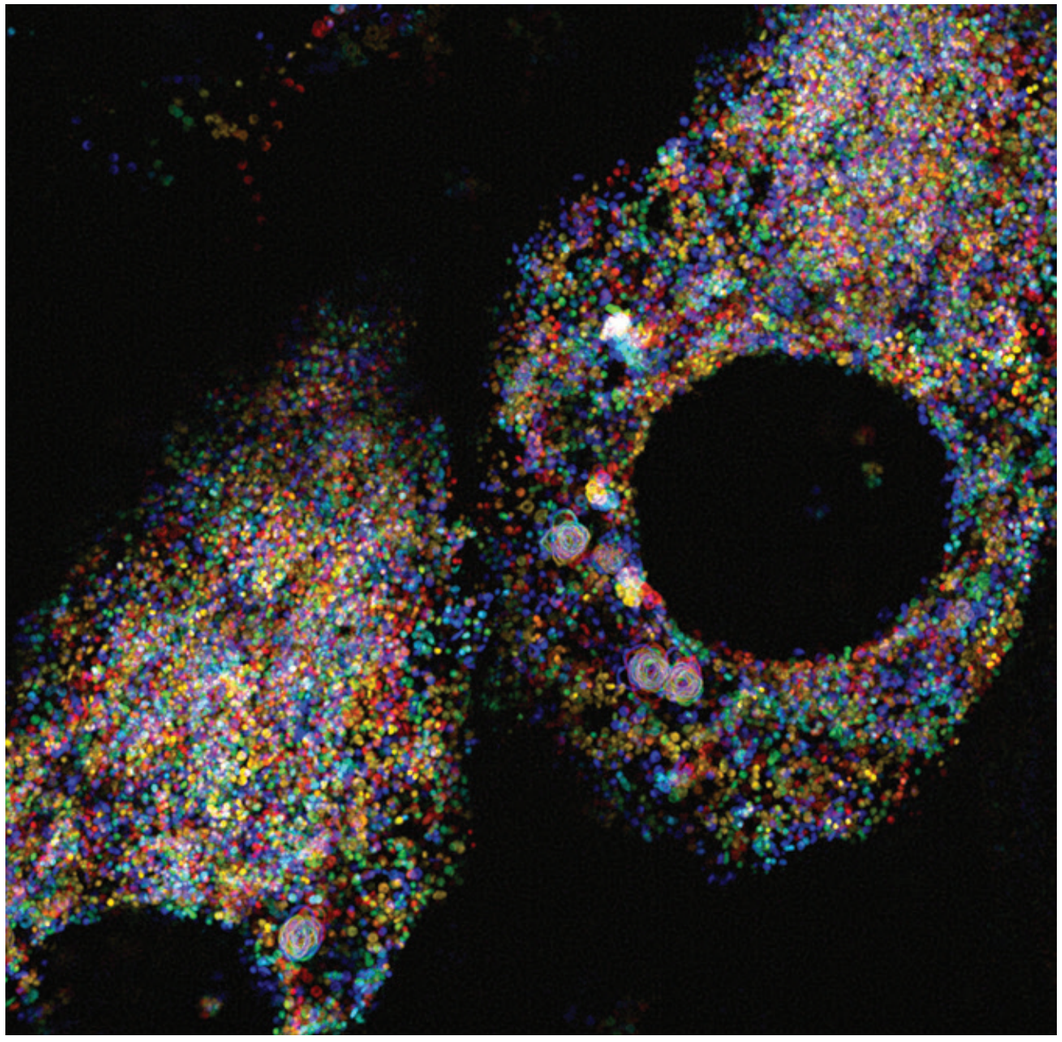
The facility's core aim is to provide access to state-of-the-art LC-MS technology that is optimised for the detection of polar metabolites and lipids. We maintain and operate the instrumentation, providing both standard metabolite profiling and custom analysis when needed. We offer expertise and assistance in data analysis, data interpretation and experimental design. We also offer training in data analysis of targeted metabolomics experiments. To learn as much as possible from the data generated, we collaborate with users to make use of more complex untargeted analysis approaches. We are continuously striving to further develop both our mass spectrometric and data analysis methods.

We work closely with the groups of Saverio Tardito, Owen Sansom, Jim Norman, Sara Zanivan, Vignir Helgason and support several other research groups within the Institute who have specific interests in cancer metabolism.

This year, Engy Shokry joined the facility as a scientific officer. Engy brings a wealth of experience and knowledge in a range of metabolomics techniques which will complement our current methodology. Engy is currently using the facilities GC and LC-MS triple quad instruments to develop novel targeted metabolomics approaches to aid ongoing projects. These include the measurement of methylamine, a volatile derivative of ammonia and the natural substrates and products of 2'-deoxycytidine kinase alongside the metabolites of the drug Cladribine, a nucleoside analogue.

Rachel's ongoing PhD project in collaboration with Saverio Tardito's group continues to study the metabolism of BRAF mutant melanoma for therapeutic gain. BRAF inhibitors provide an excellent first line of therapy for melanoma, however, resistance frequently develops. It has been shown that melanoma cells resistant to BRAF inhibitors have increased mitochondrial oxidative metabolism that depends on glutamine anaplerosis. We are using a glutaminase inhibitor in combination with the BRAF inhibitor to assess whether this metabolic rewiring constitutes a therapeutic liability. To identify which metabolic reactions are required by melanoma cells to proliferate upon BRAF and glutaminase inhibition, we are applying targeted and untargeted metabolomics to melanoma cells grown in culture and tumours *in vivo*.

Publications listed on page 121



Lysosomes Time Projection Cancer cells transfected with LAMP1-mCherry and imaged live with timelapse fluorescence microscopy using Structured Illumination Microscopy (SIM) with a Zeiss Elyra 7 microscope. Imaged every 30s for 15minutes. Images projected over time using colour-coded projection.

Image by Nikki R. Paul

PROTEOMICS

Head
Sara Zanivan

Scientific Officers
Kelly Hodge
Grigorios Koulouras
Sergio Lilla

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

The proteomics team has an outstanding expertise in high-resolution, Orbitrap-based mass spectrometry (MS) proteomics, accurate quantification approaches and MS data analysis. We work in collaboration with research groups within and outside of the Institute, and we actively develop MS-based proteomic platforms to address a variety of questions to help scientists to increase their understanding of the mechanisms that regulate various aspects of cancer.

To achieve this, we are well equipped with three nano liquid chromatography (nLC)-MS systems, of which the Orbitrap Fusion-Lumos is our most recent addition. 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. Moreover, we use Skyline for the analysis of PRM data. Finally, we use Perseus for data analysis and dissemination.

We have a competitive portfolio of techniques available, which span from single protein to sub-proteomes and global proteome analyses. We have strong expertise in quantitative analysis of secretomes (extracellular matrix, extracellular vesicles and conditioned media) and protein translation, 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 to quantify cysteine oxidation levels at global scale with no enrichment steps required (van der Reest, Lilla *et al.*, 2018 *Nat Commun*) and that has been fundamental to answer different biological questions (Port *et al.*, 2018, *Cancer Discov*; Hernandez-Fernaud, Ruengeler *et al.*, 2017, *Nat Commun*; Cao X *et al.*, 2020, *J Cell Sci*).

This year, we have worked to expand our portfolio, including developing the SICyLIA technology to establish an innovative platform for plasma redox proteomics for early detection of cancer in collaboration with the CRUK-BI groups Tumour Microenvironment and Proteomics and Liver Disease and Regeneration (Tom Bird).

During 2021, we have worked with many of the groups at the Institute and significantly contributed to the success of their research (see publications). We are continuously striving to develop new methods to answer more complex biological questions using proteomics and to improve the methods currently in place enriching the quality of the data that the facility can provide.

Publications listed on page 123

www.beatson.gla.ac.uk/transgenic_models

TRANSGENIC MODELS OF CANCER

Head

Karen Blyth

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 our 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 our colleagues across the strategic themes of the Institute probing metabolism as a cancer vulnerability, and studying the interplay within the tumour microenvironment that drives metastasis and recurrence.

Cancer cells have an increased capability to grow and proliferate, and so require to adapt and modify their cellular metabolism to survive in growth-limiting conditions. Therefore, targeting cancer cell metabolism presents an important opportunity for novel therapeutic means. Restricting amino acids such as serine and glycine for example is one exciting avenue that we have explored in preclinical models through dietary intervention (Maddocks *et al*, *Nature* 2017) as well as pharmacologically (Tajan *et al*, *Nature Communications* 2021). Continuing our long-standing collaboration with Oliver Maddocks (University of Glasgow), we have engaged in several projects looking at opportunities to manipulate cancer cell metabolism. In one recent project, we showed that high expression of the IDO1 enzyme in pancreatic cancer models facilitated utilisation of tryptophan as a nutrient source under growth limiting conditions, and that by specifically targeting IDO1 we could slow tumour cell growth (Newman *et al*, *Molecular Cell*, 2021). This was an important observation since pancreatic cancer cells were insensitive to serine/glycine restriction and this revealed that tryptophan was able to substitute for serine as a one-carbon source. Also with Oliver, and in collaboration with our Glasgow RadNet colleagues (https://www.gla.ac.uk/researchinstitutes/cancersciences/radnet/), we have been exploring the potential of sensitising tumour cells to radiotherapy using amino acid restriction.

Metabolic rewiring is not only intrinsic to the tumour cells but can also be a key modulator within the tumour microenvironment. Applying *in vivo* breast cancer models, it has been exciting to collaborate 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, thus promoting breast cancer progression (Kay *et al*, *Nature Metabolism*). Ongoing projects with Sara and her lab will further explore the crosstalk between the CAFs and tumour cells in an endeavour to exploit these signalling pathways within the microenvironment as a novel means to target tumour growth and metastasis.

Using our expertise in prostate cancer models and *in vivo* imaging, we have had the pleasure of contributing to various projects with David Bryant and his lab. Published this year was evidence of how the ARF GTPase Exchange Factor, IQSEC1 promotes cancer cell invasion through regulating phosphoinositide metabolism and that this drives metastasis *in vivo* in orthotopic prostate cancer models (Nacke *et al*, *Nature Communications* 2021). The power of using sensitive fluorescence imaging has also allowed us to refine these models enabling us to garner subtle information on metastatic spread of the tumours at an earlier clinical endpoint by imaging the organs *ex vivo* (Figure 1).

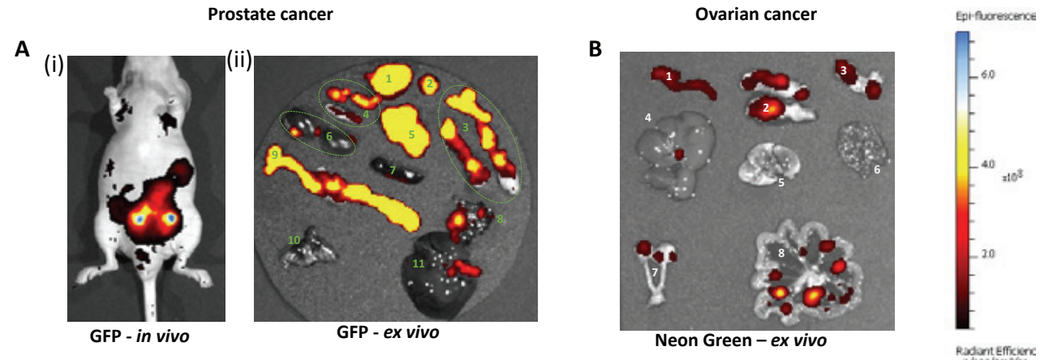
In other studies we worked closely with Tim Humpton and Karen Vousden (Francis Crick Institute) to demonstrate that while loss of MDM2 E3 activity was lethal during embryonic development, it was tolerated in adult tissues providing proof-of-concept evidence of how p53 might be activated in tumours for therapeutic gain (Humpton *et al*, *Genes & Development*, 2021). Also with Tim and Karen, we showed the importance of p53 in protecting and repairing the liver during carcinogen-induced damage as a means to protect against hepatocellular carcinoma (Humpton *et al*, *Cell Death & Differentiation*, 2021).

Meanwhile we explored how FGF might protect neighbouring cells from dying with Stephen Tait's lab (Bock *et al*, *Nature Communications* 2021) and showed that inhibition of the cytoskeleton regulator MICAL1 reduced tumour growth in a breast cancer model with Michael Olson's lab (McGarry *et al*., *Cancer Letters*, 2021).

Resources & News

In addition to our exciting collaborative projects some of which are briefly discussed above; our lab trains and supervises researchers at the Institute in many complex cancer models (e.g. breast cancer, pancreatic cancer, lung cancer, and prostate cancer). Our Senior Scientific Officers are responsible for curating and training our scientists in key equipment used for preclinical modelling such as the IVIS Spectrum 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. In particular, our lab provides expertise in surgical procedures such as orthotopic prostate delivery, mammary intraductal delivery and primary tumour removal. In all our approaches we continually promote the 3Rs refining our models exemplified with the use of *in vivo* imaging (Figure 1), and exploring replacement models such as mammary organoids. Finally, some news for 2021, we were delighted to welcome Dale Watt to the lab to cover maternity absence and congratulate Laura on the arrival of a beautiful baby boy in November.

Figure 1
Fluorescence imaging reveals metastatic cells in a model of prostate cancer (A) and ovarian cancer (B)
A (i) 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).
A (ii) 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.
B *Ex vivo* imaging of indicated tissues collected from a model of ovarian cancer, using ID8 cell-derivative expressing neon-green fluorescent protein. In addition to the tumour deposits in the omentum, splenoportal area and mesentery that could be seen macroscopically, additional deposits can be observed in the uterine fat and in (or around) the ovaries. Both projects carried out in collaboration with David Bryant.



www.beatson.gla.ac.uk/transgenic_technology

TRANSGENIC TECHNOLOGY

Head
Douglas Strathdee

Research Scientist
Eve Anderson

Scientific Officers
Cecilia Langhorne
Farah Naz Ghaffar

The Transgenic Technology Laboratory uses molecular genetic techniques to analyse gene function during the onset and progression of cancers. By using gene targeting or genome editing, we can make accurate genetic changes at precise locations in specialised stem cells. It allows us to make accurate models of clinically relevant cancers by introducing changes identical to those discovered in human cancer cells. Moreover, recent advances in these technologies give the possibility to introduce multiple genetic changes, simultaneously. Thereby, we can study how combinations of mutant alleles interact and thus, enable the development and progression of cancer.

Making better models of clinically relevant cancers

Stem cells have a number of useful properties, which make them suitable for studying the role of gene mutations in cancer. Firstly, homologous recombination, a process where endogenous genes can be replaced with copies carrying precise genetic changes, works relatively efficiently in these cells. This allows us to introduce genetic changes into genes in stem cells, identical to those uncovered in human tumours. We can, therefore, study how these genetic alterations affect the function of the altered proteins in intact cells and tissues. Secondly, stem cells can develop or differentiate to produce a wide variety of different cell types found in various tissues. Having introduced genetic changes into stem cells, we can then differentiate the cells to analyse the role of these alterations in relevant cells from the tissue where the original mutation was identified.

This year, we have collaborated with a number of groups at the Institute to generate a variety of different allele types in stem cells, including, for example, conditional knockouts and point mutations. Furthermore, advances in techniques have enabled us to replace the original genes in stem cells with their human genomic equivalents. This means that we can now introduce mutations directly within the appropriate genetic setting, ensuring that these changes more precisely reflect the mutations associated with human disease.

Recycling old broken alleles

The continued development of genome editing techniques presents an opportunity for us to approach projects in a different way. Gene targeting requires a DNA intermediate to make alterations in endogenous genes. However, with genome editing approaches, alterations can be made directly to endogenous genes without the requirement for a DNA intermediate. This enables us to modify genes in ways that are not feasible using standard gene targeting techniques.

One circumstance where genome editing provides a significant advantage over gene targeting is the introduction of small modifications to already existing targeted alleles. In the examples outlined below, the use of genome editing allowed us to modify targeted loci already in use, enabling them to function better, or in one case to work in the way that they were originally intended.

The first allele where we were used gene editing to repair, was a conditional allele of *Runx2*. This allele had been correctly targeted, introducing loxP sites on either side of exon 3, but also had introduced a selectable neomycin (Neo) marker cassette as part of the gene targeting procedure. This cassette had been impossible to remove. Furthermore, as this cassette had included a strong transcriptional promoter and had been inserted directly in the middle of the *Runx2* gene between exons 3 and 4, it had interfered with the expression of the endogenous *Runx2* gene. This had resulted in a hypomorphic allele that had displayed some of the mutant phenotypes even before Cre recombinase expression disrupted the gene by recombination at the loxP sites.

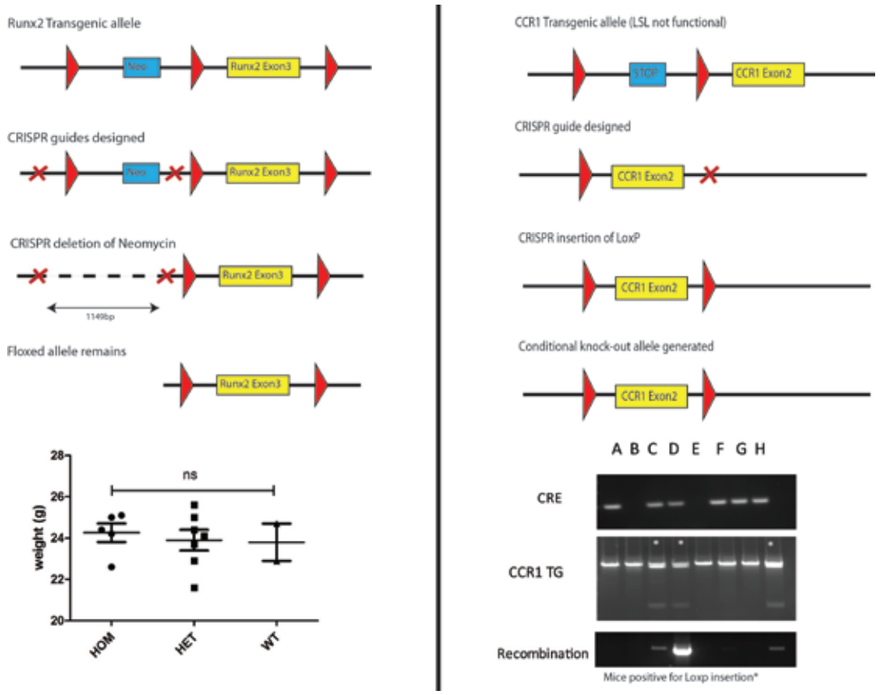


Figure 1
Repairing alleles by genome editing
(A) Diagram of the repair of the condition allele of *Runx2*. Small guide RNAs were designed on either side of the Neo selectable cassette. The guides direcedt Cas9 to excise the selectable cassette but leaving the loxP sites intact. (B) Graph of weights of mice homozygous or heterozygous for the repaired *Runx2* allele and their wild-type littermates. Following removal of the cassette the mice no longer displayed the hypomorphic phenotype and were the same size as their wild-type littermate controls. (C) Diagram of the insertion of an additional loxP site into the *Ccr1* locus. This was achieved by designing a small guide RNA directing Cas9 to cut the DNA downstream of exon 2. A repair template was also introduced which allowed incorporation of an additional loxP into the cut site by the DNA repair pathway. (D) Recombination of the modified *Ccr1* locus in response to Cre recombinase. (i) PCR of genomic DNA for Cre recombinase (ii) PCR identifying mice carrying the modified *Ccr1* allele (iii) Recombination was seen in mice carrying both the modified *Ccr1* allele and Cre recombinase, demonstrating that the additional loxP has been inserted into the *Ccr1* gene.

Altering this, would have meant to start again and create a new allele from scratch. However, using gene targeting, we had the opportunity to remove the selectable marker cassette from the already existing *Runx2* allele. This was achieved by designing two small guide RNAs which directed Cas9 nuclease activity to either side of the selectable marker cassette, taking care to leave the two loxP sites intact. The Cas9 nuclease cut precisely on either side of the selectable marker cassette, effectively excising the cassette and leaving the resultant gap to be filled by the DNA repair pathway.

This way, we removed the selectable marker from the *Runx2* allele, leaving the loxP sites intact, but the allele could also function as a conditional allele. Furthermore, we showed that animals carrying the newly engineered allele did not display any of the hypomorphic phenotypes of the original allele, but instead were the same size as their wild-type counterparts (Figure 1B).

In the second example, we modified an allele of the gene *Ccr1*. This gene was missing an essential loxP site at the 3' end of exon 2, that would have allowed the gene to work as a conditional allele. Again, to repair this allele by gene targeting would have required generating a new allele from scratch. However, we repaired the existing allele using genome editing by inserting a small 34 base pair loxP site downstream of exon 2. This was achieved by designing a small guide RNA, directing Cas9 nuclease to cleave DNA just downstream of exon 2 of the *Ccr1* gene. In order to facilitate the incorporation of the additional loxP site, we introduced a small repair template which included the loxP site of exon 2, allowing the allele to be used as a conditional allele. Furthermore, we were able to show that the expression of Cre recombinase allowed Cre recombination at the locus, which should alter the expression of the *Ccr1* gene.

Taken together, these examples showed how genome editing broadened the range of tools we have available to generate functional alleles for cancer modelling. We are now able to approach projects in new ways, and accomplish them in a more effective and ethical manner, ultimately saving valuable time and resources.

Publications listed on page 123

TRANSLATIONAL MOLECULAR IMAGING

Head
David Lewis

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

Staff Scientist
Gaurav Malviya

Medical Physicist
Caroline Findlay[1]

Scientific Officers
Emma Johnson
Agata Mrowinska

[1]Beatson Cancer Charity / Beatson Endowment

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 visualising and understanding 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. The unique research environment at the Beatson Institute enables collaboration using its world-class cancer models to develop imaging biomarkers for new applications such as tumour classification and personalised cancer therapy.

PET radiochemistry

The R&D radiochemistry platform is fully equipped for developing novel carbon-11 and fluorine 18 labelled PET probes from a range of radiolabelled precursors. This platform has allowed us to develop a panel of fluorine-18 and carbon-11 labelled radiotracers for *in vivo* metabolic studies. We have continued to support the extensive imaging programmes in the TMI with radiotracers such as [^{11}C]acetate, [^{18}F]fluoro-ethyl-tyrosine (FET), [^{18}F]terafluoroborate (TFB), [^{18}F] fluorodeoxyglucose (FDG), [^{11}C]methionine, (4S)-4-(3-[^{18}F]Fluoropropyl)-L-glutamate (FSPG) and [^{11}C]leucine.

Through collaboration with Edinburgh Imaging, we have supported radiosynthesis and quality control methods for production of [^{18}F]fluoroproline and [^{18}F]LW233, which target collagen synthesis and translocator protein (TSPO), respectively and are now available for cancer imaging studies in Glasgow.

To facilitate clinical research programmes at the West of Scotland Glasgow PET Centre we have trained a member of staff in quality control procedures of [^{18}F]FMISO. We have continued to support PhD students from the Department of Chemistry at the University of Glasgow in their radiopharmaceutical development.

Preclinical and translational imaging

In 2021, we participated in a collaborative KRAS-mutant colorectal cancer study with Prof Owen Sansom's laboratory. We 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).

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.

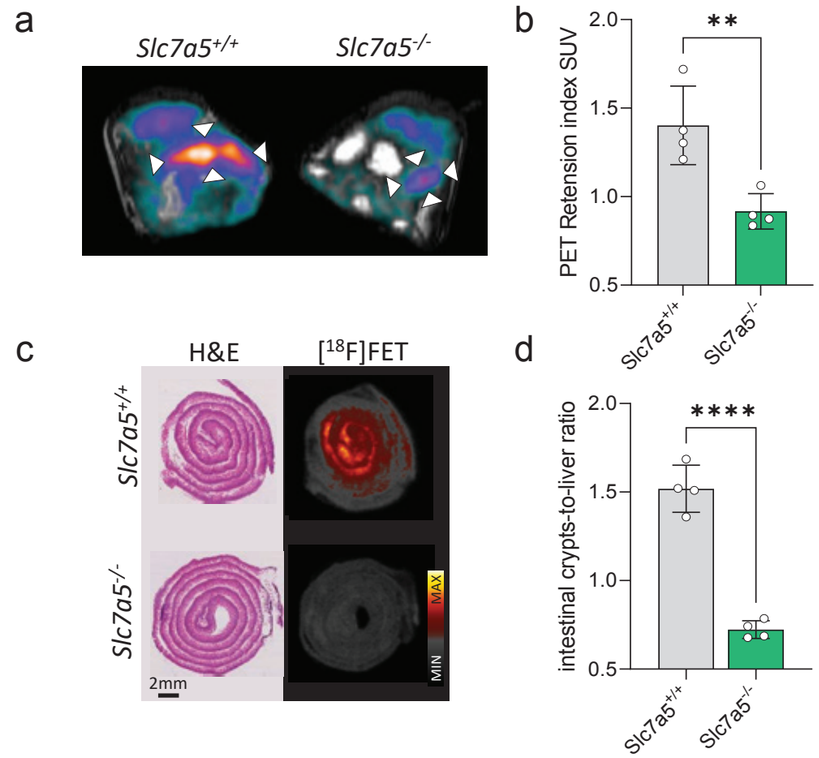


Figure 1
(a) [^{18}F]fluoro-ethyl-l-tyrosine (FET) PET/MRI in Apc$^{-/-}$ Kras$^{G12D/+}$ Slc7a5$^{+/+}$ and Apc$^{-/-}$ Kras$^{G12D/+}$ Slc7a5$^{-/-}$ mice. (b) Plots show reduced retention of FET in the absence of Slc7a5 (LAT1). (c) H&E and autoradiograms of FET uptake and distribution in Apc$^{-/-}$ Kras$^{G12D/+}$ Slc7a5$^{+/+}$ and Apc$^{-/-}$ Kras$^{G12D/+}$ Slc7a5$^{-/-}$ mice. Representative images from four biologically independent mice for each genotype. (d) Plots show specific uptake of FET in the intestinal epithelial crypts normalised to the uptake in the liver.
**** p value, < 0.0001, unpaired t-test, two-tailed.

We have supported the Glasgow CRUK Radiation Centre of Excellence (RadNet) in obtaining 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 have also supported Dr Tom Bird's group to identify and target mouse models of hepatocellular carcinoma with radiotherapy, including validation of CT contrast agents. Members of the TMI are also contributing to a number of UK-wide projects through the CRUK RadNet Molecular Imaging and Radiotherapy Working Group.

Publications listed on page 124

HISTOLOGY

Colin Nixon

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

The Histology Service performs processing of tissue samples and cellular material from the wide range of cancer models developed within the Institute. This allows material to be evaluated at a cellular level in order to understand the disease mechanics. The service offers processing for tissue samples (murine, human and drosophila) fixed in an array of different types of fixative dependent on required subsequent analysis. Once received the tissue samples are trimmed, appropriately processed and then orientated into paraffin wax blocks to facilitate tissue sectioning and staining. The tissue samples are processed according to type and necessity using previously designated specific and specialised processing cycles. We have four large-capacity automated tissue processors allowing large-scale consistent processing, but when required specialised processing cycles can be designed. Other material such as organotypic assays, cell pellets, spheroids and agar plugs can also be processed to provide a wax block to allow sectioning and further investigation. All paraffin wax blocks sectioned are stained with haematoxylin and eosin in order to allow general analysis of cell morphology and structure. After initial analysis more specialised histology stains can be performed if required to investigate specific tissue structures.

The Histology Service performs processing of tissue and cellular material from the wide range of cancer models developed within the Institute. This allows material to be evaluated at a cellular level allowing insight into disease mechanics. The service offers processing for tissue samples fixed in different types of fixative dependent on subsequent analysis. Once received, the tissue samples are trimmed, appropriately processed and then orientated into paraffin wax blocks to facilitate tissue sectioning and staining. The tissue samples are processed according to type and necessity using previously designated specific, specialised processing cycles. We have four large capacity automated tissue processors, allowing large scale consistent processing, but when required specialised processing cycles can be designed. Other material such as organotypic assays, cell pellets, spheroids and agar plugs can also be processed to provide a wax block allowing sectioning and further investigation. All paraffin wax blocks sectioned are stained with haematoxylin and eosin in order to allow 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. Frozen tissue, embryos or cells can be sectioned and when required stained for examination using routine histological stains, immuno-histochemical/immunofluorescence staining methods or *in situ* hybridisation techniques.

A comprehensive immunohistochemistry service is offered where the histology service can provide a large repertoire of previously validated antibodies that can be stained on one of our five autostainers providing consistent high-quality staining. We are continually expanding 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 optimised to produce a working protocol that allows the antibody to be used either on an autostainer or for hand staining by the researcher. Training can be provided in order that an individual scientist can understand the rationale and techniques available to allow them to perform the staining to an acceptable and consistent standard.

Where there is no antibody available for immunohistochemistry analysis or a more specific conclusive technique is required, the service can provide an *in situ* hybridisation technique using a reagent system designed to visualise cellular RNA targets using bright-field or fluorescent microscopy. This technique can be performed for single, dual or multiple staining of targets on formalin-fixed paraffin-embedded sections, cytospin preparations, other cellular material sections 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 performed. If a probe has to be designed, prior consultation with the histology service is a must in order 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 that when 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. This technique is automated, allowing for improved quality and reproducibility of the results.

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

The histology service provides a slide scanning service using a fully automated large capacity Leica Aperio AT2 slide scanner which captures bright-field images. This allows high-quality digital images to be scanned, stored and if required, automated quantitative interpretation can be performed. For digital analysis, we are able to 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 in order for the researcher to be able to use the image analysis software.

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 are able to 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

Breast tumour slice: Cyan – aSMA, Yellow – CD31, Purple – S100A9, Gray – autofluorescence.

Image by Frédéric Fercoq

LABORATORY OPERATIONS

Head
Scott Kelso

Laboratory operations cover a number of different functions which all have a remit to ensure the smooth operation of the building, facilities and support services, providing support to all the research groups housed within the Institute, giving them the freedom to focus on delivering their world class research.

The last year has continued to be a challenge in dealing with the impacts of the COVID-19 pandemic. This has resulted in staffing shortages from self-isolation rules, managing and applying the varying restrictions from Scottish Government and also created numerous supply chain issues for basic laboratory consumables. We have, however, still managed to progress with a number of improvements around the Institute by starting a phased replacement of old incubators, upgrading old building pipework and upgrading some areas of our core IT hardware. We are continuing to focus on replacing some of our core routine equipment in the laboratories whilst also trying to ensure we can remain at the forefront in some of our key technologies.

Despite the challenges, our staff have been incredible in maintaining services within the institute and we are now starting to see a future which is much more free of restrictions, allowing us to return back to more normal ways of working and delivering on our strategy of continuous improvement in laboratory operations.

Building Facilities

Alistair Wilson, Andrew Hosie, Mark Deegan, William Anderson

Building Facilities 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. We 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 maintenance and repair.

This year, we have continued to maintain COVID measures within our facilities. Additionally, we have joined the University of Edinburgh "TestEd" program of saliva testing for COVID-19. This gives our staff the opportunity to test twice per week within the Institute and contribute to University of Edinburgh's research programme in this area.

The structure within the department has altered with Andy Hosie taking direct responsibility for maintenance with a direct line report to the Head of Laboratory Operations. Andy is supported by two technicians Mark Deegan and William Anderson.

Our catering service, which was suspended during the first year of the pandemic, has now restarted albeit with a reduced service commensurate with the number of staff returning to the Institute following furlough and maintaining working from home.

Laboratory Management & Health and Safety

Caroline O'Connell, Richard Selkirk, Karen Thomas, James Dyball

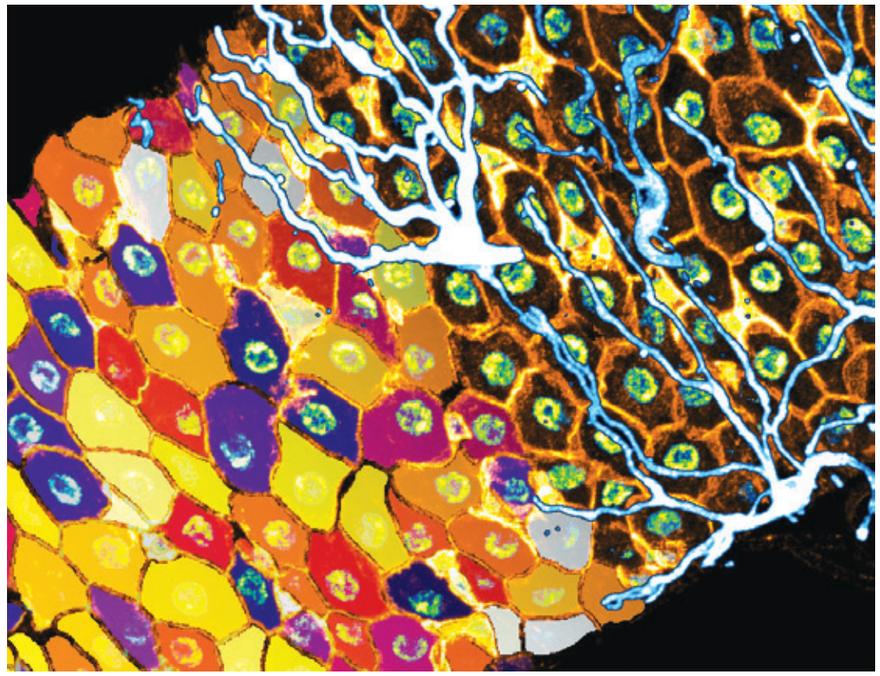
The Laboratory Management team (Caroline O'Connell, Karen Thomas and James Dyball) is responsible for providing a number of vital support roles to the Institute and ensures the smooth running of the laboratories, aiding researchers to perform their research as efficiently and effectively as possible. The laboratory management team works very closely with Richard Selkirk, the Health and Safety adviser, to also ensure that all staff, students and visitors work in a safe environment.

As safety plays an important 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. We 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. Fire safety is also managed. Lab management also monitor all outgoing orders to ensure compliance with Institute safety procedures, particularly those relating to COSHH. The provision of gas supplies, such as carbon dioxide or nitrogen, to equipment is also carefully managed and coordinated to ensure gas safety is maintained and that systems meet with regulatory compliance.

Laboratory Management also coordinates the service and maintenance of core equipment and of any systems that these require. We are proactive in ensuring that equipment breakdowns are kept to a minimum and are being dealt with as quickly as possible. We identify areas of improvement and oversee the replacement and purchase of equipment to facilitate the needs of researchers. For example, in 2021, we have purchased 16 new incubators in a wider effort to upgrade our cell culture facilities. We engage with sales and technical representatives of companies to arrange demos of new equipment, some showing new technology, to enable researchers to see first-hand the latest state-of-the-art technology. Although 2021 has been a challenging year with COVID restrictions, we have adapted by arranging a number of online training seminars and webinars to ensure researchers can still have access to new and improved techniques using the equipment and technologies already present within the Institute.

Developing and adult self-renewing epithelia undergo dynamic changes, which are essential to establish or restore tissue patterning. In the Cordero Lab, we use the adult Drosophila intestine combined with high-resolution imaging, advanced image analysis tools and molecular genetics to understand how chemical and mechanical changes within the adult intestinal epithelium (polygonal multi-coloured cells) influence the vascular-like tracheal microenvironment (white/blue cells) during intestinal regeneration and disease.

Image and Research by André Carvalho & Jessica Perochon.



Lab management 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. Lab Management also liaises with Stores to acquire free samples of new products to ensure the best and most appropriate products are used by the researchers.

Laboratory Support Services

Angela Miller, **Tracy Shields**, Elizabeth Cheetham, Dilhani Kahawela, Kirstie McPherson, Jonny Sawers, Linda Scott, Conor Gilbey and Nicola O'Hagan

Laboratory 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 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, Michael McTaggart, George Monteith, Emma Walker

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 contingency planning for a number of high-use tissue culture items, and other essential items impacted by the COVID-19 pandemic.

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 changed their way of working due to the COVID-19 restrictions, and by doing so have increased communications with research groups and been able to incorporate homeworking into their routine. 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 changes, as stock items held will be reviewed and new kits and reagents brought in in conjunction with the changes in the research needs.

Molecular Technology Services

Graeme Clark, Andrew Keith, Jillian Murray

The Molecular Technology Service provides a number of services. Routine sequencing of plasmids and PCR products is performed on an Applied Biosystems® 3130xl (16 capillary) Sequencer that provides good sample throughput, long read lengths and a sample turnaround time of 24 hours. Post-PCR products can now be purified for sequencing by the addition of USB ExoSAP-IT™ (Applied Biosystems®). In recent years, DNA sequencing has been revolutionised by the introduction of next-generation sequencing (NGS) technologies offering large-scale sequencing in a matter of hours. An Illumina® NextSeq™500 platform has enabled us to sequence libraries at a lower cost with increased data output and a faster turnaround time. Protocols currently used are ChIP-seq and RNA-seq. Upgrading of a Beckman® FXp workstation has enabled us to increase library throughput for NGS. Quality Control of libraries is carried out using a Qubit™ fluorometic quantification assay and an Agilent Tapestation 2200.

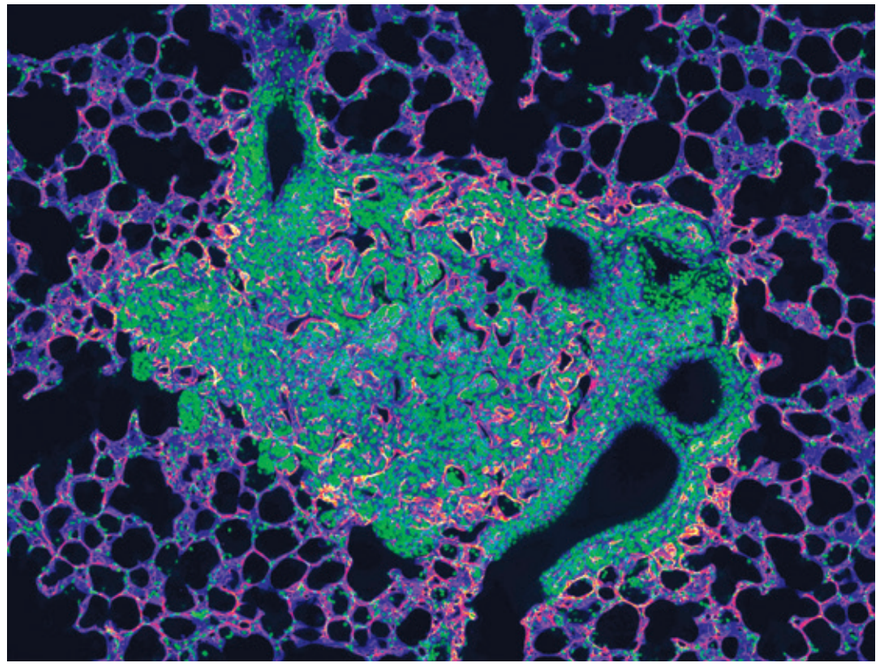
Small-scale DNA purification is performed on a Qiagen® Universal BioRobot®. Researchers provide overnight bacterial cultures that are processed by the facility. Sample numbers are consistently in the region of 17,000–19,000 per year. We continue to provide a very popular large-scale DNA purification (maxiprep) service from bacterial cultures.

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.

There are other additional services which are offered by the group. The mycoplasma screening service offers testing of each research group's cells every three to four months. Researchers are also encouraged to have newly imported cell lines tested as soon as possible after arrival, as we have found that a significant number of newly imported cell lines are infected with mycoplasma. 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. Cell-derived matrices from Tiff 5 cells are prepared to order for the research groups and have proved very popular and we also continue to coordinate batch testing of serum.

Spectral imaging of a murine lung section colonized by metastatic pancreatic ductal adenocarcinoma cells (nuclei in green) and the blue to yellow structure is a fire LUT (lookup table) representing different mean fluorescent intensities of the immune adhesion molecule CD54 close and far away from the metastases.

Image by Marco De Donatis



PUBLICATIONS

Imran Ahmad (page 10)
Models of Advanced Prostate Cancer

Primary Research Papers

Galbraith LCA, Mui E, Nixon C, Hedley A, Strachan D, MacKay G, Sumpton D, Sansom OJ, Leung HY, Ahmad I.
PPAR-gamma induced AKT3 expression increases levels of mitochondrial biogenesis driving prostate cancer. ***Oncogene***. 2021;40:2355-2366.

Ntala C, Salji M, Salmond J, Officer L, Teodosio AV, Blomme A, McGhee EJ, Powley I, Ahmad I, Kruithof-de Julio M, Thalmann G, Roberts E, Goodyear CS, Jamaspishvili T, Berman DM, Carlin LM, Le Quesne J, Leung HY.
Analysis of Prostate Cancer Tumor Microenvironment Identifies Reduced Stromal CD4 Effector T-cell Infiltration in Tumors with Pelvic Nodal Metastasis. ***Eur Urol Open Sci***. 2021;29:19-29.

Tan WS, Arianayagam R, Khetrapal P, Rowe E, Kearley S, Mahrous A, Pal R, Fowler W, Heer R, Elajnaf M, Douglas-Moore J, Leyshon Griffiths TR, Voss J, Wilby D, Al Kadhi O, Noel J, Vasdev N, McKay A, Ahmad I, Abu-Nayla I, Lamb B, Hill GT, Narahari K, Kynaston H, Yousuf A, Kusuma VRM, Cresswell J, Cooke P, Chakravarti A, Barod R, Bex A, Kelly JD, a multicentre collaborator
Major Urological Cancer Surgery for Patients is Safe and Surgical Training Should Be Encouraged During the COVID-19 Pandemic: A Multicentre Analysis of 30-day Outcomes. ***Eur Urol Open Sci***. 2021;25:39-43.

Other Publications

Hartley A, Leung HY, Ahmad I.
Targeting the BAF complex in advanced prostate cancer. ***Expert Opin Drug Discov***. 2021; 16:173-181.

Tom Bird (page 12)
Liver Disease and Regeneration

Primary Research Papers

Barthet VJA, Brucoli M, Ladds M, Nossing C, Kiourtis C, Baudot AD, O'Prey J, Zunino B, Muller M, May S, Nixon C, Long JS, Bird TG, Ryan KM.
Autophagy suppresses the formation of hepatocyte-derived cancer-initiating ductular progenitor cells in the liver. ***Sci Adv***. 2021;7.

Burton A, Tataru D, Driver RJ, Bird TG, Huws D, Wallace D, Cross TJS, Rowe IA, Alexander G, Marshall A, Group H-UBNPS.
Primary liver cancer in the UK: Incidence, incidence-based mortality, and survival by subtype, sex, and nation. ***JHEP Rep***. 2021;3:100232.

Haq MI, Drake TM, Goh TL, Ahmed A, Forrest E, Barclay S, Gillespie R, Priest M, Evans J, Graham J, Ballantyne S, McMillan DC, Hayes PC, Bird TG, Stanley AJ.
Effect of Hepatocellular Carcinoma Surveillance Programmes on Overall Survival in a Mixed Cirrhotic UK Population: A Prospective, Longitudinal Cohort Study. ***J Clin Med***. 2021;10.

Kiourtis C, Wilczynska A, Nixon C, Clark W, May S, Bird TG.
Specificity and off-target effects of AAV8-TBG viral vectors for the manipulation of hepatocellular gene expression in mice. ***Biol Open***. 2021;10.

Other Publications

Müller M, May S, Bird TG.
Ploidy dynamics increase the risk of liver cancer initiation. ***Nat Commun***. 2021; 12:1896.

Karen Blyth (page 14)
In Vivo Cancer Biology

Primary Research Papers

Bock FJ, Sedov E, Koren E, Koessinger AL, Cloix C, Zerbst D, Athineos D, Anand J, Campbell KJ, Blyth K, Fuchs Y, Tait SWG.
Apoptotic stress-induced FGF signalling promotes non-cell autonomous resistance to cell death. ***Nat Commun***. 2021;12:6572.

Campbell KJ, Mason SM, Winder ML, Willemsen RBE, Cloix C, Lawson H, Rooney N, Dhayade S, Sims AH, Blyth K, Tait SWG.
Breast cancer dependence on MCL-1 is due to its canonical anti-apoptotic function. ***Cell Death Differ***. 2021;28:2589-2600.

Dayal JHS, Mason SM, Salas-Alanis JC, McGrath JA, Taylor RG, Mellerio JE, Blyth K, South AP, Inman GJ.
Heterogeneous addiction to transforming growth factor-beta signalling in recessive dystrophic epidermolysis bullosa-associated cutaneous squamous cell carcinoma. ***Br J Dermatol***. 2021;184:697-708.

Floerchinger A, Murphy KJ, Latham SL, Warren SC, McCulloch AT, Lee YK, Stoehr J, Melenec P, Guaman CS, Metcalf XL, Lee V, Zaratzian A, Da Silva A, Tayao M, Rolo S, Phimmachanh M, Sultani G, McDonald L, Mason SM, Ferrari N, Ooms LM, Johnsson AE, Spence HJ, Olson MF, Machesky LM, Sansom OJ, Morton JP, Mitchell CA, Samuel MS, Croucher DR, Welch HCE, Blyth K, Caldon CE, Herrmann D, Anderson KI, Timpson P, Nobis M.
Optimizing metastatic-cascade-dependent Rac1 targeting in breast cancer: Guidance using optical window intravital FRET imaging. ***Cell Rep***. 2021;36:109689.

Humpton TJ, Nomura K, Weber J, Magnussen HM, Hock AK, Nixon C, Dhayade S, Stevenson D, Huang DT, Strathdee D, Blyth K, Vousden KH.
Differential requirements for MDM2 E3 activity during embryogenesis and in adult mice. ***Genes Dev***. 2021;35:117-132.

Johnson SA, Ormsby MJ, Wessel HM, Hulme HE, Bravo-Blas A, McIntosh A, Mason S, Coffelt SB, Tait SWG, Mowat AM, Milling SWF, Blyth K, Wall DM.
Monocytes mediate Salmonella Typhimurium-induced tumor growth inhibition in a mouse melanoma model. ***Eur J Immunol***. 2021;51:3228-3238.

Latif AL, Newcombe A, Li S, Gilroy K, Robertson NA, Lei X, Stewart HJS, Cole J, Terradas MT, Rishi L, McGarry L, McKeeve C, Reid C, Clark W, Campos J, Kirschner K, Davis A, Lopez J, Sakamaki JI, Morton JP, Ryan KM, Tait SWG, Abraham SA, Holyoake T, Higgins B, Huang X, Blyth K, Copland M, Chevassut TJT, Keeshan K, Adams PD.
BRD4-mediated repression of p53 is a target for combination therapy in AML. ***Nat Commun***. 2021;12:241.

McGarry DJ, Armstrong G, Castino G, Mason S, Clark W, Shaw R, McGarry L, Blyth K, Olson MF.
MICAL1 regulates actin cytoskeleton organization, directional cell migration and the growth of human breast cancer cells as orthotopic xenograft tumours. ***Cancer Lett***. 2021;519:226-236.

Nacke M, Sandilands E, Nikolatou K, Roman-Fernandez A, Mason S, Patel R, Lilla S, Yelland T, Galbraith LCA, Freckmann EC, McGarry L, Morton JP, Shanks E, Leung HY, Markert E, Ismail S, Zanivan S, Blyth K, Bryant DM.
An ARF GTPase module promoting invasion and metastasis through regulating phosphoinositide metabolism. ***Nat Commun***. 2021;12:1623.1

Newman AC, Falcone M, Huerta Uribe A, Zhang T, Athineos D, Pietzke M, Vazquez A, Blyth K, Maddocks ODK.
Immune-regulated IDO1-dependent tryptophan metabolism is source of one-carbon units for pancreatic cancer and stellate cells. ***Mol Cell***. 2021;81:2290-2302 e2297.

Rudzka DA, Mason S, Neilson M, McGarry L, Kalna G, Hedley A, Blyth K, Olson MF.
Selection of established tumour cells through narrow diameter micropores enriches for elevated Ras/Raf/MEK/ERK MAPK signalling and enhanced tumour growth. ***Small GTPases***. 2021;12:294-310.

Tajan M, Hennequart M, Cheung EC, Zani F, Hock AK, Legrave N, Maddocks ODK, Ridgway RA, Athineos D, Suarez-Bonnet A, Ludwig RL, Novellasdemunt L, Angelis N, Li VSW, Vlachogiannis G, Valeri N, Mainolfi N, Suri V, Friedman A, Manfredi M, Blyth K, Sansom OJ, Vousden KH.
Serine synthesis pathway inhibition cooperates with dietary serine and glycine limitation for cancer therapy. ***Nat Commun***. 2021;12:366.

Teresa Borrello M, Rita Emma M, Listi A, Rubis M, Coslet S, Augello G, Cusimano A, Cabibi D, Porcasi R, Giannitrapani L, Soresi M, Pantuso G, Blyth K, Montalto G, Pin C, Cervello M, Iovanna J.
NUPR1 protects liver from lipotoxic injury by improving the endoplasmic reticulum stress response. ***FASEB J***. 2021;35:e21395.

David Bryant (page 16)
Epithelial Polarity

Primary Research Papers
Nacke M, Sandilands E, Nikolatou K, Roman-Fernandez A, Mason S, Patel R, Lilla S, Yelland T, Galbraith LCA, Freckmann EC, McGarry L, Morton JP, Shanks E, Leung HY, Markert E, Ismail S, Zanivan S, Blyth K, Bryant DM.
An ARF GTPase module promoting invasion and metastasis through regulating phosphoinositide metabolism. ***Nat Commun***. 2021;12:1623.

Naszai M, Bellec K, Yu Y, Roman-Fernandez A, Sandilands E, Johansson J, Campbell AD, Norman JC, Sansom OJ, Bryant DM, Cordero JB. RAL
GTPases mediate EGFR-driven intestinal stem cell proliferation and tumourigenesis. ***Elife***. 2021;10.

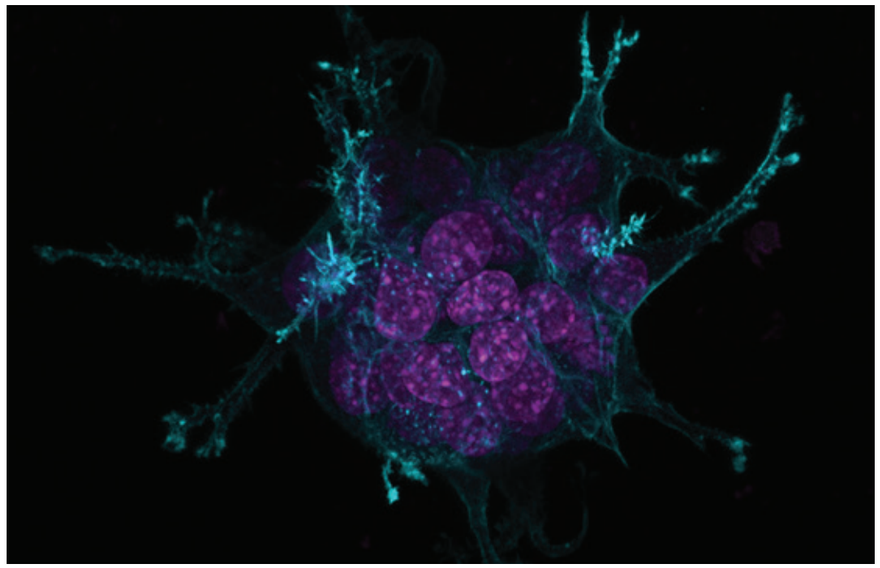
Martin Bushell (page 18)
RNA and Translational Control in Cancer

Primary Research Papers
Bader AS, Bushell M.
Damage-Net: A program for DNA repair meta-analysis identifies a network of novel repair genes that facilitate cancer evolution. ***DNA Repair (Amst)***. 2021;105:103158.

de Almeida-Faria J, Duque-Guimaraes DE, Ong TP, Pantaleao LC, Carpenter AA, Loche E, Kusinski LC, Ashmore TJ, Antrobus R, Bushell M, Fernandez-Twinn DS, Ozanne SE.
Maternal obesity during pregnancy leads to adipose tissue ER stress in mice via miR-126-mediated reduction in Lunapark. ***Diabetologia***. 2021;64:890-902.

Gillen SL, Giacomelli C, Hodge K, Zanivan S, Bushell M, Wilczynska A.
Differential regulation of mRNA fate by the human Ccr4-Not complex is driven by coding sequence composition and mRNA localization. ***Genome Biol***. 2021;22:284.

Grosso S, Marini A, Gyuraszova K, Voorde JV, Sfakianos A, Garland GD, Tenor AR, Mordue R, Chernova T, Morone N, Sereno M, Smith CP, Officer L, Farahmand P, Rooney C, Sumpton D, Das M, Teodosio A, Ficken C, Martin MG, Spriggs RV, Sun XM, Bushell M, Sansom OJ, Murphy D, MacFarlane M, Le Quesne JPC, Willis AE.



Ovarian cancer mini-tumour invading through the Extracellular Matrix. Stained with Phalloidin (F-actin, cyan) and with a nuclear stain (HOECHST, magenta).

Image by Konstantina Nikolatou

The pathogenesis of mesothelioma is driven by a dysregulated translatome. ***Nat Commun***. 2021;12:4920.

Knight JRP, Alexandrou C, Skalka GL, Vlahov N, Pennel K, Officer L, Teodosio A, Kanellos G, Gay DM, May-Wilson S, Smith EM, Najumudeen AK, Gilroy K, Ridgway RA, Flanagan DJ, Smith RCL, McDonald L, MacKay C, Cheasty A, McArthur K, Stanway E, Leach JD, Jackstadt R, Waldron JA, Campbell AD, Vlachogiannis G, Valeri N, Haigis KM, Sonenberg N, Proud CG, Jones NP, Swarbrick ME, McKinnon HJ, Faller WJ, Le Quesne J, Edwards J, Willis AE, Bushell M, Sansom OJ.
MNK Inhibition Sensitizes KRAS-Mutant Colorectal Cancer to mTORC1 Inhibition by Reducing eIF4E Phosphorylation and c-MYC Expression. ***Cancer Discov***. 2021;11:1228-1247.

Najumudeen AK, Ceteci F, Fey SK, Hamm G, Steven RT, Hall H, Nikula CJ, Dexter A, Murta T, Race AM, Sumpton D, Vlahov N, Gay DM, Knight JRP, Jackstadt R, Leach JDG, Ridgway RA, Johnson ER, Nixon C, Hedley A, Gilroy K, Clark W, Malla SB, Dunne PD, Rodriguez-Blanco G, Critchlow SE, Mrowinska A, Malviya G, Solovyev D, Brown G, Lewis DY, Mackay GM, Strathdee D, Tardito S, Gottlieb E, Consortium CRGC, Takats Z, Barry ST, Goodwin RJA, Bunch J, Bushell M, Campbell AD, Sansom OJ.
The amino acid transporter SLC7A5 is required for efficient growth of KRAS-mutant colorectal cancer. ***Nat Genet***. 2021;53:16-26.

Smith EM, Benbahouche NEH, Morris K, Wilczynska A, Gillen S, Schmidt T, Meijer HA, Jukes-Jones R, Cain K, Jones C, Stoneley M, Waldron JA, Bell C, Fonseca BD, Blagden S, Willis AE, Bushell M.
The mTOR regulated RNA-binding protein LARP1 requires PABPC1 for guided mRNA interaction. ***Nucleic Acids Res***. 2021;49:458-478.

Other Publications
Gillen SL, Waldron JA, Bushell M.
Codon optimality in cancer. ***Oncogene***. 2021; 10.1038/s41388-021-02022-x

Ross Cagan (page 20)
Biology of Therapeutics

Primary Research Papers
Bangi E, Smibert P, Uzilov AV, Teague AG, Gopinath S, Antipin Y, Chen R, Hecht C, Gruszczynski N, Yon WJ, Malyshev D, Laspina D, Selkridge I, Wang H, Gomez J, Mascarenhas J, Moe AS, Lau CY, Taik P, Pandya C, Sung M, Kim S, Yum K, Sebra R,

Donovan M, Misiukiewicz K, Ang C, Schadt EE, Posner MR, Cagan RL.
A Drosophila platform identifies a novel, personalized therapy for a patient with adenoid cystic carcinoma. ***iScience***. 2021;24:102212.

Das TK, Gatto J, Mirmira R, Hourizadeh E, Kaufman D, Gelb BD, Cagan R.
Drosophila RASopathy models identify disease subtype differences and biomarkers of drug efficacy. ***iScience.*** 2021;24:102306.

Padash Barmchi M, Thomas M, Thatte JV, Vats A, Zhang B, Cagan RL, Banks L.
Inhibition of kinase IKKbeta suppresses cellular abnormalities induced by the human papillomavirus oncoprotein HPV 18E6. ***Sci Rep***. 2021;11:1111.

Xiong Z, Jeon M, Allaway RJ, Kang J, Park D, Lee J, Jeon H, Ko M, Jiang H, Zheng M, Tan AC, Guo X, Multi-Targeting Drug DCC, Dang KK, Tropsha A, Hecht C, Das TK, Carlson HA, Abagyan R, Guinney J, Schlessinger A, Cagan R.
Crowdsourced identification of multi-target kinase inhibitors for RET- and TAU- based disease: The Multi-Targeting Drug DREAM Challenge. ***PLoS Comput Biol***. 2021;17:e1009302.

Other Publications
Posner M, Cagan R.
Interdisciplinary case study: from fly-to-bedside, translating basic research to the clinic. ***iScience***. 2021; 24:102279.

Leo Carlin (page 22)
Leukocyte Dynamics

Primary Research Papers
Brownlie D, Doughty-Shenton D, Yh Soong D, Nixon C, N OC, L MC, Kitamura T.
Metastasis-associated macrophages constrain antitumor capability of natural killer cells in the metastatic site at least partially by membrane bound transforming growth factor beta. J ***Immunother Cancer***. 2021;9.

Juzenaite G, Secklehner J, Vuononvirta J, Helbawi Y, Mackey JBG, Dean C, Harker JA, Carlin LM, Rankin S, De Filippo K.
Lung Marginated and Splenic Murine Resident Neutrophils Constitute Pioneers in Tissue-Defense During Systemic E. coli Challenge. ***Front Immunol***. 2021;12:597595.

McCowan J, Fercoq F, Kirkwood PM, T'Jonck W, Hegarty LM, Mawer CM, Cunningham R, Mirchandani AS, Hoy A, Humphries DC, Jones

GR, Hansen CG, Hirani N, Jenkins SJ, Henri S, Malissen B, Walmsley SR, Dockrell DH, Saunders PTK, Carlin LM, Bain CC.
The transcription factor EGR2 is indispensable for tissue-specific imprinting of alveolar macrophages in health and tissue repair. ***Sci Immunol***. 2021;6:eabj2132.

Ntala C, Salji M, Salmond J, Officer L, Teodosio AV, Blomme A, McGhee EJ, Powley I, Ahmad I, Kruithof-de Julio M, Thalmann G, Roberts E, Goodyear CS, Jamaspishvili T, Berman DM, Carlin LM, Le Quesne J, Leung HY.
Analysis of Prostate Cancer Tumor Microenvironment Identifies Reduced Stromal CD4 Effector T-cell Infiltration in Tumors with Pelvic Nodal Metastasis. ***Eur Urol Open Sci***. 2021;29:19-29.

Other Publications

Fercoq F, Carlin LM.
"Mind the GAP": RGS1 hinders antitumor lymphocytes. ***Nat Immunol***. 2021; 22:802-804.

McFarlane AJ, Fercoq F, Coffelt SB, Carlin LM.
Neutrophil dynamics in the tumor microenvironment. ***J Clin Invest***. 2021; 131: ed2021.

Seth Coffelt (page 24)

Immune Cells and Metastasis

Primary Research Papers

Johnson SA, Ormsby MJ, Wessel HM, Hulme HE, Bravo-Blas A, McIntosh A, Mason S, Coffelt SB, Tait SWG, Mowat AM, Milling SWF, Blyth K, Wall DM.
Monocytes mediate Salmonella Typhimurium-induced tumor growth inhibition in a mouse melanoma model. ***Eur J Immunol***. 2021;51:3228-3238.

Paterson K, Zanivan S, Glasspool R, Coffelt SB, Zagnoni M.
Microfluidic technologies for immunotherapy studies on solid tumours. ***Lab Chip***. 2021; 21: 2306-2329.

Other Publications

Edwards SC, Hoevenaar WHM, Coffelt SB.
Emerging immunotherapies for metastasis. ***Br J Cancer***. 2021; 124:37-48.

McFarlane AJ, Fercoq F, Coffelt SB, Carlin LM.
Neutrophil dynamics in the tumor microenvironment. ***J Clin Invest***. 2021; 131: ed2021.

Julia Cordero (page 26)

Local and systemic functions of the adult intestine in health and disease

Primary Research Papers

Chen Z, Cordero J, Alqarni AM, Slack C, Zeidler MP, Bellantuono I.
Zoledronate extends healthspan and survival via the mevalonate pathway in a FOXO-dependent manner. ***J Gerontol A Biol Sci Med Sci***. 2021;10.1093/gerona/glab172.

Guillermin O, Angelis N, Sidor CM, Ridgway R, Baulies A, Kucharska A, Antas P, Rose MR, Cordero J, Sansom O, Li VSW, Thompson BJ.
Wnt and Src signals converge on YAP-TEAD to drive intestinal regeneration. ***EMBO J***. 2021;40:e105770.

Hodgson JA, Parvy JP, Yu Y, Vidal M, Cordero JB.
Drosophila Larval Models of Invasive Tumorigenesis for In Vivo Studies on Tumour/Peripheral Host Tissue Interactions during Cancer Cachexia. ***Int J Mol Sci***. 2021;22.

Naszai M, Bellec K, Yu Y, Roman-Fernandez A, Sandilands E, Johansson J, Campbell AD, Norman JC, Sansom OJ, Bryant DM, Cordero JB.
RAL GTPases mediate EGFR-driven intestinal stem cell proliferation and tumourigenesis. ***Elife***. 2021;10.

Perochon J, Yu Y, Aughey GN, Medina AB, Southall TD, Cordero JB.
Dynamic adult tracheal plasticity drives stem cell adaptation to changes in intestinal homeostasis in Drosophila. ***Nat Cell Biol***. 2021;23:485-496.

Other Publications

Cell scientist to watch - Julia Cordero. ***Journal of Cell Science***. Vol 1342021.

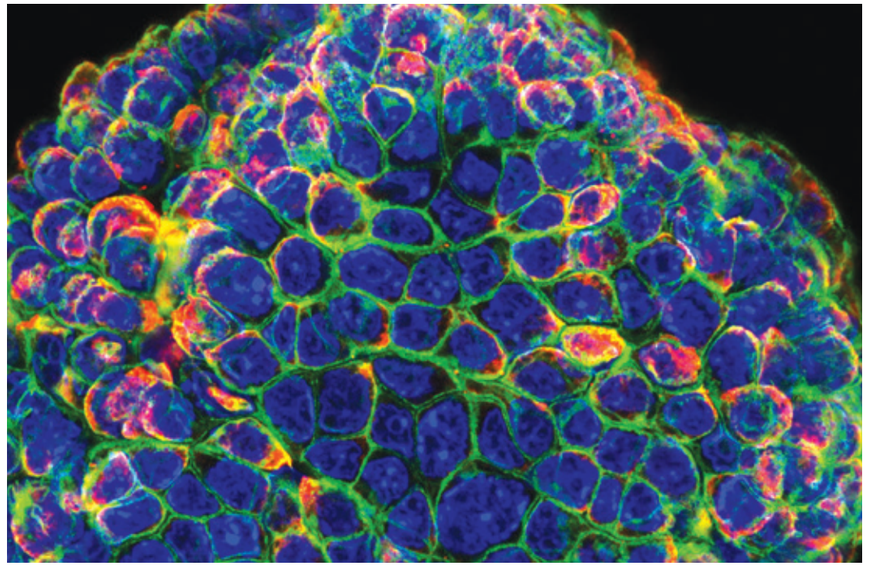
Jeff Evans

Primary Research Papers

Annese VF, Patil SB, Hu C, Giagkoulovits C, Al-Rawhani MA, Grant J, Macleod M, Clayton DJ, Heaney LM, Daly R, Accarino C, Shah YD,

Organoid Tumouroid in 3D grown from intestinal cancer cells. Red: shows expression of Notum, Green: cell junctions and Blue: Nuclei. Imaged using a Zeiss 710 LSM, maximum intensity projection of Z-stack.

Image by Dustin Flanagan and Nikki R. Paul



Cheah BC, Beeley J, Evans TRJ, Jones R, Barrett MP, Cumming DRS.
A monolithic single-chip point-of-care platform for metabolomic prostate cancer detection. ***Microsyst Nanoeng***. 2021;7:21.

Finn RS, Kudo M, Cheng AL, Wyrwicz L, Ngan RKC, Blanc JF, Baron AD, Vogel A, Ikeda M, Piscaglia F, Han KH, Qin S, Minoshima Y, Kanekiyo M, Ren M, Dairiki R, Tamai T, Dutcus CE, Ikezawa H, Funahashi Y, Evans TRJ.
Pharmacodynamic Biomarkers Predictive of Survival Benefit with Lenvatinib in Unresectable Hepatocellular Carcinoma: From the Phase III REFLECT Study. ***Clin Cancer Res***. 2021; 27: 4848-4858.

Haq MI, Drake TM, Goh TL, Ahmed A, Forrest E, Barclay S, Gillespie R, Priest M, Evans J, Graham J, Ballantyne S, McMillan DC, Hayes PC, Bird TG, Stanley AJ.
Effect of Hepatocellular Carcinoma Surveillance Programmes on Overall Survival in a Mixed Cirrhotic UK Population: A Prospective, Longitudinal Cohort Study. ***J Clin Med***. 2021;10.

Lei M, Siemers NO, Pandya D, Chang H, Sanchez T, Harbison C, Szabo PM, Janjigian Y, Ott PA, Sharma P, Bendell J, Evans TRJ, de Braud F, Chau I, Boyd Z.
Analyses of PD-L1 and Inflammatory Gene Expression Association with Efficacy of Nivolumab +/- Ipilimumab in Gastric Cancer/Gastroesophageal Junction Cancer. ***Clin Cancer Res***. 2021; 27: 3926-3935.

Marks DK, Kucharczyk J, Kim P, Chyong DI, Gartrell RD, Lu Y, Hibshoosh H, Guo H, Evans TRJ, Lopez J, Kristeleit R, Connolly E, Saenger Y, Kalinsky K.
Quantitative Multiplex Immunofluorescence Evaluation of the Tumor Microenvironment in Pretreatment Tumors of Patients with Metastatic Breast Cancer and Serous Ovarian Carcinoma Treated with Liposomal Eribulin. ***Cancer Invest***. 2021;39:466-472.

McNamara MG, Bridgewater J, Palmer DH, Faluyi O, Wasan H, Patel A, Ryder WD, Barber S, Gnanaranjan C, Ghazaly E, Evans TRJ, Valle JW.
A Phase Ib Study of NUC-1031 in Combination with Cisplatin for the First-Line Treatment of Patients with Advanced Biliary Tract Cancer (ABC-08). ***Oncologist***. 2021;26:e669-e678.

Middleton MR, Dean E, Evans TRJ, Shapiro GI, Pollard J, Hendriks BS, Falk M, Diaz-Padilla I, Plummer R.
Phase 1 study of the ATR inhibitor berzosertib (formerly M6620, VX-970) combined with gemcitabine +/- cisplatin in patients with advanced solid tumours. ***Br J Cancer***. 2021; 125: 510-519.

Fieke Froeling (page 28)

Pancreatic Cancer Evolution and Therapeutic Development

Primary Research Papers

Casolino R, Paiella S, Azzolina D, Beer PA, Corbo V, Lorenzoni G, Gregori D, Golan T, Braconi C, Froeling FEM, Milella M, Scarpa A, Pea A, Malleo G, Salvia R, Bassi C, Chang DK, Biankin AV.

Homologous Recombination Deficiency in Pancreatic Cancer: A Systematic Review and Prevalence Meta-Analysis. ***J Clin Oncol.*** 2021;39:2617-2631.

Cortés-Charry R, Hennah L, Froeling FEM, Short D, Aguiar X, Tin T, Harvey R, Unsworth N, Kaur B, Savage P, Sarwar N, Seckl MJ.
Increasing the human chorionic gonadotrophin cut-off to ≤1000 IU/L for starting actinomycin D in post-molar gestational trophoblastic neoplasia developing resistance to methotrexate spares more women multi-agent chemotherapy. ***ESMO Open***. 2021;6(3):100110.

Dreyer SB, Upstill-Goddard R, Paulus-Hock V, Paris C, Lampraki EM, Dray E, Serrels B, Caligiuri G, Rebus S, Plenker D, Galluzzo Z, Brunton H, Cunningham R, Tesson M, Nourse C, Bailey UM, Jones M, Moran-Jones K, Wright DW, Duthie F, Oien K, Evers L, McKay CJ, McGregor GA, Gulati A, Brough R, Bajrami I, Pettitt S, Dziubinski ML, Candido J, Balkwill F, Barry ST, Grutzmann R, Rahib L, Glasgow Precision Oncology L, Australian Pancreatic Cancer Genome I, Johns A, Pajic M, Froeling FEM, Beer P, Musgrove EA, Petersen GM, Ashworth A, Frame MC, Crawford HC, Simeone DM, Lord C, Mukhopadhyay D, Pilarsky C, Tuveson DA, Cooke SL, Jamieson NB, Morton JP, Sansom OJ, Bailey PJ, Biankin AV, Chang DK.
Targeting DNA Damage Response and Replication Stress in Pancreatic Cancer. ***Gastroenterology***. 2021;160:362-377 e313.

Other Publications

Casolino R, Braconi C, Malleo G, Paiella S, Bassi C, Milella M, Dreyer SB, Froeling FEM, Chang DK, Biankin AV, Golan T.
Reshaping preoperative treatment of pancreatic cancer in the era of precision medicine. ***Ann Oncol***. 2021; 31:183-196.

Froeling FEM, Casolino R, Pea A, Biankin AV, Chang DK.
Molecular Subtyping and Precision Medicine for Pancreatic Cancer. ***J Clin Med***. 2021; 10:ed2021.

Payam Gammage (page 30)

Mitochondrial Oncogenetics

Primary Research Papers

Gorelick AN, Kim M, Chatila WK, La K, Hakimi AA, Berger MF, Taylor BS, Gammage PA, Reznik E.
Respiratory complex and tissue lineage drive recurrent mutations in tumour mtDNA. ***Nat Metab***. 2021;3:558-570.

Rabas N, Palmer S, Mitchell L, Ismail S, Gohlke A, Riley JS, Tait SWG, Gammage P, Soares LL, Macpherson IR, Norman JC.
PINK1 drives production of mtDNA-containing extracellular vesicles to promote invasiveness. ***J Cell Biol***. 2021;220.

Danny Huang (page 32)

Ubiquitin Signalling

Primary Research Papers

Ahmed SF, Buetow L, Gabrielsen M, Lilla S, Sibbet GJ, Sumpton D, Zanivan S, Hedley A, Clark W, Huang DT.
E3 ligase-inactivation rewires CBL interactome to elicit oncogenesis by hijacking RTK-CBL-CIN85 axis. ***Oncogene***. 2021;40:2149-2164.

Humpton TJ, Nomura K, Weber J, Magnussen HM, Hock AK, Nixon C, Dhayade S, Stevenson D, Huang DT, Strathdee D, Blyth K, Vousden KH.
Differential requirements for MDM2 E3 activity during embryogenesis and in adult mice. ***Genes Dev***. 2021;35:117-132.

Magnussen HM, Huang DT.
Identification of a Catalytic Active but Non-Aggregating MDM2 RING Domain Variant. ***J Mol Biol***. 2021;433:166807.

Gareth Inman (page 34)

Growth Factor Signalling and Squamous Cancers

Primary Research Papers

Cosgarea I, McConnell AT, Ewen T, Tang D, Hill DS, Anagnostou M, Elias M, Ellis RA, Murray A, Spender LC, Giglio P, Gagliardi M, Greenwood A, Piacentini M, Inman GJ, Fimia GM, Corazzari M, Armstrong JL, Lovat PE.
Melanoma secretion of transforming growth factor-beta2 leads to loss of epidermal AMBRA1 threatening epidermal integrity and facilitating tumour ulceration. ***Br J Dermatol***. 2021; 10.1111/bjd.20889.

Dayal JHS, Mason SM, Salas-Alanis JC, McGrath JA, Taylor RG, Mellerio JE, Blyth K, South AP, Inman GJ.
Heterogeneous addiction to transforming growth factor-beta signalling in recessive dystrophic epidermolysis bullosa-associated cutaneous squamous cell carcinoma. ***Br J Dermatol***. 2021;184:697-708.

Hollis RL, Stillie LJ, Hopkins S, Bartos C, Churchman M, Rye T, Nussey F, Fegan S, Nirsimloo R, Inman GJ, Herrington CS, Gourley C.
Clinicopathological Determinants of Recurrence Risk and Survival in Mucinous Ovarian Carcinoma. ***Cancers (Basel)***. 2021;13.

Leach JDG, Vlahov N, Tsantoulis P, Ridgway RA, Flanagan DJ, Gilroy K, Sphyris N, Vazquez EG, Vincent DF, Faller WJ, Hodder MC, Raven A, Fey S, Najumudeen AK, Strathdee D, Nixon C, Hughes M, Clark W, Shaw R, consortium SC, van Hooff SR, Huels DJ, Medema JP, Barry ST, Frame MC, Unciti-Broceta A, Leedham SJ, Inman GJ, Jackstadt R, Thompson BJ, Campbell AD, Tejpar S, Sansom OJ.
Oncogenic BRAF, unrestrained by TGFbeta-receptor signalling, drives right-sided colonic tumorigenesis. ***Nat Commun***. 2021;12:3464.

Thomson J, Bewicke-Copley F, Anene CA, Gulati A, Nagano A, Purdie K, Inman GJ, Proby CM, Leigh IM, Harwood CA, Wang J.
The Genomic Landscape of Actinic Keratosis. ***J Invest Dermatol***. 2021;141:1664-1674 e1667.

Other Publications

Kumari A, Shonibare Z, Monavarian M, Arend RC, Lee NY, Inman GJ, Mythreye K.
TGFβ signaling networks in ovarian cancer progression and plasticity. ***Clin Exp Metastasis***. 2021; 38: 139-161.

Robert Insall (page 36)

Cell Migration and Chemotaxis

Primary Research Papers

Singh SP, Thomason PA, Insall RH.
Extracellular Signalling Modulates Scar/WAVE Complex Activity through Abi Phosphorylation. ***Cells***. 2021;10: 2021.2010.2009.462405.

Yang Y, Li D, Chao X, Singh SP, Thomason P, Yan Y, Dong M, Li L, Insall RH, Cai H.
Leep1 interacts with PIP3 and the Scar/WAVE complex to regulate cell migration and macropinocytosis. ***J Cell Biol***. 2021;220.

Yelland T, Le AH, Nikolaou S, Insall R, Machesky L, Ismail S.
Structural Basis of CYRI-B Direct Competition with Scar/WAVE Complex for Rac1. ***Structure***. 2021; 29: 226-237 e224.

Other Publications

Insall R.
Actin in 2021. ***Curr Biol.*** 2021; 31:R496-r498.
Machesky LM, Insall RH.
WAVE complex regulation by force. ***Nat Cell Biol***. 2021; 10.1038/s41556-021-00790-y.

Kristina Kirschner (page 38)

Stem Cell Ageing & Cancer

Primary Research Papers

Flanagan DJ, Pentinmikko N, Luopajarvi K, Willis NJ, Gilroy K, Raven AP, McGarry L, Englund JI, Webb AT, Scharaw S, Nasreddin N, Hodder MC, Ridgway RA, Minnee E, Sphyris N, Gilchrist E, Najumudeen AK, Romagnolo B, Perret C, Williams AC, Clevers H, Nummela P, Lahde M, Alitalo K, Hietakangas V, Hedley A, Clark W, Nixon C, Kirschner K, Jones EY, Ristimaki A, Leedham SJ, Fish PV, Vincent JP, Katajisto P, Sansom OJ.
NOTUM from Apc-mutant cells biases clonal competition to initiate cancer. ***Nature***. 2021;594:430-435.

Latif AL, Newcombe A, Li S, Gilroy K, Robertson NA, Lei X, Stewart HJS, Cole J, Terradas MT, Rishi L, McGarry L, McKeeve C, Reid C, Clark W, Campos J, Kirschner K, Davis A, Lopez J, Sakamaki JI, Morton JP, Ryan KM, Tait SWG, Abraham SA, Holyoake T, Higgins B, Huang X, Blyth K, Copland M, Chevassut TJT, Keeshan K, Adams PD.
BRD4-mediated repression of p53 is a target for combination therapy in AML. ***Nat Commun***. 2021;12:241.

Other Publications

Hsieh YC, Kirschner K, Copland M.
Improving outcomes in chronic myeloid leukemia through harnessing the immunological landscape. ***Leukemia***; 2021; 10.1038/s41375-021-01238-w.

Mooney L, Goodyear CS, Chandra T, Kirschner K, Copland M, Petrie MC, Lang NN.
Clonal haematopoiesis of indeterminate potential: intersections between inflammation, vascular disease and heart failure. ***Clin Sci (Lond)***. 2021; 135: 991-1007.

Rattanavirotkul N, Kirschner K, Chandra T.
Induction and transmission of oncogene-induced senescence. ***Cell Mol Life Sci***. 2021;78: 843-852.

John Le Quesne (page 40)

Deep Phenotyping of Solid Tumours

Primary Research Papers

Ghaddar N, Wang S, Woodvine B, Krishnamoorthy J, van Hoef V, Darini C, Kazimierczak U, Ah-Son N, Popper H,

Johnson M, Officer L, Teodosio A, Broggini M, Mann KK, Hatzoglou M, Topisirovic I, Larsson O, Le Quesne J, Koromilas AE.
The integrated stress response is tumorigenic and constitutes a therapeutic liability in KRAS-driven lung cancer. ***Nat Commun***. 2021; 12: 4651.

Grosso S, Marini A, Gyuraszova K, Voorde JV, Sfakianos A, Garland GD, Tenor AR, Mordue R, Chernova T, Morone N, Sereno M, Smith CP, Officer L, Farahmand P, Rooney C, Sumpton D, Das M, Teodosio A, Ficken C, Martin MG, Spriggs RV, Sun XM, Bushell M, Sansom OJ, Murphy D, MacFarlane M, Le Quesne JPC, Willis AE.
The pathogenesis of mesothelioma is driven by a dysregulated translatome. ***Nat Commun***. 2021; 12: 4920.

Knight JRP, Alexandrou C, Skalka GL, Vlahov N, Pennel K, Officer L, Teodosio A, Kanellos G, Gay DM, May-Wilson S, Smith EM, Najumudeen AK, Gilroy K, Ridgway RA, Flanagan DJ, Smith RCL, McDonald L, MacKay C, Cheasty A, McArthur K, Stanway E, Leach JD, Jackstadt R, Waldron JA, Campbell AD, Vlachogiannis G, Valeri N, Haigis KM, Sonenberg N, Proud CG, Jones NP, Swarbrick ME, McKinnon HJ, Faller WJ, Le Quesne J, Edwards J, Willis AE, Bushell M, Sansom OJ.
MNK Inhibition Sensitizes KRAS-Mutant Colorectal Cancer to mTORC1 Inhibition by Reducing eIF4E Phosphorylation and c-MYC Expression. ***Cancer Discov***. 2021; 11: 1228-1247.

Ntala C, Salji M, Salmond J, Officer L, Teodosio AV, Blomme A, McGhee EJ, Powley I, Ahmad I, Kruithof-de Julio M, Thalmann G, Roberts E, Goodyear CS, Jamaspishvili T, Berman DM, Carlin LM, Le Quesne J, Leung HY.
Analysis of Prostate Cancer Tumor Microenvironment Identifies Reduced Stromal CD4 Effector T-cell Infiltration in Tumors with Pelvic Nodal Metastasis. ***Eur Urol Open Sci***. 2021; 29: 19-29.

Phatak V, von Grabowiecki Y, Janus J, Officer L, Behan C, Aschauer L, Pinon L, Mackay H, Zanivan S, Norman JC, Kelly M, Le Quesne J, Muller PAJ.
Mutant p53 promotes RCP-dependent chemoresistance coinciding with increased delivery of P-glycoprotein to the plasma membrane. ***Cell Death Dis***. 2021; 12: 207.

Hing Leung (page 42)
Prostate Cancer Biology

Primary Research Papers
Cangiano M, Grudniewska M, Salji MJ, Nykter M, Jenster G, Urbanucci A, Granchi Z, Janssen B, Hamilton G, Leung HY, Beumer IJ.
Gene Regulation Network Analysis on Human Prostate Orthografts Highlights a Potential Role for the JMJD6 Regulon in Clinical Prostate Cancer. ***Cancers (Basel)***. 2021; 13.

Clement KD, Day L, Rooney H, Neilson M, Birrell F, Salji M, Norman E, Clark R, Patel A, Morrison J, Leung HY.
Developing a coordinate-based strategy to support cognitive targeted prostate biopsies and correlative spatial-histopathological outcome analysis. ***Asian J Androl.*** 2021;23:231-235.

Galbraith LCA, Mui E, Nixon C, Hedley A, Strachan D, MacKay G, Sumpton D, Sansom OJ, Leung HY, Ahmad I.
PPAR-gamma induced AKT3 expression increases levels of mitochondrial biogenesis driving prostate cancer. ***Oncogene.*** 2021;40:2355-2366.

Laskar P, Somani S, Mullin M, Tate RJ, Warzecha M, Bowering D, Keating P, Irving C, Leung HY, Dufes C.
Octadecyl chain-bearing PEGylated poly(propyleneimine)-based dendrimersomes: physicochemical studies, redox-responsiveness, DNA condensation, cytotoxicity and gene delivery to cancer cells. ***Biomater Sci***. 2021; 9: 1431-1448.

Martinez RS, Salji MJ, Rushworth L, Ntala C, Rodriguez Blanco G, Hedley A, Clark W, Peixoto P, Hervouet E, Renaude E, Kung SHY, Galbraith LCA, Nixon C, Lilla S, MacKay GM, Fazli L, Gaughan L, Sumpton D, Gleave ME, Zanivan S, Blomme A, Leung HY.
SLFN5 Regulates LAT1-Mediated mTOR Activation in Castration-Resistant Prostate Cancer. ***Cancer Res***. 2021; 81: 3664-3678.

McAteer MA, O'Connor JPB, Koh DM, Leung HY, Doran SJ, Jauregui-Osoro M, Muirhead N, Brew-Graves C, Plummer ER, Sala E, Ng T, Aboagye EO, Higgins GS, Punwani S.
Introduction to the National Cancer Imaging Translational Accelerator (NCITA): a UK-wide infrastructure for multicentre clinical translation of cancer imaging biomarkers. ***Br J Cancer.*** 2021; 125: 1462-1465.

Nacke M, Sandilands E, Nikolatou K, Roman-Fernandez A, Mason S, Patel R, Lilla S, Yelland T, Galbraith LCA, Freckmann EC, McGarry L, Morton JP, Shanks E, Leung HY, Markert E, Ismail S, Zanivan S, Blyth K, Bryant DM.
An ARF GTPase module promoting invasion and metastasis through regulating phosphoinositide metabolism. ***Nat Commun***. 2021; 12: 1623.

Ntala C, Salji M, Salmond J, Officer L, Teodosio AV, Blomme A, McGhee EJ, Powley I, Ahmad I, Kruithof-de Julio M, Thalmann G, Roberts E, Goodyear CS, Jamaspishvili T, Berman DM, Carlin LM, Le Quesne J, Leung HY.
Analysis of Prostate Cancer Tumor Microenvironment Identifies Reduced Stromal CD4 Effector T-cell Infiltration in Tumors with Pelvic Nodal Metastasis. ***Eur Urol Open Sci***. 2021; 29: 19-29.

Other Publications
Campbell KJ, Leung HY.
Evasion of cell death: A contributory factor in prostate cancer development and treatment resistance. ***Cancer Lett***. 2021; 520: 213-221.

Hartley A, Leung HY, Ahmad I.
Targeting the BAF complex in advanced prostate cancer. ***Expert Opin Drug Discov.*** 2021; 16: 173-181.

David Lewis (page 44)
Molecular Imaging

Primary Research Papers
Najumudeen AK, Ceteci F, Fey SK, Hamm G, Steven RT, Hall H, Nikula CJ, Dexter A, Murta T, Race AM, Sumpton D, Vlahov N, Gay DM, Knight JRP, Jackstadt R, Leach JDG, Ridgway RA, Johnson ER, Nixon C, Hedley A, Gilroy K, Clark W, Malla SB, Dunne PD, Rodriguez-Blanco G, Critchlow SE, Mrowinska A, Malviya G, Solovyev D, Brown G, Lewis DY, Mackay GM, Strathdee D, Tardito S, Gottlieb E, Consortium CRGC, Takats Z, Barry ST, Goodwin RJA, Bunch J, Bushell M, Campbell AD, Sansom OJ.
The amino acid transporter SLC7A5 is required for efficient growth of KRAS-mutant colorectal cancer. ***Nat Genet.*** 2021; 53: 16-26.

Woitek R, McLean MA, Ursprung S, Rueda OM, Manzano Garcia R, Locke MJ, Beer L, Baxter G, Rundo L, Provenzano E, Kaggie J, Patterson A, Frary A, Field-Rayner J, Papalouka V, Kane J, Benjamin AJV, Gill AB, Priest AN, Lewis DY, Russell R, Grimmer A, White B, Latimer-Bowman B, Patterson I, Schiller A, Carmo B, Slough R, Lanz T, Wason J, Schulte RF, Chin SF, Graves MJ, Gilbert FJ, Abraham JE, Caldas C, Brindle KM, Sala E, Gallagher FA.
Hyperpolarized Carbon-13 MRI for Early Response Assessment of Neoadjuvant Chemotherapy in Breast Cancer Patients. ***Cancer Res***. 2021; 81: 6004-6017.

Laura Machesky (page 46)
Migration, Invasion and Metastasis

Primary Research Papers
Callenberg C, Lyons A, Brok DD, Fatima A, Turpin A, Zickus V, Machesky L, Whitelaw J, Faccio D, Hullin MB.
Super-resolution time-resolved imaging using computational sensor fusion. ***Sci Rep***. 2021; 11: 1689.

Floerchinger A, Murphy KJ, Latham SL, Warren SC, McCulloch AT, Lee YK, Stoehr J, Melenec P, Guaman CS, Metcalf XL, Lee V, Zaratzian A, Da Silva A, Tayao M, Rolo S, Phimmachanh M, Sultani G, McDonald L, Mason SM, Ferrari N, Ooms LM, Johnsson AE, Spence HJ, Olson MF, Machesky LM, Sansom OJ, Morton JP, Mitchell CA, Samuel MS, Croucher DR, Welch HCE, Blyth K, Caldon CE, Herrmann D, Anderson KI, Timpson P, Nobis M.
Optimizing metastatic-cascade-dependent Rac1 targeting in breast cancer: Guidance using optical window intravital FRET imaging. ***Cell Rep***. 2021; 36: 109689.

Le AH, Yelland T, Paul NR, Fort L, Nikolaou S, Ismail S, Machesky LM.
CYRI-A limits invasive migration through macropinosome formation and integrin uptake regulation. ***J Cell Biol***. 2021;220.

Pickering KA, Gilroy K, Cassidy JW, Fey SK, Najumudeen AK, Zeiger LB, Vincent DF, Gay DM, Johansson J, Fordham RP, Miller B, Clark W, Hedley A, Unal EB, Kiel C, McGhee E, Machesky LM, Nixon C, Johnsson AE, Bain M, Strathdee D, van Hoof SR, Medema JP, Anderson KI, Brachmann SM, Stucke VM, Malliri A, Drysdale M, Turner M, Serrano L, Myant K, Campbell AD, Sansom OJ.

A RAC-GEF network critical for early intestinal tumourigenesis. ***Nat Commun.*** 2021; 12: 56.

Swaminathan K, Campbell A, Papalazarou V, Jaber-Hijazi F, Nixon C, McGhee E, Strathdee D, Sansom OJ, Machesky LM.
The RAC1 Target NCKAP1 Plays a Crucial Role in the Progression of Braf;Pten-Driven Melanoma in Mice. ***J Invest Dermatol***. 2021; 141: 628-637 e615.

Yelland T, Le AH, Nikolaou S, Insall R, Machesky L, Ismail S.
Structural Basis of CYRI-B Direct Competition with Scar/WAVE Complex for Rac1. ***Structure***. 2021; 29: 226-237 e224.

Other Publications

Drew J, Machesky LM.
The liver metastatic niche: modelling the extracellular matrix in metastasis. ***Dis Model Mech***. 2021; 14:

Machesky LM, Insall RH.
WAVE complex regulation by force. ***Nat Cell Biol***. 2021; 10.1038/s41556-021-00790-y.

Olson MF, Machesky LM.
MICAL2 fine-tunes Arp2/3 for actin branching. ***J Cell Biol***. 2021; 220

Papalazarou V, Machesky LM.
The cell pushes back: The Arp2/3 complex is a key orchestrator of cellular responses to environmental forces. ***Curr Opin Cell Biol***. 2021; 68: 37-44.

Crispin Miller (page 48)

Computational Biology

Primary Research Papers

Tsim S, Alexander L, Kelly C, Shaw A, Hinsley S, Clark S, Evison M, Holme J, Cameron EJ, Sharma D, Wright A, Grundy S, Grieve D, Ionescu A, Breen DP, Paramasivam E, Psallidas I, Mukherjee D, Chetty M, Cox G, Hart-Thomas A, Naseer R, Edwards J, Daneshvar C, Panchal R, Munavvar M, Ostroff R, Alexander L, Hall H, Neilson M, Miller C, McCormick C, Thomson F, Chalmers AJ, Maskell NA, Blyth KG.
Serum Proteomics and Plasma Fibulin-3 in Differentiation of Mesothelioma From Asbestos-Exposed Controls and Patients With Other Pleural Diseases. ***J Thorac Oncol.*** 2021; 16: 1705-1717.

Jennifer Morton (page 50)

Precision-Panc Preclinical Lab

Primary Research Papers

Below CR, Kelly J, Brown A, Humphries JD, Hutton C, Xu J, Lee BY, Cintas C, Zhang X, Hernandez-Gordillo V, Stockdale L, Goldsworthy MA, Geraghty J, Foster L, O'Reilly DA, Schedding B, Askari J, Burns J, Hodson N, Smith DL, Lally C, Ashton G, Knight D, Mironov A, Banyard A, Eble JA, Morton JP, Humphries MJ, Griffith LG, Jorgensen C.
A microenvironment-inspired synthetic three-dimensional model for pancreatic ductal adenocarcinoma organoids. ***Nat Mater.*** 2022; 21: 110-119.

Dreyer SB, Upstill-Goddard R, Paulus-Hock V, Paris C, Lampraki EM, Dray E, Serrels B, Caligiuri G, Rebus S, Plenker D, Galluzzo Z, Brunton H, Cunningham R, Tesson M, Nourse C, Bailey UM, Jones M, Moran-Jones K, Wright DW, Duthie F, Oien K, Evers L, McKay CJ, McGregor GA, Gulati A, Brough R, Bajrami I, Pettitt S, Dziubinski ML, Candido J, Balkwill F, Barry ST, Grutzmann R, Rahib L, Glasgow Precision Oncology L, Australian Pancreatic Cancer Genome I, Johns A, Pajic M, Froeling FEM, Beer P, Musgrove EA, Petersen GM, Ashworth A, Frame MC, Crawford HC, Simeone DM, Lord C, Mukhopadhyay D, Pilarsky C, Tuveson DA, Cooke SL, Jamieson NB, Morton JP, Sansom OJ, Bailey PJ, Biankin AV, Chang DK.
Targeting DNA Damage Response and Replication Stress in Pancreatic Cancer. ***Gastroenterology***. 2021; 160: 362-377 e313.

Falcomata C, Barthel S, Ulrich A, Diersch S, Veltkamp C, Rad L, Boniolo F, Solar M, Steiger K, Seidler B, Zukowska M, Madej J, Wang M, Ollinger R, Maresch R, Barenboim M, Eser S, Tschurtschenthaler M, Mehrabi A, Roessler S, Goeppert B, Kind A, Schnieke A, Robles MS, Bradley A, Schmid RM, Schmidt-Supprian M, Reichert M, Weichert W, Sansom OJ, Morton JP, Rad R, Schneider G, Saur D.
Genetic Screens Identify a Context-Specific PI3K/p27(Kip1) Node Driving Extrahepatic Biliary Cancer. ***Cancer Discov***. 2021; 10.1158/2159-8290.CD-21-0209.

Floerchinger A, Murphy KJ, Latham SL, Warren SC, McCulloch AT, Lee YK, Stoehr J, Melenec P, Guaman CS, Metcalf XL, Lee V, Zaratzian A, Da Silva A, Tayao M, Rolo S, Phimmachanh M, Sultani G, McDonald L, Mason SM, Ferrari N, Ooms LM, Johnsson AE, Spence HJ, Olson MF, Machesky LM, Sansom OJ, Morton JP, Mitchell CA, Samuel MS, Croucher DR, Welch HCE, Blyth K, Caldon CE, Herrmann D, Anderson KI, Timpson P, Nobis M.
Optimizing metastatic-cascade-dependent Rac1 targeting in breast cancer: Guidance using optical window intravital FRET imaging. ***Cell Rep.*** 2021; 36: 109689.

Hill W, Zaragkoulias A, Salvador-Barbero B, Parfitt GJ, Alatsatianos M, Padilha A, Porazinski S, Woolley TE, Morton JP, Sansom OJ, Hogan C.
EPHA2-dependent outcompetition of KRASG12D mutant cells by wild-type neighbors in the adult pancreas. ***Curr Biol***. 2021; 31: 2550-2560 e2555.

Hutton C, Heider F, Blanco-Gomez A, Banyard A, Kononov A, Zhang X, Karim S, Paulus-Hock V, Watt D, Steele N, Kemp S, Hogg EKJ, Kelly J, Jackstadt RF, Lopes F, Menotti M, Chisholm L, Lamarca A, Valle J, Sansom OJ, Springer C, Malliri A, Marais R, Pasca di Magliano M, Zelenay S, Morton JP, Jorgensen C.
Single-cell analysis defines a pancreatic fibroblast lineage that supports anti-tumor immunity. ***Cancer Cell***. 2021; 39: 1227-1244 e1220.

Latif AL, Newcombe A, Li S, Gilroy K, Robertson NA, Lei X, Stewart HJS, Cole J, Terradas MT, Rishi L, McGarry L, McKeeve C, Reid C, Clark W, Campos J, Kirschner K, Davis A, Lopez J, Sakamaki JI, Morton JP, Ryan KM, Tait SWG, Abraham SA, Holyoake T, Higgins B, Huang X, Blyth K, Copland M, Chevassut TJT, Keeshan K, Adams PD.
BRD4-mediated repression of p53 is a target for combination therapy in AML. ***Nat Commun***. 2021; 12: 241.

Murphy KJ, Reed DA, Vennin C, Conway JRW, Nobis M, Yin JX, Chambers CR, Pereira BA, Lee V, Filipe EC, Trpceski M, Ritchie S, Lucas MC, Warren SC, Skhinas JN, Magenau A, Metcalf XL, Stoehr J, Major G, Parkin A, Bidanel R, Lyons RJ, Zaratzian A, Tayao M, Da Silva A, Abdulkhalek L, Australian Pancreatic Genome I, Australian Pancreatic Cancer Matrix A, Gill AJ, Johns AL, Biankin AV, Samra J, Grimmond SM, Chou A, Goetz JG, Samuel MS, Lyons JG, Burgess A, Caldon CE, Horvath LG, Daly RJ, Gadegaard N, Wang Y, Sansom OJ, Morton JP, Cox TR, Pajic M, Herrmann D, Timpson P.
Intravital imaging technology guides FAK-mediated priming in pancreatic cancer precision medicine according to Merlin status. ***Sci Adv***. 2021;7:eabh0363.

Nacke M, Sandilands E, Nikolatou K, Roman-Fernandez A, Mason S, Patel R, Lilla S, Yelland T, Galbraith LCA, Freckmann EC, McGarry L, Morton JP, Shanks E, Leung HY, Markert E, Ismail S, Zanivan S, Blyth K, Bryant DM.
An ARF GTPase module promoting invasion and metastasis through regulating phosphoinositide metabolism. ***Nat Commun.*** 2021; 12: 1623.

Nielsen SR, Strobech JE, Horton ER, Jackstadt R, Laitala A, Bravo MC, Maltese G, Jensen ARD, Reuten R, Rafaeva M, Karim SA, Hwang CI, Arnes L, Tuveson DA, Sansom OJ, Morton JP, Erler JT.
Suppression of tumor-associated neutrophils by lorlatinib attenuates pancreatic cancer growth and improves treatment with immune checkpoint blockade. ***Nat Commun.*** 2021; 12: 3414.

Race AM, Sutton D, Hamm G, Maglennon G, Morton JP, Strittmatter N, Campbell A, Sansom OJ, Wang Y, Barry ST, Takats Z, Goodwin RJA, Bunch J.
Deep Learning-Based Annotation Transfer between Molecular Imaging Modalities: An Automated Workflow for Multimodal Data Integration. ***Anal Chem.*** 2021; 93: 3061-3071.

Sharbeen G, McCarroll JA, Akerman A, Kopecky C, Youkhana J, Kokkinos J, Holst J, Boyer C, Erkan M, Goldstein D, Timpson P, Cox TR, Pereira BA, Chitty JL, Fey SK, Najumudeen AK, Campbell AD, Sansom OJ, Ignacio RMC, Naim S, Liu J, Russia N, Lee J, Chou A, Johns A, Gill AJ, Gonzales-Aloy E, Gebski V, Guan YF, Pajic M, Turner N, Apte MV, Davis TP, Morton JP, Haghighi KS, Kasparian J, McLean BJ, Setargew YF, Phillips PA, Australian Pancreatic Cancer Genome I.
Cancer-Associated Fibroblasts in Pancreatic Ductal Adenocarcinoma Determine Response to SLC7A11 Inhibition. ***Cancer Res.*** 2021; 81: 3461-3479.

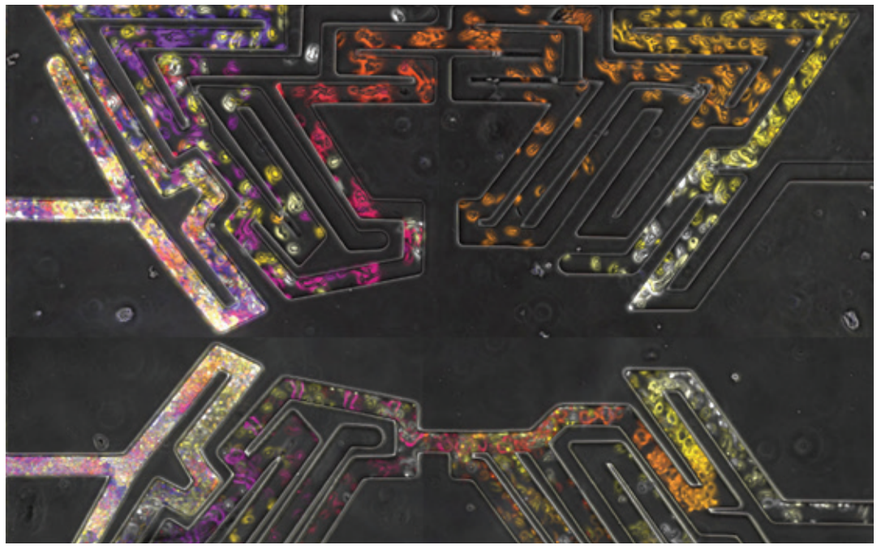
Other Publications

Heinrich MA, Mostafa A, Morton JP, Hawinkels L, Prakash J.
Translating complexity and heterogeneity of pancreatic tumor: 3D in vitro to in vivo models. ***Adv Drug Deliv Rev***. 2021; 174: 265-293.

Watt DM, Morton JP.
Heterogeneity in Pancreatic Cancer Fibroblasts-TGFβ as a Master Regulator? ***Cancers (Basel)***. 2021:13

Cells in maze

Image by Cell Migration and Chemotaxis lab



Daniel Murphy (page 52)

Myc-Induced Vulnerabilities/Thoracic Cancer Research

Primary Research Papers

Grosso S, Marini A, Gyuraszova K, Voorde JV, Sfakianos A, Garland GD, Tenor AR, Mordue R, Chernova T, Morone N, Sereno M, Smith CP, Officer L, Farahmand P, Rooney C, Sumpton D, Das M, Teodosio A, Ficken C, Martin MG, Spriggs RV, Sun XM, Bushell M, Sansom OJ, Murphy D, MacFarlane M, Le Quesne JPC, Willis AE.
The pathogenesis of mesothelioma is driven by a dysregulated translatome. ***Nat Commun***. 2021; 12: 4920.

Yun S, Vincelette ND, Yu X, Watson GW, Fernandez MR, Yang C, Hitosugi T, Cheng CH, Freischel AR, Zhang L, Li W, Hou H, Schaub FX, Vedder AR, Cen L, McGraw KL, Moon J, Murphy DJ, Ballabio A, Kaufmann SH, Berglund AE, Cleveland JL.
TFEB links MYC signaling to epigenetic control of myeloid differentiation and acute myeloid leukemia. ***Blood Cancer Discov***. 2021; 2: 162-185.

Jim Norman (page 54)

Integrin Cell Biology

Primary Research Papers

Marco S, Neilson M, Moore M, Perez-Garcia A, Hall H, Mitchell L, Lilla S, Blanco GR, Hedley A, Zanivan S, Norman JC.
Nuclear-capture of endosomes depletes nuclear G-actin to promote SRF/MRTF activation and cancer cell invasion. ***Nat Commun***. 2021; 12: 6829.

Naszai M, Bellec K, Yu Y, Roman-Fernandez A, Sandilands E, Johansson J, Campbell AD, Norman JC, Sansom OJ, Bryant DM, Cordero JB.
RAL GTPases mediate EGFR-driven intestinal stem cell proliferation and tumourigenesis. ***Elife***. 2021; 10.

Phatak V, von Grabowiecki Y, Janus J, Officer L, Behan C, Aschauer L, Pinon L, Mackay H, Zanivan S, Norman JC, Kelly M, Le Quesne J, Muller PAJ.
Mutant p53 promotes RCP-dependent chemoresistance coinciding with increased delivery of P-glycoprotein to the plasma membrane. ***Cell Death Dis***. 2021; 12: 207.

Rabas N, Palmer S, Mitchell L, Ismail S, Gohlke A, Riley JS, Tait SWG, Gammage P, Soares LL, Macpherson IR, Norman JC.
PINK1 drives production of mtDNA-containing extracellular vesicles to promote invasiveness. ***J Cell Biol***. 2021; 220.

Other Publications

Jackstadt R, Norman JC.
Stromal WNTer Keeps the Tumor Cold and Drives Metastasis. ***Dev Cell***. 2021; 56: 3-4.

Ed Roberts (page 56)

Immune Priming and Tumour Microenvironment

Primary Research Papers

Ntala C, Salji M, Salmond J, Officer L, Teodosio AV, Blomme A, McGhee EJ, Powley I, Ahmad I, Kruithof-de Julio M, Thalmann G, Roberts E, Goodyear CS, Jamaspishvili T, Berman DM, Carlin LM, Le Quesne J, Leung HY.
Analysis of Prostate Cancer Tumor Microenvironment Identifies Reduced Stromal CD4 Effector T-cell Infiltration in Tumors with Pelvic Nodal Metastasis. ***Eur Urol Open Sci***. 2021; 29: 19-29.

Kevin Ryan (page 58)

Tumour Cell Death

Primary Research Papers

Barthet VJA, Brucoli M, Ladds M, Nossing C, Kiourtis C, Baudot AD, O'Prey J, Zunino B, Muller M, May S, Nixon C, Long JS, Bird TG, Ryan KM.
Autophagy suppresses the formation of hepatocyte-derived cancer-initiating ductular progenitor cells in the liver. ***Sci Adv.*** 2021;7.

Latif AL, Newcombe A, Li S, Gilroy K, Robertson NA, Lei X, Stewart HJS, Cole J, Terradas MT, Rishi L, McGarry L, McKeeve C, Reid C, Clark W, Campos J, Kirschner K, Davis A, Lopez J, Sakamaki JI, Morton JP, Ryan KM, Tait SWG, Abraham SA, Holyoake T, Higgins B, Huang X, Blyth K, Copland M, Chevassut TJT, Keeshan K, Adams PD.
BRD4-mediated repression of p53 is a target for combination therapy in AML. ***Nat Commun***. 2021; 12: 241.

Other Publication

Klionsky DJ *et al.*
Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition) (1). ***Autophagy***. 2021; 17: 1-382.

Klionsky DJ, Petroni G, Amaravadi RK, Baehrecke EH, Ballabio A, Boya P, Bravo-San Pedro JM, Cadwell K, Cecconi F, Choi AMK, Choi ME, Chu CT, Codogno P, Colombo MI, Cuervo AM, Deretic V, Dikic I, Elazar Z, Eskelinen EL, Fimia GM, Gewirtz DA, Green DR, Hansen M, Jaattela M, Johansen T, Juhasz G, Karantza V, Kraft C, Kroemer G, Ktistakis NT, Kumar S, Lopez-Otin C, Macleod KF, Madeo F, Martinez J, Melendez A, Mizushima N, Munz C, Penninger JM, Perera RM, Piacentini M, Reggiori F, Rubinsztein DC, Ryan KM, Sadoshima J, Santambrogio L, Scorrano L, Simon HU, Simon AK, Simonsen A, Stolz A, Tavernarakis N, Tooze SA, Yoshimori T, Yuan J, Yue Z, Zhong Q, Galluzzi L, Pietrocola F.
Autophagy in major human diseases. ***EMBO J***. 2021; 40: e108863.

McEwan DG, Ryan KM.
ATG2 and VPS13 proteins: molecular highways transporting lipids to drive membrane expansion and organelle communication. ***Febs j***. 2021; 10.1111/febs.16280.

Rosenfeldt MT, O'Prey J, Lindsay CR, Nixon C, Roth S, Sansom OJ, Ryan KM.
Loss of autophagy affects melanoma development in a manner dependent on PTEN status. ***Cell Death Differ***. 2021; 28: 1437-1439.

Owen Sansom (page 60)

Colorectal Cancer and Wnt Signalling

Primary Research Papers

Alexander PG, Roseweir AK, Pennel KAF, van Wyk HC, Powell A, McMillan DC, Horgan PG, Kelly C, Hay J, Sansom O, Harkin A, Roxburgh CSD, Graham J, Church DN, Tomlinson I, Saunders M, Iveson TJ, Edwards J, Park JH.
The Glasgow Microenvironment Score associates with prognosis and adjuvant chemotherapy response in colorectal cancer. ***Br J Cancer***. 2021; 124: 786-796.

Dreyer SB, Upstill-Goddard R, Paulus-Hock V, Paris C, Lampraki EM, Dray E, Serrels B, Caligiuri G, Rebus S, Plenker D, Galluzzo Z, Brunton H, Cunningham R, Tesson M, Nourse C, Bailey UM, Jones M, Moran-Jones K, Wright DW, Duthie F, Oien K, Evers L, McKay CJ, McGregor GA, Gulati A, Brough R, Bajrami I, Pettitt S, Dziubinski ML, Candido J, Balkwill F, Barry ST, Grutzmann R, Rahib L, Glasgow Precision Oncology L, Australian Pancreatic Cancer Genome I, Johns A, Pajic M, Froeling FEM, Beer P, Musgrove EA, Petersen GM, Ashworth A, Frame MC, Crawford HC, Simeone DM, Lord C, Mukhopadhyay D, Pilarsky C, Tuveson DA, Cooke SL, Jamieson NB, Morton JP, Sansom OJ, Bailey PJ, Biankin AV, Chang DK.
Targeting DNA Damage Response and Replication Stress in Pancreatic Cancer. ***Gastroenterology***. 2021; 160: 362-377 e313.

Dunbar K, Valanciute A, Lima ACS, Vinuela PF, Jamieson T, Rajasekaran V, Blackmur J, Ochocka-Fox AM, Guazzelli A, Cammareri P, Arends MJ, Sansom OJ, Myant KB, Farrington SM, Dunlop MG, Din FVN.

Aspirin Rescues Wnt-Driven Stem-like Phenotype in Human Intestinal Organoids and Increases the Wnt Antagonist Dickkopf-1. ***Cell Mol Gastroenterol Hepatol.*** 2021; 11: 465-489.

Falcomata C, Barthel S, Ulrich A, Diersch S, Veltkamp C, Rad L, Boniolo F, Solar M, Steiger K, Seidler B, Zukowska M, Madej J, Wang M, Ollinger R, Maresch R, Barenboim M, Eser S, Tschurtschenthaler M, Mehrabi A, Roessler S, Goeppert B, Kind A, Schnieke A, Robles MS, Bradley A, Schmid RM, Schmidt-Supprian M, Reichert M, Weichert W, Sansom OJ, Morton JP, Rad R, Schneider G, Saur D.
Genetic Screens Identify a Context-Specific PI3K/p27(Kip1) Node Driving Extrahepatic Biliary Cancer. ***Cancer Discov.*** 2021; 10.1158/2159-8290.CD-21-0209.

Flanagan DJ, Pentinmikko N, Luopajarvi K, Willis NJ, Gilroy K, Raven AP, McGarry L, Englund JI, Webb AT, Scharaw S, Nasreddin N, Hodder MC, Ridgway RA, Minnee E, Sphyris N, Gilchrist E, Najumudeen AK, Romagnolo B, Perret C, Williams AC, Clevers H, Nummela P, Lahde M, Alitalo K, Hietakangas V, Hedley A, Clark W, Nixon C, Kirschner K, Jones EY, Ristimaki A, Leedham SJ, Fish PV, Vincent JP, Katajisto P, Sansom OJ.
NOTUM from Apc-mutant cells biases clonal competition to initiate cancer. ***Nature.*** 2021; 594: 430-435.

Floerchinger A, Murphy KJ, Latham SL, Warren SC, McCulloch AT, Lee YK, Stoehr J, Melenec P, Guaman CS, Metcalf XL, Lee V, Zaratzian A, Da Silva A, Tayao M, Rolo S, Phimmachanh M, Sultani G, McDonald L, Mason SM, Ferrari N, Ooms LM, Johnsson AE, Spence HJ, Olson MF, Machesky LM, Sansom OJ, Morton JP, Mitchell CA, Samuel MS, Croucher DR, Welch HCE, Blyth K, Caldon CE, Herrmann D, Anderson KI, Timpson P, Nobis M.
Optimizing metastatic-cascade-dependent Rac1 targeting in breast cancer: Guidance using optical window intravital FRET imaging. ***Cell Rep.*** 2021;36:109689.

Galbraith LCA, Mui E, Nixon C, Hedley A, Strachan D, MacKay G, Sumpton D, Sansom OJ, Leung HY, Ahmad I.
PPAR-gamma induced AKT3 expression increases levels of mitochondrial biogenesis driving prostate cancer. ***Oncogene.*** 2021; 40: 2355-2366.

Grosso S, Marini A, Gyuraszova K, Voorde JV, Sfakianos A, Garland GD, Tenor AR, Mordue R,

Chernova T, Morone N, Sereno M, Smith CP, Officer L, Farahmand P, Rooney C, Sumpton D, Das M, Teodosio A, Ficken C, Martin MG, Spriggs RV, Sun XM, Bushell M, Sansom OJ, Murphy D, MacFarlane M, Le Quesne JPC, Willis AE.
The pathogenesis of mesothelioma is driven by a dysregulated translatome. ***Nat Commun.*** 2021; 12: 4920.

Gudino V, Pohl SO, Billard CV, Cammareri P, Bolado A, Aitken S, Stevenson D, Hall AE, Agostino M, Cassidy J, Nixon C, von Kriegsheim A, Freile P, Popplewell L, Dickson G, Murphy L, Wheeler A, Dunlop M, Din F, Strathdee D, Sansom OJ, Myant KB.
RAC1B modulates intestinal tumourigenesis via modulation of WNT and EGFR signalling pathways. ***Nat Commun.*** 2021; 12: 2335.

Guillermin O, Angelis N, Sidor CM, Ridgway R, Baulies A, Kucharska A, Antas P, Rose MR, Cordero J, Sansom O, Li VSW, Thompson BJ.
Wnt and Src signals converge on YAP-TEAD to drive intestinal regeneration. ***EMBO J.*** 2021; 40: e105770.

Heino S, Fang S, Lahde M, Hogstrom J, Nassiri S, Campbell A, Flanagan D, Raven A, Hodder M, Nasreddin N, Xue HH, Delorenzi M, Leedham S, Petrova TV, Sansom O, Alitalo K.
Lef1 restricts ectopic crypt formation and tumor cell growth in intestinal adenomas. ***Sci Adv.*** 2021; 7: eabj0512.

Hill W, Zaragkoulias A, Salvador-Barbero B, Parfitt GJ, Alatsatianos M, Padilha A, Porazinski S, Woolley TE, Morton JP, Sansom OJ, Hogan C.
EPHA2-dependent outcompetition of KRASG12D mutant cells by wild-type neighbors in the adult pancreas. ***Curr Biol.*** 2021; 31: 2550-2560 e2555.

Hutton C, Heider F, Blanco-Gomez A, Banyard A, Kononov A, Zhang X, Karim S, Paulus-Hock V, Watt D, Steele N, Kemp S, Hogg EKJ, Kelly J, Jackstadt RF, Lopes F, Menotti M, Chisholm L, Lamarca A, Valle J, Sansom OJ, Springer C, Malliri A, Marais R, Pasca di Magliano M, Zelenay S, Morton JP, Jorgensen C.
Single-cell analysis defines a pancreatic fibroblast lineage that supports anti-tumor immunity. ***Cancer Cell.*** 2021; 39: 1227-1244 e1220.

Knight JRP, Alexandrou C, Skalka GL, Vlahov N, Pennel K, Officer L, Teodosio A, Kanellos G,

Gay DM, May-Wilson S, Smith EM, Najumudeen AK, Gilroy K, Ridgway RA, Flanagan DJ, Smith RCL, McDonald L, MacKay C, Cheasty A, McArthur K, Stanway E, Leach JD, Jackstadt R, Waldron JA, Campbell AD, Vlachogiannis G, Valeri N, Haigis KM, Sonenberg N, Proud CG, Jones NP, Swarbrick ME, McKinnon HJ, Faller WJ, Le Quesne J, Edwards J, Willis AE, Bushell M, Sansom OJ.
MNK Inhibition Sensitizes KRAS-Mutant Colorectal Cancer to mTORC1 Inhibition by Reducing eIF4E Phosphorylation and c-MYC Expression. ***Cancer Discov.*** 2021; 11: 1228-1247.

Krotenberg Garcia A, Fumagalli A, Le HQ, Jackstadt R, Lannagan TRM, Sansom OJ, van Rheenen J, Suijkerbuijk SJE.
Active elimination of intestinal cells drives oncogenic growth in organoids. ***Cell Rep.*** 2021; 36: 109307.

Lahde M, Heino S, Hogstrom J, Kaijalainen S, Anisimov A, Flanagan D, Kallio P, Leppanen VM, Ristimaki A, Ritvos O, Wu K, Tammela T, Hodder M, Sansom OJ, Alitalo K.
Expression of R-Spondin 1 in Apc(Min/+) Mice Suppresses Growth of Intestinal Adenomas by Altering Wnt and Transforming Growth Factor Beta Signaling. ***Gastroenterology.*** 2021; 160: 245-259.

Lampis A, Hahne JC, Gasparini P, Cascione L, Hedayat S, Vlachogiannis G, Murgia C, Fontana E, Edwards J, Horgan PG, Terracciano L, Sansom OJ, Martins CD, Kramer-Marek G, Croce CM, Braconi C, Fassan M, Valeri N.
MIR21-induced loss of junctional adhesion molecule A promotes activation of oncogenic pathways, progression and metastasis in colorectal cancer. ***Cell Death Differ.*** 2021; 28: 2970-2982.

Leach JDG, Vlahov N, Tsantoulis P, Ridgway RA, Flanagan DJ, Gilroy K, Sphyris N, Vazquez EG, Vincent DF, Faller WJ, Hodder MC, Raven A, Fey S, Najumudeen AK, Strathdee D, Nixon C, Hughes M, Clark W, Shaw R, consortium SC, van Hooff SR, Huels DJ, Medema JP, Barry ST, Frame MC, Unciti-Broceta A, Leedham SJ, Inman GJ, Jackstadt R, Thompson BJ, Campbell AD, Tejpar S, Sansom OJ.
Oncogenic BRAF, unrestrained by TGFbeta-receptor signalling, drives right-sided colonic tumorigenesis. ***Nat Commun.*** 2021; 12: 3464.

Minnis-Lyons SE, Ferreira-Gonzalez S, Aleksieva N, Man TY, Gadd VL, Williams MJ, Guest RV, Lu WY, Dwyer BJ, Jamieson T, Nixon C, Van Hul N, Lemaigre FP, McCafferty J, Leclercq IA, Sansom OJ, Boulter L, Forbes SJ.
Notch-IGF1 signaling during liver regeneration drives biliary epithelial cell expansion and inhibits hepatocyte differentiation. ***Sci Signal.*** 2021; 14.

Murphy KJ, Reed DA, Vennin C, Conway JRW, Nobis M, Yin JX, Chambers CR, Pereira BA, Lee V, Filipe EC, Trpceski M, Ritchie S, Lucas MC, Warren SC, Skhinas JN, Magenau A, Metcalf XL, Stoehr J, Major G, Parkin A, Bidanel R, Lyons RJ, Zaratzian A, Tayao M, Da Silva A, Abdulkhalek L, Australian Pancreatic Genome I, Australian Pancreatic Cancer Matrix A, Gill AJ, Johns AL, Biankin AV, Samra J, Grimmond SM, Chou A, Goetz JG, Samuel MS, Lyons JG, Burgess A, Caldon CE, Horvath LG, Daly RJ, Gadegaard N, Wang Y, Sansom OJ, Morton JP, Cox TR, Pajic M, Herrmann D, Timpson P.
Intravital imaging technology guides FAK-mediated priming in pancreatic cancer precision medicine according to Merlin status. ***Sci Adv.*** 2021; 7: eabh0363.

Murta T, Steven RT, Nikula CJ, Thomas SA, Zeiger LB, Dexter A, Elia EA, Yan B, Campbell AD, Goodwin RJA, Takats Z, Sansom OJ, Bunch J.
Implications of Peak Selection in the Interpretation of Unsupervised Mass Spectrometry Imaging Data Analyses. ***Anal Chem.*** 2021; 93: 2309-2316.

Najumudeen AK, Ceteci F, Fey SK, Hamm G, Steven RT, Hall H, Nikula CJ, Dexter A, Murta T, Race AM, Sumpton D, Vlahov N, Gay DM, Knight JRP, Jackstadt R, Leach JDG, Ridgway RA, Johnson ER, Nixon C, Hedley A, Gilroy K, Clark W, Malla SB, Dunne PD, Rodriguez-Blanco G, Critchlow SE, Mrowinska A, Malviya G, Solovyev D, Brown G, Lewis DY, Mackay GM, Strathdee D, Tardito S, Gottlieb E, Consortium CRGC, Takats Z, Barry ST, Goodwin RJA, Bunch J, Bushell M, Campbell AD, Sansom OJ.
The amino acid transporter SLC7A5 is required for efficient growth of KRAS-mutant colorectal cancer. ***Nat Genet.*** 2021; 53: 16-26.

Naszai M, Bellec K, Yu Y, Roman-Fernandez A, Sandilands E, Johansson J, Campbell AD, Norman JC, Sansom OJ, Bryant DM, Cordero JB.
RAL GTPases mediate EGFR-driven intestinal stem cell proliferation and tumourigenesis. ***Elife.*** 2021;10.

Nielsen SR, Strobech JE, Horton ER, Jackstadt R, Laitala A, Bravo MC, Maltese G, Jensen ARD, Reuten R, Rafaeva M, Karim SA, Hwang CI, Arnes L, Tuveson DA, Sansom OJ, Morton JP, Erler JT.

Suppression of tumor-associated neutrophils by lorlatinib attenuates pancreatic cancer growth and improves treatment with immune checkpoint blockade. ***Nat Commun.*** 2021; 12: 3414.

Pickering KA, Gilroy K, Cassidy JW, Fey SK, Najumudeen AK, Zeiger LB, Vincent DF, Gay DM, Johansson J, Fordham RP, Miller B, Clark W, Hedley A, Unal EB, Kiel C, McGhee E, Machesky LM, Nixon C, Johnsson AE, Bain M, Strathdee D, van Hoof SR, Medema JP, Anderson KI, Brachmann SM, Stucke VM, Malliri A, Drysdale M, Turner M, Serrano L, Myant K, Campbell AD, Sansom OJ.
A RAC-GEF network critical for early intestinal tumourigenesis. ***Nat Commun.*** 2021; 12: 56.

Race AM, Sutton D, Hamm G, Maglennon G, Morton JP, Strittmatter N, Campbell A, Sansom OJ, Wang Y, Barry ST, Takats Z, Goodwin RJA, Bunch J.
Deep Learning-Based Annotation Transfer between Molecular Imaging Modalities: An Automated Workflow for Multimodal Data Integration. ***Anal Chem.*** 2021; 93: 3061-3071.

Ramesh P, Lannagan TRM, Jackstadt R, Atencia Taboada L, Lansu N, Wirapati P, van Hooff SR, Dekker D, Pritchard J, Kirov AB, van Neerven SM, Tejpar S, Kops G, Sansom OJ, Medema JP.
BCL-XL is crucial for progression through the adenoma-to-carcinoma sequence of colorectal cancer. ***Cell Death Differ.*** 2021; 28: 3282-3296.

Sacchetti A, Teeuwssen M, Verhagen M, Joosten R, Xu T, Stabile R, van der Steen B, Watson MM, Gusinac A, Kim WK, Ubink I, Van de Werken HJ, Fumagalli A, Paauwe M, Van Rheenen J, Sansom OJ, Kranenburg O, Fodde R.
Phenotypic plasticity underlies local invasion and distant metastasis in colon cancer. ***Elife.*** 2021; 10.

Sharbeen G, McCarroll JA, Akerman A, Kopecky C, Youkhana J, Kokkinos J, Holst J, Boyer C, Erkan M, Goldstein D, Timpson P, Cox TR, Pereira BA, Chitty JL, Fey SK, Najumudeen AK, Campbell AD, Sansom OJ, Ignacio RMC, Naim S, Liu J, Russia N, Lee J, Chou A, Johns A, Gill AJ, Gonzales-Aloy E, Gebski V, Guan YF, Pajic M, Turner N, Apte MV, Davis TP, Morton JP, Haghighi KS, Kasparian J, McLean BJ, Setargew YF, Phillips PA, Australian Pancreatic Cancer Genome I.
Cancer-Associated Fibroblasts in Pancreatic Ductal Adenocarcinoma Determine Response to SLC7A11 Inhibition. ***Cancer Res.*** 2021; 81: 3461-3479.

Swaminathan K, Campbell A, Papalazarou V, Jaber-Hijazi F, Nixon C, McGhee E, Strathdee D, Sansom OJ, Machesky LM.
The RAC1 Target NCKAP1 Plays a Crucial Role in the Progression of Braf;Pten-Driven Melanoma in Mice. ***J Invest Dermatol.*** 2021; 141: 628-637 e615.

Tajan M, Hennequart M, Cheung EC, Zani F, Hock AK, Legrave N, Maddocks ODK, Ridgway RA, Athineos D, Suarez-Bonnet A, Ludwig RL, Novellasdemunt L, Angelis N, Li VSW, Vlachogiannis G, Valeri N, Mainolfi N, Suri V, Friedman A, Manfredi M, Blyth K, Sansom OJ, Vousden KH.
Serine synthesis pathway inhibition cooperates with dietary serine and glycine limitation for cancer therapy. ***Nat Commun.*** 2021; 12: 366.

van der Weyden L, Harle V, Turner G, Offord V, Iyer V, Droop A, Swiatkowska A, Rabbie R, Campbell AD, Sansom OJ, Pardo M, Choudhary JS, Ferreira I, Tullett M, Arends MJ, Speak AO, Adams DJ.
CRISPR activation screen in mice identifies novel membrane proteins enhancing pulmonary metastatic colonisation. ***Commun Biol.*** 2021; 4: 395.

Other Publications

Gillespie MA, Steele CW, Lannagan TRM, Sansom OJ, Roxburgh CSD.
Pre-clinical modelling of rectal cancer to develop novel radiotherapy-based treatment strategies. ***Oncol Rev.*** 2021; 25: 511.

Knight JRP, Sansom OJ.
Tuning protein synthesis for cancer therapy. ***Mol Cell Oncol.*** 2021; 8: 1884034.

Lannagan TR, Jackstadt R, Leedham SJ, Sansom OJ.
Advances in colon cancer research: in vitro and animal models. ***Curr Opin Genet Dev.*** 2021; 66: 50-56.

Rosenfeldt MT, O'Prey J, Lindsay CR, Nixon C, Roth S, Sansom OJ, Ryan KM.
Loss of autophagy affects melanoma development in a manner dependent on PTEN status. ***Cell Death Differ.*** 2021; 28: 1437-1439.

Smith RCL, Kanellos G, Vlahov N, Alexandrou C, Willis AE, Knight JRP, Sansom OJ.
Translation initiation in cancer at a glance. ***J Cell Sci.*** 2021; 134

Sphyris N, Hodder MC, Sansom OJ.
Subversion of Niche-Signalling Pathways in Colorectal Cancer: What Makes and Breaks the Intestinal Stem Cell. ***Cancers (Basel).*** 2021; 13

Colin Steele (page 64)

Advanced Colorectal Cancer

Primary Research Papers

Han J, Tao M, Wu X, Li D, Ma Y, Dawood S, Steele CW, Tan KK, Wang Q.
Reporting quality of practice guidelines on colorectal cancer: evaluation using the RIGHT reporting checklist. ***Ann Transl Med.*** 2021; 9: 1175.

Other Publications

Galbraith NJ, Wood C, Steele CW.
Targeting Metastatic Colorectal Cancer with Immune Oncological Therapies. ***Cancers (Basel).*** 2021; 13

Gillespie MA, Steele CW, Lannagan TRM, Sansom OJ, Roxburgh CSD.
Pre-clinical modelling of rectal cancer to develop novel radiotherapy-based treatment strategies. ***Oncol Rev.*** 2021; 15: 511.

McSorley ST, Steele CW, Anderson JH, McKinlay S.
Comment on 'Preoperative intravenous iron therapy and survival after colorectal cancer surgery: long-term results from the IVICA randomized controlled trial'. ***Colorectal Dis.*** 2021; 21: 555-556.

Stephen Tait (page 66)

Mitochondria and Cancer Cell Death

Primary Research Papers

Bock FJ, Sedov E, Koren E, Koessinger AL, Cloix C, Zerbst D, Athineos D, Anand J, Campbell KJ, Blyth K, Fuchs Y, Tait SWG.
Apoptotic stress-induced FGF signalling promotes non-cell autonomous resistance to cell death. ***Nat Commun.*** 2021; 12: 6572.

Campbell KJ, Mason SM, Winder ML, Willemsen RBE, Cloix C, Lawson H, Rooney N, Dhayade S, Sims AH, Blyth K, Tait SWG.
Breast cancer dependence on MCL-1 is due to its canonical anti-apoptotic function. ***Cell Death Differ.*** 2021; 28: 2589-2600.

Johnson SA, Ormsby MJ, Wessel HM, Hulme HE, Bravo-Blas A, McIntosh A, Mason S, Coffelt SB, Tait SWG, Mowat AM, Milling SWF, Blyth K, Wall DM.
Monocytes mediate Salmonella Typhimurium-induced tumor growth inhibition in a mouse melanoma model. ***Eur J Immunol.*** 2021; 51: 3228-3238.

Latif AL, Newcombe A, Li S, Gilroy K, Robertson NA, Lei X, Stewart HJS, Cole J, Terradas MT, Rishi L, McGarry L, McKeeve C, Reid C, Clark W, Campos J, Kirschner K, Davis A, Lopez J, Sakamaki JI, Morton JP, Ryan KM, Tait SWG, Abraham SA, Holyoake T, Higgins B, Huang X, Blyth K, Copland M, Chevassut TJT, Keeshan K, Adams PD.
BRD4-mediated repression of p53 is a target for combination therapy in AML. ***Nat Commun.*** 2021; 12: 241.

Rabas N, Palmer S, Mitchell L, Ismail S, Gohlke A, Riley JS, Tait SWG, Gammage P, Soares LL, Macpherson IR, Norman JC.
PINK1 drives production of mtDNA-containing extracellular vesicles to promote invasiveness. ***J Cell Biol.*** 2021; 220.

Other Publications

Roca-Portoles A, Tait SWG.
Mitochondrial quality control: from molecule to organelle. ***Cell Mol Life Sci.*** 2021; 10.1007/s00018-021-03775-0.

Saverio Tardito (page 68)

Oncometabolism

Primary Research Papers

Cano-Galiano A, Oudin A, Fack F, Allega MF, Sumpton D, Martinez-Garcia E, Dittmar G, Hau AC, De Falco A, Herold-Mende C, Bjerkvig R, Meiser J, Tardito S, Niclou SP.
Cystathionine-gamma-lyase drives antioxidant defense in cysteine-restricted IDH1-mutant astrocytomas. ***Neurooncol Adv.*** 2021; 3: vdab057.

Chiu M, Taurino G, Dander E, Bardelli D, Fallati A, Andreoli R, Bianchi MG, Carubbi C, Pozzi G, Galuppo L, Mirandola P, Rizzari C, Tardito S, Biondi A, D'Amico G, Bussolati O.
ALL blasts drive primary mesenchymal stromal cells to increase asparagine availability during asparaginase treatment. ***Blood Adv.*** 2021; 5: 5164-5178.

Gonzalez-Menendez P, Romano M, Yan H, Deshmukh R, Papoin J, Oburoglu L, Daumur M, Dume AS, Phadke I, Mongellaz C, Qu X, Bories PN, Fontenay M, An X, Dardalhon V, Sitbon M, Zimmermann VS, Gallagher PG, Tardito S, Blanc L, Mohandas N, Taylor N, Kinet S.
An IDH1-vitamin C crosstalk drives human erythroid development by inhibiting pro-oxidant mitochondrial metabolism. ***Cell Rep.*** 2021; 34: 108723.

Matias MI, Yong CS, Foroushani A, Goldsmith C, Mongellaz C, Sezgin E, Levental KR, Talebi A, Perrault J, Riviere A, Dehairs J, Delos O, Bertand-Michel J, Portais JC, Wong M, Marie JC, Kelekar A, Kinet S, Zimmermann VS, Levental I, Yvan-Charvet L, Swinnen JV, Muljo SA, Hernandez-Vargas H, Tardito S, Taylor N, Dardalhon V.
Regulatory T cell differentiation is controlled by alphaKG-induced alterations in mitochondrial metabolism and lipid homeostasis. ***Cell Rep.*** 2021; 37: 109911.

Najumudeen AK, Ceteci F, Fey SK, Hamm G, Steven RT, Hall H, Nikula CJ, Dexter A, Murta T, Race AM, Sumpton D, Vlahov N, Gay DM, Knight JRP, Jackstadt R, Leach JDG, Ridgway RA, Johnson ER, Nixon C, Hedley A, Gilroy K, Clark W, Malla SB, Dunne PD, Rodriguez-Blanco G, Critchlow SE, Mrowinska A, Malviya G, Solovyev D, Brown G, Lewis DY, Mackay GM, Strathdee D, Tardito S, Gottlieb E, Consortium CRGC, Takats Z, Barry ST, Goodwin RJA, Bunch J, Bushell M, Campbell AD, Sansom OJ.
The amino acid transporter SLC7A5 is required for efficient growth of KRAS-mutant colorectal cancer. ***Nat Genet.*** 2021; 53: 16-26.

Other Publications

Deshmukh R, Allega MF, Tardito S.
A map of the altered glioma metabolism. ***Trends Mol Med.*** 2021; 10.1016/j.molmed.2021.07.011.

Sara Zanivan (page 70)

Tumour Microenvironment and Proteomics

Primary Research Papers

Ahmed SF, Buetow L, Gabrielsen M, Lilla S, Sibbet GJ, Sumpton D, Zanivan S, Hedley A, Clark W, Huang DT.
E3 ligase-inactivation rewires CBL interactome to elicit oncogenesis by hijacking RTK-CBL-CIN85 axis. ***Oncogene.*** 2021; 40: 2149-2164.

Budden T, Gaudy-Marqueste C, Porter A, Kay E, Gurung S, Earnshaw CH, Roeck K, Craig S, Traves V, Krutmann J, Muller P, Motta L, Zanivan S, Malliri A, Furney SJ, Nagore E, Viros A.
Ultraviolet light-induced collagen degradation inhibits melanoma invasion. ***Nat Commun.*** 2021; 12: 2742.

Gillen SL, Giacomelli C, Hodge K, Zanivan S, Bushell M, Wilczynska A.
Differential regulation of mRNA fate by the human Ccr4-Not complex is driven by coding sequence composition and mRNA localization. ***Genome Biol.*** 2021; 22: 284.

Kugeratski FG, Hodge K, Lilla S, McAndrews KM, Zhou X, Hwang RF, Zanivan S, Kalluri R.
Quantitative proteomics identifies the core proteome of exosomes with syntenin-1 as the highest abundant protein and a putative universal biomarker. ***Nat Cell Biol.*** 2021; 23: 631-641.

Marco S, Neilson M, Moore M, Perez-Garcia A, Hall H, Mitchell L, Lilla S, Blanco GR, Hedley A, Zanivan S, Norman JC.
Nuclear-capture of endosomes depletes nuclear G-actin to promote SRF/MRTF activation and cancer cell invasion. ***Nat Commun.*** 2021; 12: 6829.

Martinez RS, Salji MJ, Rushworth L, Ntala C, Rodriguez Blanco G, Hedley A, Clark W, Peixoto P, Hervouet E, Renaude E, Kung SHY, Galbraith LCA, Nixon C, Lilla S, MacKay GM, Fazli L, Gaughan L, Sumpton D, Gleave ME, Zanivan S, Blomme A, Leung HY.
SLFN5 Regulates LAT1-Mediated mTOR Activation in Castration-Resistant Prostate Cancer. ***Cancer Res.*** 2021; 81: 3664-3678.

Nacke M, Sandilands E, Nikolatou K, Roman-Fernandez A, Mason S, Patel R, Lilla S, Yelland T, Galbraith LCA, Freckmann EC, McGarry L, Morton JP, Shanks E, Leung HY, Markert E, Ismail S, Zanivan S, Blyth K, Bryant DM.
An ARF GTPase module promoting invasion and metastasis through regulating phosphoinositide metabolism. ***Nat Commun.*** 2021; 12: 1623.

Phatak V, von Grabowiecki Y, Janus J, Officer L, Behan C, Aschauer L, Pinon L, Mackay H, Zanivan S, Norman JC, Kelly M, Le Quesne J, Muller PAJ.
Mutant p53 promotes RCP-dependent chemoresistance coinciding with increased delivery of P-glycoprotein to the plasma membrane. ***Cell Death Dis.*** 2021; 12: 207.

Other Publications

Domen A, Quatannens D, Zanivan S, Deben C, Van Audenaerde J, Smits E, Wouters A, Lardon F, Roeyen G, Verhoeven Y, Janssens A, Vandamme T, van Dam P, Peeters M, Prenen H.
Cancer-Associated Fibroblasts as a Common Orchestrator of Therapy Resistance in Lung and Pancreatic Cancer. ***Cancers (Basel).*** 2021; 13

Kay EJ, Koulouras G, Zanivan S.
Regulation of Extracellular Matrix Production in Activated Fibroblasts: Roles of Amino Acid Metabolism in Collagen Synthesis. ***Front Oncol.*** 2021; 11: 719922.

Kay EJ, Zanivan S.
Metabolic pathways fuelling protumourigenic cancer-associated fibroblast functions. ***Current Opinion in Systems Biology.*** 2021; 282021: 100377.

Kay EJ, Zanivan S.
Two opposing sub-populations of fibroblasts decide progression of pancreatic cancer. ***Cancer Cell.*** 2021; 39: 1175-1177.

Klionsky DJ, et al.
Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition) (1). ***Autophagy.*** 2021; 17: 1-382.

Paterson K, Zanivan S, Glasspool R, Coffelt SB, Zagnoni M.
Microfluidic technologies for immunotherapy studies on solid tumours. ***Lab Chip.*** 2021; 21: 2306-2329.

Justin Bower & Heather McKinnon (page 74)

Drug Discovery

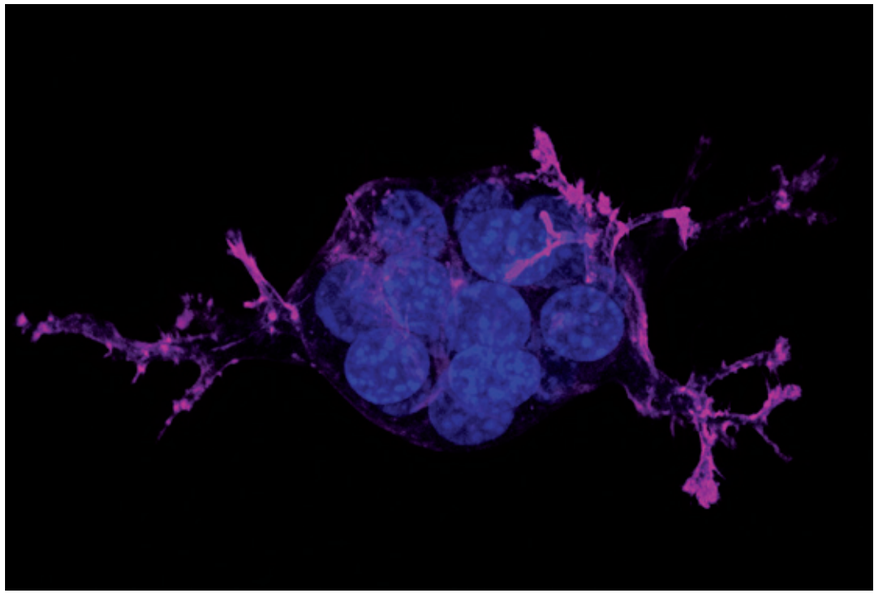
Primary Research Papers

Bilsland AE, McAulay K, West R, Pugliese A, Bower J.
Automated Generation of Novel Fragments Using Screening Data, a Dual SMILES Autoencoder, Transfer Learning and Syntax Correction. ***J Chem Inf Model.*** 2021; 61: 2547-2559.

Knight JRP, Alexandrou C, Skalka GL, Vlahov N, Pennel K, Officer L, Teodosio A, Kanellos G, Gay DM, May-Wilson S, Smith EM, Najumudeen AK, Gilroy K, Ridgway RA, Flanagan DJ, Smith RCL, McDonald L, MacKay C, Cheasty A, McArthur K, Stanway E, Leach JD, Jackstadt R, Waldron JA, Campbell AD, Vlachogiannis G, Valeri N, Haigis KM, Sonenberg N, Proud CG, Jones NP, Swarbrick ME, McKinnon HJ, Faller WJ, Le Quesne J, Edwards J, Willis AE, Bushell M, Sansom OJ.
MNK Inhibition Sensitizes KRAS-Mutant Colorectal Cancer to mTORC1 Inhibition by Reducing eIF4E Phosphorylation and c-MYC Expression. ***Cancer Discov.*** 2021; 11: 1228-1247.

Ovarian cancer mini-tumour invading through the Extracellular Matrix. Stained with Phalloidin (F-actin, magenta) and with a nuclear stain (HOECHST, blue).

Image by Konstantina Nikolatou



Leo Carlin (page 78)
Beatson Advanced Imaging Resources

Primary Research Papers

Flanagan DJ, Pentinmikko N, Luopajarvi K, Willis NJ, Gilroy K, Raven AP, McGarry L, Englund JI, Webb AT, Scharaw S, Nasreddin N, Hodder MC, Ridgway RA, Minnee E, Sphyris N, Gilchrist E, Najumudeen AK, Romagnolo B, Perret C, Williams AC, Clevers H, Nummela P, Lahde M, Alitalo K, Hietakangas V, Hedley A, Clark W, Nixon C, Kirschner K, Jones EY, Ristimaki A, Leedham SJ, Fish PV, Vincent JP, Katajisto P, Sansom OJ.
NOTUM from Apc-mutant cells biases clonal competition to initiate cancer. ***Nature.*** 2021; 594: 430-435.

Galbraith LCA, Mui E, Nixon C, Hedley A, Strachan D, MacKay G, Sumpton D, Sansom OJ, Leung HY, Ahmad I.
PPAR-gamma induced AKT3 expression increases levels of mitochondrial biogenesis driving prostate cancer. ***Oncogene.*** 2021; 40: 2355-2366.

Julian L, Naylor G, Wickman GR, Rath N, Castino G, Stevenson D, Bryson S, Munro J, McGarry L, Mullin M, Rice A, Del Rio Hernandez A, Olson MF.
Defective apoptotic cell contractility provokes sterile inflammation, leading to liver damage and tumour suppression. ***Elife.*** 2021; 10.

Latif AL, Newcombe A, Li S, Gilroy K, Robertson NA, Lei X, Stewart HJS, Cole J, Terradas MT, Rishi L, McGarry L, McKeeve C, Reid C, Clark W, Campos J, Kirschner K, Davis A, Lopez J, Sakamaki JI, Morton JP, Ryan KM, Tait SWG, Abraham SA, Holyoake T, Higgins B, Huang X, Blyth K, Copland M, Chevassut TJT, Keeshan K, Adams PD.
BRD4-mediated repression of p53 is a target for combination therapy in AML. ***Nat Commun.*** 2021; 12: 241.

Le AH, Yelland T, Paul NR, Fort L, Nikolaou S, Ismail S, Machesky LM.
CYRI-A limits invasive migration through macropinosome formation and integrin uptake regulation. ***J Cell Biol.*** 2021; 220.

McGarry DJ, Armstrong G, Castino G, Mason S, Clark W, Shaw R, McGarry L, Blyth K, Olson MF.
MICAL1 regulates actin cytoskeleton organization, directional cell migration and the growth of human breast cancer cells as orthotopic xenograft tumours. ***Cancer Lett.*** 2021; 519: 226-236.

Nacke M, Sandilands E, Nikolatou K, Roman-Fernandez A, Mason S, Patel R, Lilla S, Yelland T, Galbraith LCA, Freckmann EC, McGarry L, Morton JP, Shanks E, Leung HY, Markert E, Ismail S, Zanivan S, Blyth K, Bryant DM.
An ARF GTPase module promoting invasion and metastasis through regulating phosphoinositide metabolism. ***Nat Commun.*** 2021; 12: 1623.

Ntala C, Salji M, Salmond J, Officer L, Teodosio AV, Blomme A, McGhee EJ, Powley I, Ahmad I, Kruithof-de Julio M, Thalmann G, Roberts E, Goodyear CS, Jamaspishvili T, Berman DM, Carlin LM, Le Quesne J, Leung HY.
Analysis of Prostate Cancer Tumor Microenvironment Identifies Reduced Stromal CD4 Effector T-cell Infiltration in Tumors with Pelvic Nodal Metastasis. ***Eur Urol Open Sci.*** 2021; 29: 19-29.

Pickering KA, Gilroy K, Cassidy JW, Fey SK, Najumudeen AK, Zeiger LB, Vincent DF, Gay DM, Johansson J, Fordham RP, Miller B, Clark W, Hedley A, Unal EB, Kiel C, McGhee E, Machesky LM, Nixon C, Johnsson AE, Bain M, Strathdee D, van Hoof SR, Medema JP, Anderson KI, Brachmann SM, Stucke VM, Malliri A, Drysdale M, Turner M, Serrano L, Myant K, Campbell AD, Sansom OJ.
A RAC-GEF network critical for early intestinal tumourigenesis. ***Nat Commun.*** 2021; 12: 56.

Rudzka DA, Mason S, Neilson M, McGarry L, Kalna G, Hedley A, Blyth K, Olson MF.
Selection of established tumour cells through narrow diameter micropores enriches for elevated Ras/Raf/MEK/ERK MAPK signalling and enhanced tumour growth. ***Small GTPases***. 2021; 12: 294-310.

Swaminathan K, Campbell A, Papalazarou V, Jaber-Hijazi F, Nixon C, McGhee E, Strathdee D, Sansom OJ, Machesky LM.
The RAC1 Target NCKAP1 Plays a Crucial Role in the Progression of Braf;Pten-Driven Melanoma in Mice. J ***Invest Dermatol.*** 2021; 141: 628-637 e615.

Crispin Miller (page 80)
Bioinformatics & Data Science

Primary Research Papers

Flanagan DJ, Pentinmikko N, Luopajarvi K, Willis NJ, Gilroy K, Raven AP, McGarry L, Englund JI, Webb AT, Scharaw S, Nasreddin N, Hodder MC, Ridgway RA, Minnee E, Sphyris N, Gilchrist E, Najumudeen AK, Romagnolo B, Perret C, Williams AC, Clevers H, Nummela P, Lahde M, Alitalo K, Hietakangas V, Hedley A, Clark W, Nixon C, Kirschner K, Jones EY, Ristimaki A, Leedham SJ, Fish PV, Vincent JP, Katajisto P, Sansom OJ.
NOTUM from Apc-mutant cells biases clonal competition to initiate cancer. ***Nature.*** 2021; 594: 430-435.

Galbraith LCA, Mui E, Nixon C, Hedley A, Strachan D, MacKay G, Sumpton D, Sansom OJ, Leung HY, Ahmad I.
PPAR-gamma induced AKT3 expression increases levels of mitochondrial biogenesis driving prostate cancer. ***Oncogene.*** 2021; 40: 2355-2366.

Julian L, Naylor G, Wickman GR, Rath N, Castino G, Stevenson D, Bryson S, Munro J, McGarry L, Mullin M, Rice A, Del Rio Hernandez A, Olson MF.
Defective apoptotic cell contractility provokes sterile inflammation, leading to liver damage and tumour suppression. ***Elife.*** 2021; 10.

Latif AL, Newcombe A, Li S, Gilroy K, Robertson NA, Lei X, Stewart HJS, Cole J, Terradas MT, Rishi L, McGarry L, McKeeve C, Reid C, Clark W, Campos J, Kirschner K, Davis A, Lopez J, Sakamaki JI, Morton JP, Ryan KM, Tait SWG, Abraham SA, Holyoake T, Higgins B, Huang X, Blyth K, Copland M, Chevassut TJT, Keeshan K, Adams PD.
BRD4-mediated repression of p53 is a target for combination therapy in AML. ***Nat Commun.*** 2021; 12: 241.

Le AH, Yelland T, Paul NR, Fort L, Nikolaou S, Ismail S, Machesky LM.
CYRI-A limits invasive migration through macropinosome formation and integrin uptake regulation. ***J Cell Biol.*** 2021; 220.

McGarry DJ, Armstrong G, Castino G, Mason S, Clark W, Shaw R, McGarry L, Blyth K, Olson MF.
MICAL1 regulates actin cytoskeleton organization, directional cell migration and the growth of human breast cancer cells as orthotopic xenograft tumours. ***Cancer Lett.*** 2021; 519: 226-236.

Nacke M, Sandilands E, Nikolatou K, Roman-Fernandez A, Mason S, Patel R, Lilla S, Yelland T, Galbraith LCA, Freckmann EC, McGarry L, Morton JP, Shanks E, Leung HY, Markert E, Ismail S, Zanivan S, Blyth K, Bryant DM.
An ARF GTPase module promoting invasion and metastasis through regulating phosphoinositide metabolism. ***Nat Commun.*** 2021; 12: 1623.

Ntala C, Salji M, Salmond J, Officer L, Teodosio AV, Blomme A, McGhee EJ, Powley I, Ahmad I, Kruithof-de Julio M, Thalmann G, Roberts E, Goodyear CS, Jamaspishvili T, Berman DM, Carlin LM, Le Quesne J, Leung HY.
Analysis of Prostate Cancer Tumor Microenvironment Identifies Reduced Stromal CD4 Effector T-cell Infiltration in Tumors with Pelvic Nodal Metastasis. ***Eur Urol Open Sci.*** 2021; 29: 19-29.

Pickering KA, Gilroy K, Cassidy JW, Fey SK, Najumudeen AK, Zeiger LB, Vincent DF, Gay DM, Johansson J, Fordham RP, Miller B, Clark W, Hedley A, Unal EB, Kiel C, McGhee E, Machesky LM, Nixon C, Johnsson AE, Bain M, Strathdee D, van Hoof SR, Medema JP, Anderson KI, Brachmann SM, Stucke VM, Malliri A, Drysdale M, Turner M, Serrano L, Myant K, Campbell AD, Sansom OJ.
A RAC-GEF network critical for early intestinal tumourigenesis. ***Nat Commun.*** 2021; 12: 56.

Rudzka DA, Mason S, Neilson M, McGarry L, Kalna G, Hedley A, Blyth K, Olson MF.
Selection of established tumour cells through narrow diameter micropores enriches for elevated Ras/Raf/MEK/ERK MAPK signalling and enhanced tumour growth. ***Small GTPases.*** 2021; 12: 294-310.

Swaminathan K, Campbell A, Papalazarou V, Jaber-Hijazi F, Nixon C, McGhee E, Strathdee D, Sansom OJ, Machesky LM.
The RAC1 Target NCKAP1 Plays a Crucial Role in the Progression of Braf;Pten-Driven Melanoma in Mice. ***J Invest Dermatol.*** 2021; 141: 628-637 e615.

David Sumpton (page 82)
Metabolomics

Primary Research Papers

Ahmed SF, Buetow L, Gabrielsen M, Lilla S, Sibbet GJ, Sumpton D, Zanivan S, Hedley A, Clark W, Huang DT.
E3 ligase-inactivation rewires CBL interactome to elicit oncogenesis by hijacking RTK-CBL-CIN85 axis. ***Oncogene***. 2021; 40: 2149-2164.

Cano-Galiano A, Oudin A, Fack F, Allega MF, Sumpton D, Martinez-Garcia E, Dittmar G, Hau AC, De Falco A, Herold-Mende C, Bjerkvig R, Meiser J, Tardito S, Niclou SP.
Cystathionine-gamma-lyase drives antioxidant defense in cysteine-restricted IDH1-mutant astrocytomas. ***Neurooncol Adv.*** 2021; 3: vdab057.

Galbraith LCA, Mui E, Nixon C, Hedley A, Strachan D, MacKay G, Sumpton D, Sansom OJ, Leung HY, Ahmad I.
PPAR-gamma induced AKT3 expression increases levels of mitochondrial biogenesis driving prostate cancer. ***Oncogene.*** 2021; 40: 2355-2366.

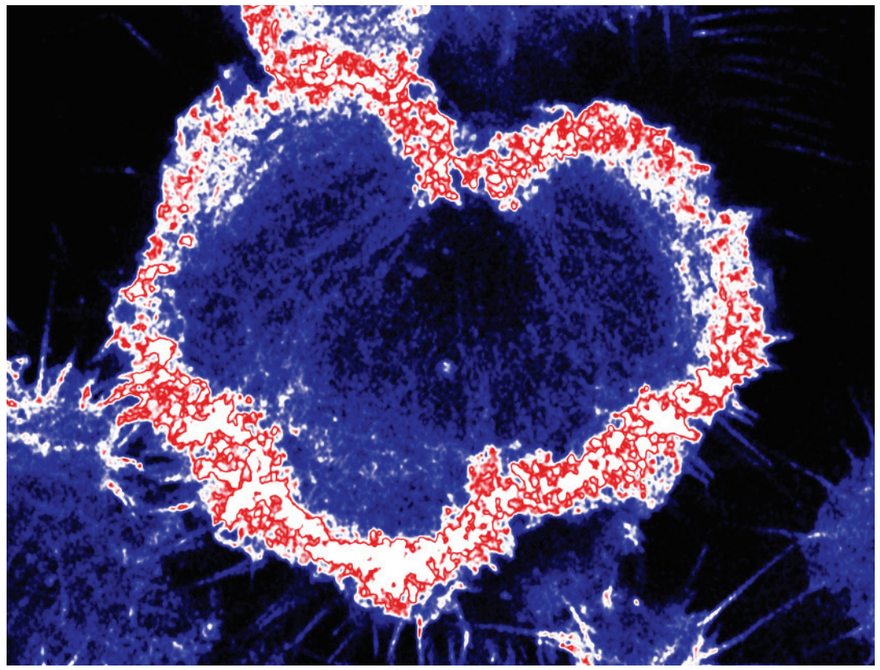
Grosso S, Marini A, Gyuraszova K, Voorde JV, Sfakianos A, Garland GD, Tenor AR, Mordue R, Chernova T, Morone N, Sereno M, Smith CP, Officer L, Farahmand P, Rooney C, Sumpton D, Das M, Teodosio A, Ficken C, Martin MG, Spriggs RV, Sun XM, Bushell M, Sansom OJ, Murphy D, MacFarlane M, Le Quesne JPC, Willis AE.
The pathogenesis of mesothelioma is driven by a dysregulated translatome. ***Nat Commun.*** 2021; 12: 4920.

Martinez RS, Salji MJ, Rushworth L, Ntala C, Rodriguez Blanco G, Hedley A, Clark W, Peixoto P, Hervouet E, Renaude E, Kung SHY, Galbraith LCA, Nixon C, Lilla S, MacKay GM, Fazli L, Gaughan L, Sumpton D, Gleave ME, Zanivan S, Blomme A, Leung HY.
SLFN5 Regulates LAT1-Mediated mTOR Activation in Castration-Resistant Prostate Cancer. ***Cancer Res.*** 2021; 81: 3664-3678.

Najumudeen AK, Ceteci F, Fey SK, Hamm G, Steven RT, Hall H, Nikula CJ, Dexter A, Murta T, Race AM, Sumpton D, Vlahov N, Gay DM, Knight JRP, Jackstadt R, Leach JDG, Ridgway RA, Johnson ER, Nixon C, Hedley A, Gilroy K, Clark W, Malla SB, Dunne PD, Rodriguez-Blanco G, Critchlow SE, Mrowinska A, Malviya G, Solovyev D, Brown G, Lewis DY, Mackay GM, Strathdee D, Tardito S, Gottlieb E, Consortium CRGC, Takats Z, Barry ST, Goodwin RJA, Bunch J, Bushell M, Campbell AD, Sansom OJ.
The amino acid transporter SLC7A5 is required for efficient growth of KRAS-mutant colorectal cancer. ***Nat Genet.*** 2021; 53: 16-26.

Actin Ruffle Pancreatic cancer cell expressing a GFP marker of the actin cytoskeleton, imaged over time using a Zeiss 880 LSM with Airyscan.

Image by Nikki R. Paul



Sara Zanivan (page 84)
Proteomics

Primary Research Papers

Ahmed SF, Buetow L, Gabrielsen M, Lilla S, Sibbet GJ, Sumpton D, Zanivan S, Hedley A, Clark W, Huang DT.
E3 ligase-inactivation rewires CBL interactome to elicit oncogenesis by hijacking RTK-CBL-CIN85 axis. ***Oncogene.*** 2021; 40: 2149-2164.

Cano-Galiano A, Oudin A, Fack F, Allega MF, Sumpton D, Martinez-Garcia E, Dittmar G, Hau AC, De Falco A, Herold-Mende C, Bjerkvig R, Meiser J, Tardito S, Niclou SP.
Cystathionine-gamma-lyase drives antioxidant defense in cysteine-restricted IDH1-mutant astrocytomas. ***Neurooncol Adv.*** 2021; 3: vdab057.

Galbraith LCA, Mui E, Nixon C, Hedley A, Strachan D, MacKay G, Sumpton D, Sansom OJ, Leung HY, Ahmad I.
PPAR-gamma induced AKT3 expression increases levels of mitochondrial biogenesis driving prostate cancer. ***Oncogene.*** 2021; 40: 2355-2366.

Grosso S, Marini A, Gyuraszova K, Voorde JV, Sfakianos A, Garland GD, Tenor AR, Mordue R, Chernova T, Morone N, Sereno M, Smith CP, Officer L, Farahmand P, Rooney C, Sumpton D, Das M, Teodosio A, Ficken C, Martin MG, Spriggs RV, Sun XM, Bushell M, Sansom OJ, Murphy D, MacFarlane M, Le Quesne JPC, Willis AE.
The pathogenesis of mesothelioma is driven by a dysregulated translatome. ***Nat Commun.*** 2021; 12: 4920.

Martinez RS, Salji MJ, Rushworth L, Ntala C, Rodriguez Blanco G, Hedley A, Clark W, Peixoto P, Hervouet E, Renaude E, Kung SHY, Galbraith LCA, Nixon C, Lilla S, MacKay GM, Fazli L, Gaughan L, Sumpton D, Gleave ME, Zanivan S, Blomme A, Leung HY.
SLFN5 Regulates LAT1-Mediated mTOR Activation in Castration-Resistant Prostate Cancer. ***Cancer Res.*** 2021; 81: 3664-3678.

Najumudeen AK, Ceteci F, Fey SK, Hamm G, Steven RT, Hall H, Nikula CJ, Dexter A, Murta T, Race AM, Sumpton D, Vlahov N, Gay DM, Knight JRP, Jackstadt R, Leach JDG, Ridgway RA, Johnson ER, Nixon C, Hedley A, Gilroy K, Clark W, Malla SB, Dunne PD, Rodriguez-Blanco G, Critchlow SE, Mrowinska A, Malviya G, Solovyev D, Brown G, Lewis DY, Mackay GM, Strathdee D, Tardito S, Gottlieb E, Consortium CRGC, Takats Z, Barry ST, Goodwin RJA, Bunch J, Bushell M, Campbell AD, Sansom OJ.
The amino acid transporter SLC7A5 is required for efficient growth of KRAS-mutant colorectal cancer. ***Nat Genet.*** 2021; 53: 16-26.

Douglas Strathdee (page 88)
Transgenic Technology

Primary Research Papers

Gudino V, Pohl SO, Billard CV, Cammareri P, Bolado A, Aitken S, Stevenson D, Hall AE, Agostino M, Cassidy J, Nixon C, von Kriegsheim A, Freile P, Popplewell L, Dickson G, Murphy L, Wheeler A, Dunlop M, Din F, Strathdee D, Sansom OJ, Myant KB.
RAC1B modulates intestinal tumourigenesis via modulation of WNT and EGFR signalling pathways. ***Nat Commun.*** 2021; 12: 2335.

Humpton TJ, Nomura K, Weber J, Magnussen HM, Hock AK, Nixon C, Dhayade S, Stevenson D, Huang DT, Strathdee D, Blyth K, Vousden KH.
Differential requirements for MDM2 E3 activity during embryogenesis and in adult mice. ***Genes Dev.*** 2021; 35: 117-132.

Julian L, Naylor G, Wickman GR, Rath N, Castino G, Stevenson D, Bryson S, Munro J, McGarry L, Mullin M, Rice A, Del Rio Hernandez A, Olson MF.
Defective apoptotic cell contractility provokes sterile inflammation, leading to liver damage and tumour suppression. ***Elife.*** 2021; 10: e61983.

Leach JDG, Vlahov N, Tsantoulis P, Ridgway RA, Flanagan DJ, Gilroy K, Sphyris N, Vazquez EG, Vincent DF, Faller WJ, Hodder MC, Raven A, Fey S, Najumudeen AK, Strathdee D, Nixon C, Hughes M, Clark W, Shaw R, consortium SC, van Hooff SR, Huels DJ, Medema JP, Barry ST, Frame MC, Unciti-Broceta A, Leedham SJ, Inman GJ, Jackstadt R, Thompson BJ, Campbell AD, Tejpar S, Sansom OJ.
Oncogenic BRAF, unrestrained by TGFbeta-receptor signalling, drives right-sided colonic tumorigenesis. ***Nat Commun***. 2021; 12: 3464.

Najumudeen AK, Ceteci F, Fey SK, Hamm G, Steven RT, Hall H, Nikula CJ, Dexter A, Murta T, Race AM, Sumpton D, Vlahov N, Gay DM, Knight JRP, Jackstadt R, Leach JDG, Ridgway RA, Johnson ER, Nixon C, Hedley A, Gilroy K, Clark W, Malla SB, Dunne PD, Rodriguez-Blanco G, Critchlow SE, Mrowinska A, Malviya G, Solovyev D, Brown G, Lewis DY, Mackay GM, Strathdee D, Tardito S, Gottlieb E,

Consortium CRGC, Takats Z, Barry ST, Goodwin RJA, Bunch J, Bushell M, Campbell AD, Sansom OJ.
The amino acid transporter SLC7A5 is required for efficient growth of KRAS-mutant colorectal cancer. ***Nat Genet.*** 2021; 53: 16-26.

Pickering KA, Gilroy K, Cassidy JW, Fey SK, Najumudeen AK, Zeiger LB, Vincent DF, Gay DM, Johansson J, Fordham RP, Miller B, Clark W, Hedley A, Unal EB, Kiel C, McGhee E, Machesky LM, Nixon C, Johnsson AE, Bain M, Strathdee D, van Hoof SR, Medema JP, Anderson KI, Brachmann SM, Stucke VM, Malliri A, Drysdale M, Turner M, Serrano L, Myant K, Campbell AD, Sansom OJ.
A RAC-GEF network critical for early intestinal tumourigenesis. ***Nat Commun.*** 2021; 12: 56.

Swaminathan K, Campbell A, Papalazarou V, Jaber-Hijazi F, Nixon C, McGhee E, Strathdee D, Sansom OJ, Machesky LM.
The RAC1 Target NCKAP1 Plays a Crucial Role in the Progression of Braf;Pten-Driven Melanoma in Mice. ***J Invest Dermatol.*** 2021; 141: 628-637 e615.

David Lewis (page 90)

Translational Molecular Imaging

Primary Research Papers

Najumudeen AK, Ceteci F, Fey SK, Hamm G, Steven RT, Hall H, Nikula CJ, Dexter A, Murta T, Race AM, Sumpton D, Vlahov N, Gay DM, Knight JRP, Jackstadt R, Leach JDG, Ridgway RA, Johnson ER, Nixon C, Hedley A, Gilroy K, Clark W, Malla SB, Dunne PD, Rodriguez-Blanco G, Critchlow SE, Mrowinska A, Malviya G, Solovyev D, Brown G, Lewis DY, Mackay GM, Strathdee D, Tardito S, Gottlieb E, Consortium CRGC, Takats Z, Barry ST, Goodwin RJA, Bunch J, Bushell M, Campbell AD, Sansom OJ.
The amino acid transporter SLC7A5 is required for efficient growth of KRAS-mutant colorectal cancer. ***Nat Genet.*** 2021; 53: 16-26.

Other Publications

Pandey S, Malviya G, Chottova Dvorakova M.
Role of Peptides in Diagnostics. ***Int J Mol Sci.*** 2021: 22

Other Beatson Publications

Azam F, Vazquez A.
Trends in Phase II Trials for Cancer Therapies. ***Cancers (Basel).*** 2021; 13.

Bernabe-Rubio M, Bosch-Fortea M, Garcia E, Bernardino de la Serna J, Alonso MA.
Adaptive Lipid Immiscibility and Membrane Remodeling Are Active Functional Determinants of Primary Ciliogenesis. ***Small Methods.*** 2021; 5: e2000711.

Bodineau C, Tome M, Courtois S, Costa ASH, Sciacovelli M, Rousseau B, Richard E, Vacher P, Parejo-Perez C, Bessede E, Varon C, Soubeyran P, Frezza C, Murdoch PDS, Villar VH, Duran RV.
Two parallel pathways connect glutamine metabolism and mTORC1 activity to regulate glutamoptosis. ***Nat Commun.*** 2021; 12: 4814.

ElMaghloob Y, Sot B, McIlwraith MJ, Garcia E, Yelland T, Ismail S.
ARL3 activation requires the co-GEF BART and effector-mediated turnover. ***Elife.*** 2021; 10.

Gillen S.
Advancing early gastric cancer detection. ***FEBS Open Bio.*** 2021; 11: 1812-1813.

Gonzalez-Menendez P, Romano M, Yan H, Deshmukh R, Papoin J, Oburoglu L, Daumur M, Dume AS, Phadke I, Mongellaz C, Qu X, Bories PN, Fontenay M, An X, Dardalhon V, Sitbon M, Zimmermann VS, Gallagher PG, Tardito S, Blanc L, Mohandas N, Taylor N, Kinet S.
An IDH1-vitamin C crosstalk drives human erythroid development by inhibiting pro-oxidant mitochondrial metabolism. ***Cell Rep.*** 2021; 34: 108723.

Griffith BGC, Upstill-Goddard R, Brunton H, Grimes GR, Biankin AV, Serrels B, Byron A, Frame MC.
FAK regulates IL-33 expression by controlling chromatin accessibility at c-Jun motifs. ***Sci Rep.*** 2021; 11: 229.

Gubas A, Karantanou C, Popovic D, Tascher G, Hoffmann ME, Platzek A, Dawe N, Dikic I, Krause DS, McEwan DG.
The endolysosomal adaptor PLEKHM1 is a direct target for both mTOR and MAPK pathways. ***FEBS Lett.*** 2021; 595: 864-880.

Heydt Q, Xintaropoulou C, Clear A, Austin M, Pislariu I, Miraki-Moud F, Cutillas P, Korfi K, Calaminici M, Cawthorn W, Suchacki K, Nagano A, Gribben JG, Smith M, Cavenagh JD, Oakervee H, Castleton A, Taussig D, Peck B, Wilczynska A, McNaughton L, Bonnet D, Mardakheh F, Patel B.
Adipocytes disrupt the translational programme of acute lymphoblastic leukaemia to favour tumour survival and persistence. ***Nat Commun.*** 2021; 12: 5507.

Ianniciello A, Zarou MM, Rattigan KM, Scott M, Dawson A, Dunn K, Brabcova Z, Kalkman ER, Nixon C, Michie AM, Copland M, Vetrie D, Ambler M, Saxty B, Helgason GV.
ULK1 inhibition promotes oxidative stress-induced differentiation and sensitizes leukemic stem cells to targeted therapy. ***Sci Transl Med.*** 2021; 13: eabd5016.

Jackson MR, Stevenson K, Chahal SK, Curley E, Finney GE, Gutierrez-Quintana R, Onwubiko E, Rupp A, Strathdee K, Williams K, MacLeod MKL, McSharry C, Chalmers AJ.
Low-Dose Lung Radiation Therapy for COVID-19 Lung Disease: A Preclinical Efficacy Study in a Bleomycin Model of Pneumonitis. ***Int J Radiat Oncol Biol Phys.*** 2022; 112: 197-211.

Julian L, Naylor G, Wickman GR, Rath N, Castino G, Stevenson D, Bryson S, Munro J, McGarry L, Mullin M, Rice A, Del Rio Hernandez A, Olson MF.
Defective apoptotic cell contractility provokes sterile inflammation, leading to liver damage and tumour suppression. ***Elife.*** 2021; 10.

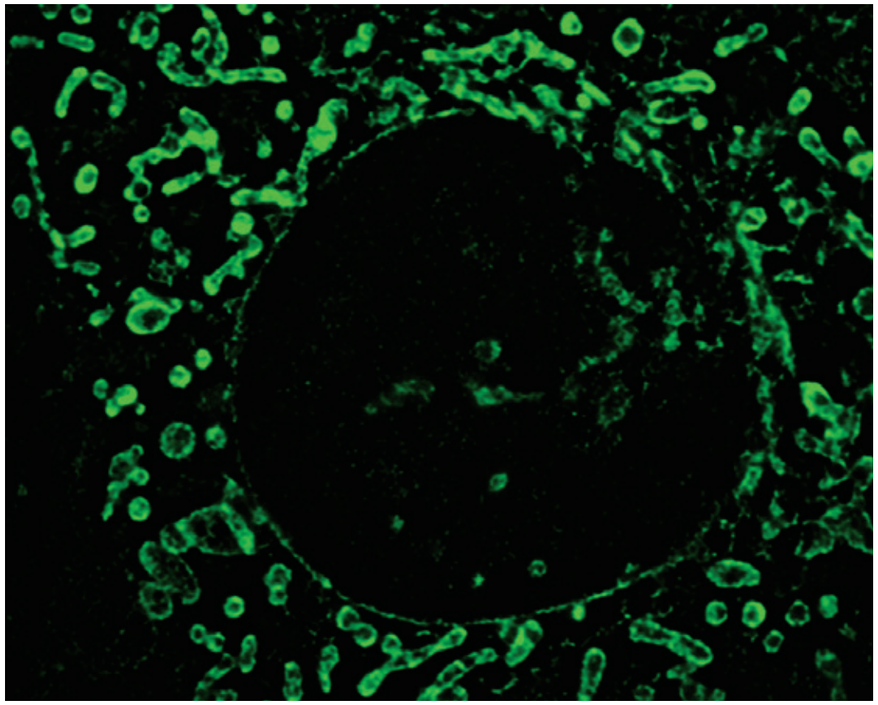
Lawson H, van de Lagemaat LN, Barile M, Tavosanis A, Durko J, Villacreces A, Bellani A, Mapperley C, Georges E, Martins-Costa C, Sepulveda C, Allen L, Campos J, Campbell KJ, O'Carroll D, Gottgens B, Cory S, Rodrigues NP, Guitart AV, Kranc KR.
CITED2 coordinates key hematopoietic regulatory pathways to maintain the HSC pool in both steady-state hematopoiesis and transplantation. ***Stem Cell Reports.*** 2021; 16: 2784-2797.

Mojsilovic SS, Mojsilovic S, Villar VH, Santibanez JF.
The Metabolic Features of Tumor-Associated Macrophages: Opportunities for Immunotherapy? ***Anal Cell Pathol (Amst).*** 2021; 2021: 5523055.

Powell L, Samarakoon YH, Ismail S, Sayer JA.
ARL3, a small GTPase with a functionally conserved role in primary cilia and immune synapses. ***Small GTPases.*** 2021; 12: 167-176.

Visualisation of the mitochondrial network. Representative images showing the mitochondria network in endothelial cells (SVEC) by means of staining cardiolipin (a mitochondria specific lipid) using dye Nonyl Acridine Orange (NAO). Images were taken with a Zeiss Elyra7 using a 63x water immersion objective.

Images taken by Nikki Pau; Image preparation by Rosalie Heilig & Jordan Lee



Rae C, Amato F, Braconi C.
Patient-Derived Organoids as a Model for Cancer Drug Discovery. ***Int J Mol Sci.*** 2021; 22.

Rossi F, Crnjar A, Comitani F, Feliciano R, Jahn L, Malim G, Southgate L, Kay E, Oakey R, Buggs R, Moir A, Kistler L, Rodriguez Mateos A, Molteni C, Schulz R.
Extraction and high-throughput sequencing of oak heartwood DNA: Assessing the feasibility of genome-wide DNA methylation profiling. ***PLoS One.*** 2021; 16: e0254971.

Schniete JK, Tinning PW, Scrimgeour RC, Robb G, Kolln LS, Wesencraft K, Paul NR, Bushell TJ, McConnell G.
An evaluation of multi-excitation-wavelength standing-wave fluorescence microscopy (TartanSW) to improve sampling density in studies of the cell membrane and cytoskeleton. ***Sci Rep.*** 2021; 11: 2903.

Spennati G, Horowitz LF, McGarry DJ, Rudzka DA, Armstrong G, Olson MF, Folch A, Yin H.
Organotypic platform for studying cancer cell metastasis. ***Exp Cell Res.*** 2021;401:112527. 21.

Toti A, Santi A, Pardella E, Nesi I, Tomasini R, Mello T, Paoli P, Caselli A, Cirri P.
Activated fibroblasts enhance cancer cell migration by microvesicles-mediated transfer of Galectin-1. ***J Cell Commun Signal.*** 2021; 15: 405-419.

Tran BM, Flanagan DJ, Phesse TJ, Vincan E.
Frizzled7 Activates beta-Catenin-Dependent and beta-Catenin-Independent Wnt Signalling Pathways During Developmental Morphogenesis: Implications for Therapeutic Targeting in Colorectal Cancer. ***Handb Exp Pharmacol.*** 2021; 269: 251-277.

Wu Y, Bai Y, McEwan DG, Bentley L, Aravani D, Cox RD.
Palmitoylated small GTPase ARL15 is translocated within Golgi network during adipogenesis. ***Biol Open.*** 2021; 10.

Yelland T, Garcia E, Samarakoon Y, Ismail S.
The Structural and Biochemical Characterization of UNC119B Cargo Binding and Release Mechanisms. ***Biochemistry.*** 2021; 60: 1952-1963.

Stott KJ, Phillips B, Parry L, May S.
Recent advancements in the exploitation of the gut microbiome in the diagnosis and treatment of colorectal cancer. ***Biosci Rep.*** 2021; 41

Zarou MM, Vazquez A, Vignir Helgason G.
Folate metabolism: a re-emerging therapeutic target in haematological cancers. ***Leukemia.*** 2021; 35: 1539-1551.

THESES

Brucoli, Martina (2021) Addressing a non-cell autonomous tumour suppressive role for Dram1 in inflammation-induced colorectal cancer. [PhD thesis, University of Glasgow, Beatson Institute]

Cangiano, Mario (2021) Network based analysis to identify master regulators in prostate carcinogenesis. [PhD thesis, University of Glasgow, Beatson Institute]

Chatrin, Chatrin (2021) Structural insights into ADP-ribosylation of ubiquitin C-terminus by Deltex E3 ligase. [PhD thesis, University of Glasgow, Beatson Institute]

Dabrowska, Adrianna (2021) Molecular characterisation of eIF4A paralogues. [PhD thesis, University of Glasgow, Beatson Institute]

Fey, Sigrid (2021) Investigating KRAS driven tumorigenesis, metabolism, and potential therapeutic targets in pancreatic cancer. [PhD thesis, University of Glasgow, Beatson Institute]

Kiourtis, Christos (2021) A study on the systemic transmission of cellular senescence and its role in acute liver failure [PhD thesis, University of Glasgow, Beatson Institute]

Kössinger, Dominik (2021) An investigation into the role of glioma-derived extracellular vesicles in infiltrative invasion. [PhD thesis, University of Glasgow, Beatson Institute]

Lapienyte, Laura (2021) Targeting the immune microenvironment in pancreatic cancer. [PhD thesis, University of Glasgow, Beatson Institute]

Mohammed, Bashir (2021) The development of ALICE-tRNA-sequencing and its use in exploring the role of tRNAs in translational control. [PhD thesis, University of Glasgow, Beatson Institute]

Nikolaou, Savvas (2021) Investigating the role of CYRI-B in pancreatic ductal adenocarcinoma. [PhD thesis, University of Glasgow, Beatson Institute]

Nössing, Christoph (2021) Investigating the regulation of cell death by p62/SQSTM1 & The role of RASAL1 in cancer. [PhD thesis, University of Glasgow, Beatson Institute]

Smith, Rachel (2021) Investig paralogues within tissue homoeostasis and colorectal cancer [PhD thesis, University of Glasgow, Beatson Institute]

Terradas Terradas, Maria (2021) Deciphering heterogeneity of the ageing haematopoietic compartment and predisposition to cancer using multi-omics approaches. [PhD thesis, University of Glasgow, Beatson Institute]

Watt, Dale (2021) Dissecting the role of TGFβ in pancreatic ductal adenocarcinoma. [PhD thesis, University of Glasgow, Beatson Institute]

CONFERENCES AND WORKSHOPS

Cancer Research UK Beatson International Cancer Conference

The Cartography of Cancer – Mapping Tumours in 3D

12–13 July 2021
Virtual Meeting
Scientific Committee: Kevin Ryan, Jim Norman, Gareth Inman, Laura Machesky, David Lewis, Leo Carlin, Owen Sansom

Recent advances in technology are revolutionising the way we can further our understanding of the molecular mechanisms of cancer initiation, progression and spread. While conventional methods often involve the dissociation of tissues, emerging technologies now enable us to preserve spatial information within the tumour or in relation to the tumour microenvironment and interrogate cancer in space and time in unprecedented breadth and depth. Along with the technology itself, this meeting aimed to focus on aspects of genetics, metabolism, microenvironment and immunology to address the cellular and metabolic rewiring that occurs during tumour development, to get a deeper understanding of how these networks are modified by cancer treatment – and ultimately help us build a 'Cartography of Cancer'.

We were delighted to welcome Josephine Bunch from the National Physical Laboratory (NPL) as our keynote speaker. Josephine is leading the Rosetta Cancer Grand Challenge that aims to develop a map of cancer similar to Google Earth, gaining resolution of tumours at the molecular, cellular and tissue level. With their unparalleled expertise in the art and science of taking precise measurements, she described the key role of the NPL in this project. She spoke about using a plethora of advanced techniques such as nanoscale secondary ion mass spectrometry and desorption electrospray ionisation to unpick the metabolic heterogeneity of tumours.

Throughout the two days we had excellent presentations from Ed Hawkins, Ed Roberts, Georgia Scita, Cristina LoCelso, Val Weaver, Rosalie Sears, Michael Sheetz, Leo Carlin, Gareth Inman, Rebecca Fitzgerald, Trevor Graham, Laura Machesky, Ralph deBerardinis, John Le Quesne and Alec Kimmelman; and Jason Andrechak, Alejandro Mayorca-Guiliani, Johan Vande Voorde and Sarah Palmer in the short talk category. Congratulations to Colinda Scheele from the VIB Centre for Cancer Biology in Leuven on the best short talk, showcasing her work describing how multi-dimensional imaging of tumour initiation in the breast can be used to answer the question of how healthy tissue structure prevents tumour formation.

In the virtual conference setting, a flash talk session with over 15 presentations from early career researchers stood in for the usual poster presentations. Toshiyasu Suzuki from the Beatson presented on how loss of BTNL1 mediates gamma delta T cell exclusion from intestinal tumours, and was announced winner in this category.

This year, we also held an insightful careers workshop with Maria Papatriantafyllou from FEBS and Daniel Klimmeck from EMBO, both editors who shared their experience of careers in publishing, as well as Beatson group leader Laura Machesky.

The meeting was generously sponsored by Cancer Research UK. Our gratitude also goes to Transnetyx, the European Association for Cancer Research, Li-Cor, Fluidigm, the National Cancer Research Institute, Merck, The EMBO Journal, FEBS Press and proteintech for their support.

Beatson Workshop 2022 – An Immunological Lens for Cancer

7-8 July 2022
Organisers: Ed Roberts, Megan Macleod

For 2022, we are looking forward to welcoming scientists back to the Institute in Glasgow for our workshop *An Immunological Lens for Cancer.* Over two days, sessions will span numerous themes including *Setting a Threshold for Immune Activation, Progress in T cell based Immunotherapies, Microenvironmental Control of Immunity* and I*mmunity at the Surface*. We are looking forward to keynote talks from Dr Jessica Strid (Imperial College London) and Prof Guido Kroemer (INSERM).

A key aim of our workshop is to provide opportunities for early career researchers to present their work and network with all attendees. We have encouraged invited speakers to share their talk with a relevant researcher from their lab. Flash talks will be selected from abstracts.

IPSCC 2021
Top: Poster session in the virtual environment of Gather.Town
Bottom: Organising Committee of the 14th IPSCC

14th International PhD Student Cancer Conference

16-18 June 2021
Virtual Meeting
Organising Committee: Francesca Alega, Valentin Barthet, Martina Brucoli, Adrianna Dabrowska, Marco de Donatis, Michalis Gounis, Konstantina Nikolatou, Christoph Nössing, Ilaria Puoti, Youhani Samarakoon, Marta Zarou, Lucas Zeiger

Since 2007, the International PhD Student Cancer Conference has brought together doctoral candidates from the top cancer institutes in Europe to interact and exchange with each other about their work, research and scientific life. This year, the Glasgow students took their turn in organising the meeting and invited attendees to a virtual Beatson Institute via the Gather.Town platform ,which recreated the main hall, lecture theatre, poster session room and social events area – The Pub and The Rooftop.

Over three days, we welcomed 160 students from 8 European institutes. Laura Machesky (CRUK Beatson Institute) opened the conference with her keynote talk where she described how cancer cells navigate their environment and respond to local stresses. We also heard from Ross Cagan (University of Glasgow) on curing 'Fly cancer and other bits' and Christian Frezza (CECAD Cologne and former Beatson postdoc) took us on a journey with fumarate hydratase, an enzyme and tumour suppressor.

A number of excellent presentations invited for stimulating discussions, including talks by Denise Grieshuber (DFKZ), Ryan Guilbert (CRUK Manchester Institute), Narissa Parry (CRUK Beatson Institute), Marianna Maniaci (SEMM: IFOM/IEO), Robert Wiesheu (CRUK Beatson Institute), Christel Ramirez, Tuyu Zheng (DFKZ) and Aadhitthya Vijayaraghayan (CRUK Cambridge Institute). Congratulations went to Vivien Veninga (NKI) who was awarded best talk for her work entitled "*Exploiting the potential of organoid co-cultures to shine light on tumor reactive immune cells from the innate and adaptive immune system*", and Eimear Flanagan (CRUK Manchester Institute) and Catarina Pechincha (DFKZ) who won best posters, selected by their peers via Mentimeter.

The third day was rounded off with a highly engaging careers session. The Glasgow-based Payam Gammage, Ed Roberts and Chris Halsey shared insights into their academic career progression. And intriguing alternatives were showcased by a medical writer, a scientist in a start-up company, medical affairs manager, bioinformatician and industry scientists.

We are grateful for generous sponsorship by New England Biolabs, Thermo Scientifics and Beckman Coulter.

SEMINARS

The following seminars were held at the Cancer Research UK Beatson Institute throughout 2021. Due to the pandemic epidemic, we continued with different set up; at the beginning of the year we had a fantastic series of seminars given by group leaders within the institute and many seminars were held virtually. Towards the end of the year, we were delighted to move to a hybrid system offering in person and virtual attendance.

January

Saverio Tardito, CRUK Beatson Institute, UK

Irene Miguel Aliaga, MRC London Institute of Medical Sciences, Imperial College London, UK

Vignir Helgason, Institute of Cancer Sciences, University of Glasgow, UK

February

Kevin Ryan, CRUK Beatson Institute, UK

Seth Coffelt, CRUK Beatson Institute, UK

Jennifer Morton, CRUK Beatson Institute, UK

March

Paul Shiels, Institute of Cancer Sciences, University of Glasgow UK

Jessica Wade, Imperial College London, UK

Jos Jonkers, Netherlands Cancer Institute, University of Leiden, Netherlands

Eileen Parkes, Department of Oncology, University of Oxford, UK

April

Joanne Edwards, Institute of Cancer Sciences, University of Glasgow, UK

Edna Cukierman, Fox Chase Cancer Center, Philadelphia, USA

Ke Yuan, School of Computing Science, University of Glasgow, UK

Carla Kim, Boston Children's Hospital, Harvard Medical School, Boston, USA

May

Frank Winkler, German Cancer Research Centre, Heidelberg, Germany

Leah Cook, University of Nebraska Medical Center, Omaha, USA

June

Alessandro Esposito, MRC Cancer Unit, University of Cambridge, UK

Adam Mead, Radcliffe Department of Medicine, University of Oxford, UK

August

Martin Kampmann, University of California, San Francisco, USA

September

Chiara Ambrogio, Molecular Biotechnology Center, University of Torino, Italy

Kristin Swanson, Mayo Clinic, Phoenix, USA

Johanna Joyce, Swiss Cancer Center Léman, Lausanne, Switzerland

October

Awen Gallimore, Systems Immunity Research Institute, University Hospital of Wales, Cardiff, UK

Tom MacVicar, Max Planck Institute for Biology of Ageing, Cologne, Germany

Lars Zender, Institute of Pathology, University Hospital Tübingen, Germany

Wendy Garrett, Harvard Chan School of Public Health, Boston, USA

Yibin Kang, Department of Molecular Biology, Princeton University, USA

November

David I. Quinn, Keck School of Medicine, University of Southern California, Los Angeles, USA

Deborah Caswell, Francis Crick Institute, London, UK

Anna Christina Obenauf, Research Institute of Molecular Pathology, Vienna, Austria

John Gribben, CRUK Barts Centre, London, UK

December

Giulia Biffi, CRUK Cambridge Institute, UK

Alessio Ciulli, School of Life Sciences, University of Dundee, UK

PhD STUDENTS, CLINICAL RESEARCH FELLOWS AND POSTDOCTORAL SCIENTISTS

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

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

PhD Students and Clinical Research Fellows

The purpose of our PhD training programme is to give graduates and trainee clinicians who are starting in research an opportunity to work in state-of-the-art laboratories with leading researchers. This enables them to assess and develop their research talents to the full and to use their period of graduate study as a springboard for their future career path. Our four-year studentships (or three-year clinical research fellowships) are designed to give graduates (or clinical trainees) who show a strong aptitude and potential for research the opportunity to complete a substantial research project resulting in high quality publications. We also support an extra year post-PhD for publication ready projects. As well as developing their laboratory skills, students receive training in safe working practices, writing project reports, research integrity and other transferable skills. Training also involves learning to be an independent scientist and students are central to the intellectual life of the Institute, attending and giving seminars and actively contributing to scientific discussions. Students are also given the opportunity to present to national and international conferences to enhance their network of scientific contacts.
Our students are fully integrated with University of Glasgow graduate school (www.gla.ac.uk/colleges/mvls/graduateschool) and are allocated primary and secondary supervisors who are jointly responsible for supporting and monitoring their progress. The primary supervisor is responsible for developing the student's research abilities, providing all practical support required for the project and dealing with any administrative matters required in relation to the University or funding body. The secondary supervisor gives additional guidance by providing independent advice on any matters concerning the studentship. Students are also assigned two independent panel reviewers to assist them in reviewing their progress and advising them on their training and career development needs. The PhD training programme is overseen by a senior member of the Institute (Professor Stephen Tait). There is also a range of support available to help ensure the health and wellbeing of students.

Postdoctoral Scientists

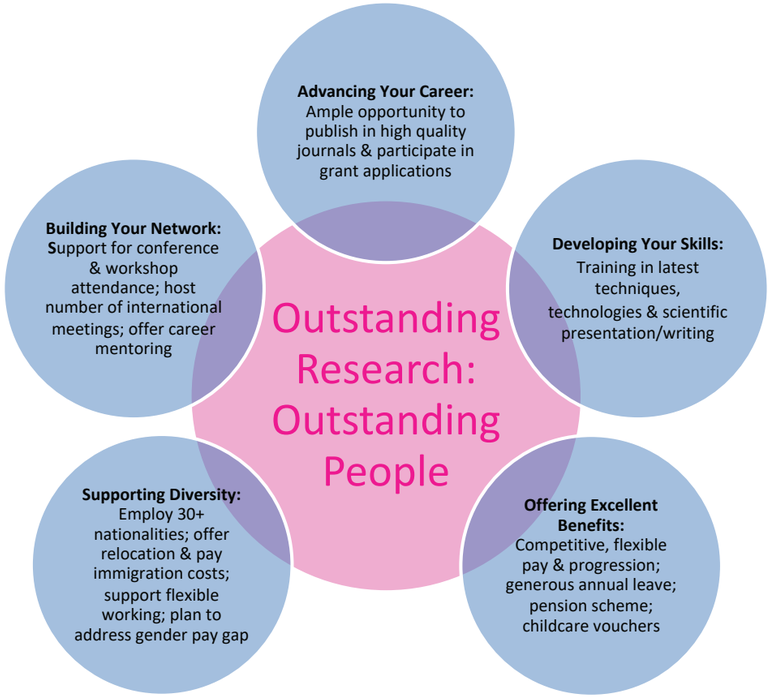
We see postdocs as pillars of the research and intellectual activities of their own groups and of the Institute as a whole. Our postdoctoral training, which is overseen by a senior member of the Institute (Professor Jennifer Morton), is designed to promote the development of outstanding and dedicated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

Postdocs are initially employed for three years but outstanding individuals who are developing into independent scientists may be given additional support and responsibility – such as more technical help or mentoring of a postgraduate student. At the discretion of their group leader, funding may also be extended for 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, Lynn Wilson, Patricia Wylie

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.

2021 continued to be a challenging year as the team had to work through the devastating impact of Covid-19 on our funding streams. Maximising our use of schemes such as Furlough and support from the Scottish Funding Council has been essential in minimising the longer term effects on our research.

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 (part-time)

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 2021, much of HR's focus was on managing the consequences of the pandemic, including staff communications, managing the furlough scheme, recruiting to key positions in very difficult circumstances and ensuring that staff wellbeing and mental health was properly supported. In addition to this, HR has been working with colleagues to focus on the Institute's Equality, Diversity and Inclusion agenda with the introduction of EDI Champions and the first EDI Survey across the Institute. Against a backdrop of difficult financial circumstances, we did manage a small pay increase for staff in 2021 and in 2022, we will be reviewing our pay ranges along with CRUK Cambridge and Manchester.

Administration

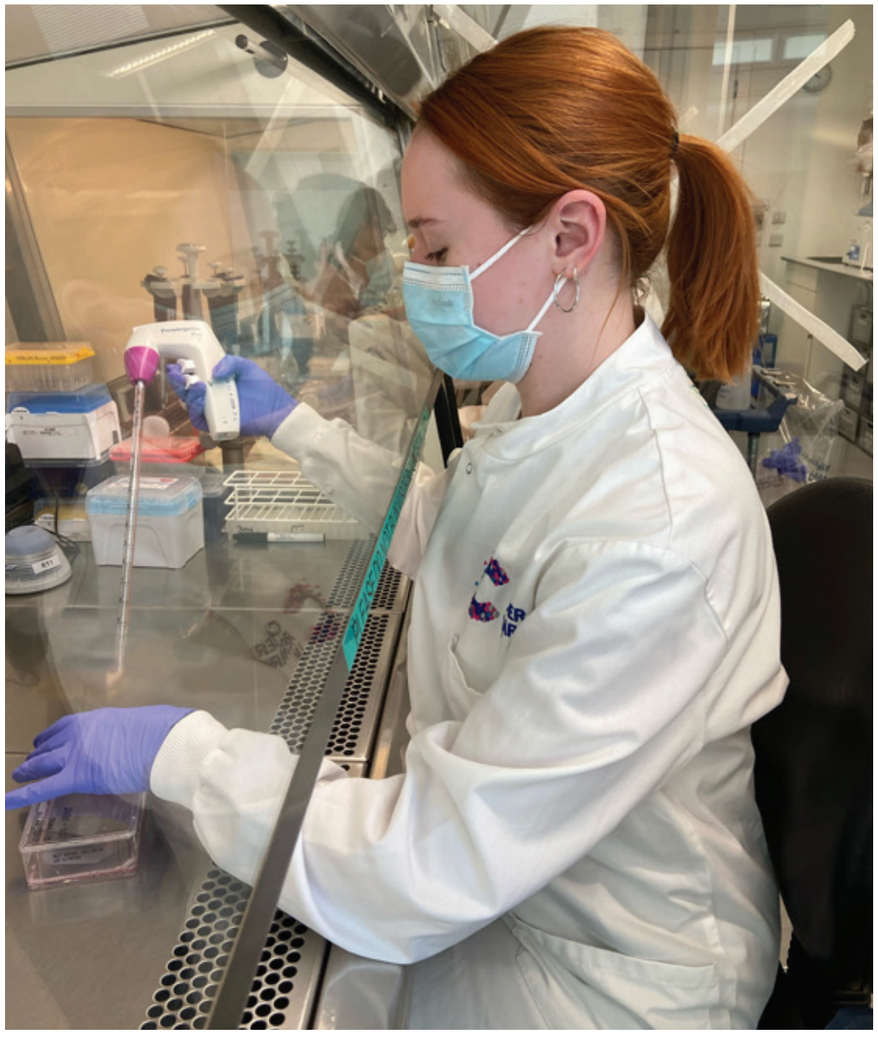
Sheila McNeill (Administration Manager), Rebecca Gebbie (PA to Director), Barbara Laing (Admin Officer), Sarah Price (Admin Officer), Catriona Entwistle (Admin Officer), Shona McCall (PA to Director)

The Administration team provides an extensive range of secretarial, admin and office services. These include assisting with staff recruitment; organising travel and accommodation; internal and external seminar arrangements; organisation of the Institute's annual conference, workshops and open evenings; database maintenance; and the operation of Reception for the Institute. 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, Angela Kelsey MSc, 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. In 2021, the team continued to work mostly from home supporting our researchers with their funding applications, manuscript submissions, student recruitment and communications as well as helping address various issues that arose during COVID. In September, Angela moved to a new role in the NHS, however we were fortunate that Kate was able to join us as her full-time replacement.



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 Institute 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. In 2021, we held International Women's Day, LGBTQ+ STEM Day and neurodiversity related events. However, reaching more diverse candidates is a priority for us for the future and this year, we established a search committee with an EDI remit for senior appointments to help us with this. We also appointed a group of EDI advocates and conducted a staff survey to better understand how we can improve EDI at the Institute. Finally, we published our gender pay gap report for 2021 (see opposite page), 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 working 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 full potential 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 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 Pay Differential Relative to Gender (PDR2G), 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. Compared to 2020, the mean hourly pay gap between females and males decreased by 2.96% points and the median hourly pay gap decreased by 1.02% points in 2021, though disappointing that our PDR2G has increased since 2019.

The figures shown here do not include Group Leaders who are employed by the University of Glasgow and who will feature in their PDR2G.

PDR2G pay gap			Calculations		
	Female	Male	**Apr-21**	Apr-20	Apr-19
Mean	17.04	18.98	**10.24%**	13.20%	9.9%
Median	16.66	18.53	**10.08%**	11.10%	7.10%

Gender pay gap vs equal pay

Equal pay has been a legal requirement 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, regardless of gender.

What is behind our gender pay gap?

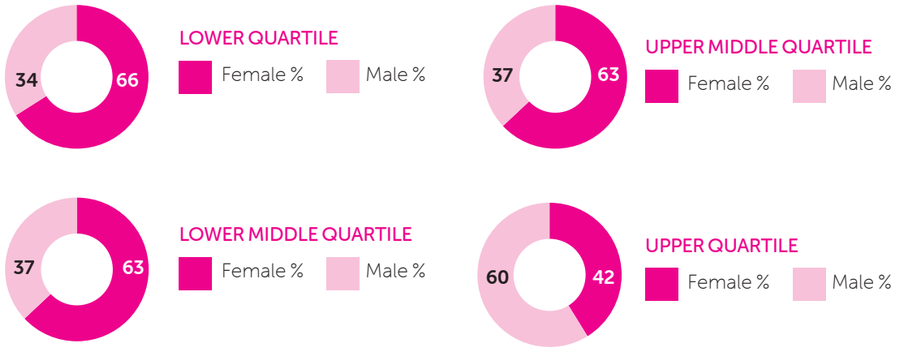
In 2021 our gender pay gap improved slightly with the mean difference between female and male salaries reducing 2.96 percentage points and the median reducing 1.02 percentage point. To understand this improvement, it's important to reflect on our recruitment activities over that period. In the past 2 years we have continued to recruit to key positions and we recruited 61% females compared to 39% males. The females we recruited were offered a starting salary of on average 3% more than newly appointed males. In addition, we also undertook a deep dive into our grades to review any discrepancies in pay between males and females and made a small number of adjustments to female salaries. This exercise was limited in 2021 by the impact of COVID on our core funding and budgets, though it is our intention to continue comparing male/female salaries by grade as part of our annual review process.

It is important to note that our most senior managers are not reflected in our PDR2G 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's important to highlight our pay gender ratios with them included. You will find these below. We will track and report on this data going forward.

PDR2G inc senior researchers on hybrid contracts April 21 pay gap			Calculations
	Female	Male	**Apr-21**
Mean	17.69	21.29	**16.88%**
Median	16.95	18.98	**10.68%**

In 2021 our workforce is 46% male and 54% female. When we rank the pay of our staff into 4 quartiles, we can see that there is a majority of females in the lower and lower middle quartiles – 66% and 63% respectively, though compared to 2020 the number of females in the lower quartile has decreased by 6% points. Interestingly the number of females in the upper middle quartile has increased – by 5%

points - suggesting that there is a shift from the lower quartile to the lower middle quartile for some females. The number of females in the upper middle quartile has remained the same and there has been only a slight change in the number of females in the upper quartile – 1% point.



Comparison of quartiles over past 3 years – 2021 to 2019

	M-2021	F-2021	M-2020	F-2020	M-2019	F-2019
Lower Quartile	34%	66%	28%	72%	29%	71%
Lower Middle Quartile	37%	63%	42%	58%	51%	49%
Upper Middle Quartile	48%	52%	48%	52%	54%	46%
Upper Quartile	59%	41%	61%	39%	58%	42%

What are we doing to close our gender pay gap?

The CRUK Beatson Institute is committed to reducing its PDR2G through actions identified in our pay gender 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 working in Science, Technology, Engineering and Mathematics (STEM) occupations has risen by 2% points in the past year to 24% of the STEM workforce, that is just over 1 million women working in STEM roles (WISE Annual Report 2019/20).

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 and either leave science completely or do not continue to pursue a research career. In 2020 and 2021, two thirds of newly appointed postdocs were female and whilst this is encouraging, we recognise that we need to translate the higher percentage of female postdocs pursuing a scientific research career into more senior positions such as Group Leader.

In recent years, an increasing number of female Postdoctoral Researchers at the Institute have taken maternity leave and we have been able to support their return to work through extension of their temporary contracts. This has provided them with more time to develop their scientific track record and potentially seek a PI position In the past there was a reluctance to request more flexible or part-time working arrangements but one of the consequences of the Covid-19 pandemic is that attitudes to more flexible and hybrid working arrangements have started to change and we have anecdotal evidence of more flexibility between lab, office and home working and we have recently introduced a hybrid working policy.

Review of areas for improvement

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.

- We have been limited in this action due to budget constraints and very flat pay increase in 2021, though we do intend to keep reviewing this on an annual basis as part of our pay review.

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

- We have introduced a hybrid working policy and one of the impacts of the COVID pandemic is that attitudes to more flexible working are changing with opportunities for staff to work from home, in the lab or office on an informal or more formal basis.

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

- We compiled our first search committee for a senior faculty position towards the end of 2021 to ensure that we were spreading our net as widely as possible and aiming for a 50/50 split in male/female applicants. More will follow on this in our 2022 report.

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.

- 87% of staff have inputted their EDI information to allow us to collate data and identify where the gaps are and inform our ongoing action plan
- We have also conducted an EDI Survey towards the end of 2021 and will report on this soon
- We have also introduced EDI Champions across the Institute to help embed good practice and behaviours towards a more inclusive and diverse workforce

In Summary

We are starting to see an improvement in our PDR2G and it's good to see that salaries for females in the lower quartile are starting to increase when compared to their male counterparts, however there is still work to be done to achieve greater equity in the lower quartiles. Whilst we have a good equilibrium in male and female pay in the Upper Middle Quartile, again we see very little improvement in the Upper Quartile where 60% are male. More work needs to be done to continue to address this such as encouraging more senior women to apply for senior positions.

Improving equality 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
AstraZeneca, University of Edinburgh, University of Glasgow Paterson Endowment Fund, Wellcome Trust

Karen Blyth
Breast Cancer Now

Martin Bushell
BBSRC, Celgene, MRC

Kirsteen Campbell
Prostate Cancer Research

Leo Carlin
Breast Cancer Now

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

Gareth Inman
British Skin Foundation, DEBRA, SANOFI

John Le Quesne
Celgene

David Lewis
Beatson Cancer Charity/Beatson Endowment

Laura Machesky

Jennifer Morton
MRC, Pancreatic Cancer UK

Kevin Ryan
The Kay Kendall Leukaemia Fund, Wellcome Trust

Owen Sansom
AstraZeneca, Celgene, McNab, MRC, NHS Greater Glasgow & Clyde Health Board Endowment Fund, Novartis, Pancreatic Cancer UK, Wellcome Trust

Sara Zanivan
Breast Cancer Now

Institute 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, Royal Society, Wellcome Trust

Beatson staff supported Andy MacKenzie in walking 22miles as for Breast Cancer Now's Wear It Pink campaign, raising funds in memory of his wife and former employee Elaine.

Robert Insall
ESPRC Physics of Life, Wellcome Trust

Kristina Kirschner
MRC, Saudi Government

Hing Leung
Horizon 2020, Prostate Cancer Foundation

Daniel Murphy
British Lung Foundation, Chief Scientist Office, Merck, Mick Knighton Mesothelioma Research Fund, Pancreatic Cancer UK, Puma Biotechnologies

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.

Allison, Tom
Better Points Ltd
Bremner, Scot
Brown, Margaret G
Byiers, A
Charities Aid Foundation
Charities Trust
Dempster, Senga
Devenny, Kathleen
Dunn, Etta – in memory of Mrs Dunn's Husband Charles
Gartley, Lesley
Goldie, Elizabeth – in memory of Mr David Goldie
Haddow, Avril
Hilton Worldwide Ltd.
James Inglis Testamentary Trust
Jaffery, Linda – raised through mask making
Kirkoswald Curling Club
Legacy from the Estate of the Late Mr Ian Boyd Holmes
Legacy from the Estate of the Late Mr Russell James Forgie
Lyke, Janet
McIntyre, GDS
McNeil, Fiona
Muir, M
Philips, Wilma
Slimming, Tom
Teevan, John – in memory of mother
The Kiltwalk
The Robertson Trust
Thomson, Jacqueline
Thornhill Gardening Society
Tommy Burns Skin Cancer Trust
Wales, JA
West of Scotland Women's Bowling Association

PATRONS AND BOARD OF DIRECTORS

Patrons

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

Board of Directors

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

Prof John Iredale (Chair)
Pro Vice Chancellor, Health and Life Sciences, University of Bristol

Mr Craig Anderson
Former Senior Partner, KPMG

Ms Rosalie Chadwick
Partner, Pinsent Masons

Dr Iain Foulkes
Executive Director, Strategy and Research Funding, CRUK

Ms Samantha Horne
Interim Finance Director, Cancer Research UK

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

Company Secretary
Mr Gary Niven
The Beatson Institute for Cancer Research

CONTACT DETAILS

The Beatson Institute for Cancer Research
Director: Professor Owen J Sansom
Tel: +44(0) 141 330 3953
Fax: +44(0) 141 942 6521

Address
Cancer Research UK Beatson Institute
Garscube Estate, Switchback Road
Bearsden, Glasgow G61 1BD

E-mail: beatson@gla.ac.uk
Website: www.beatson.gla.ac.uk

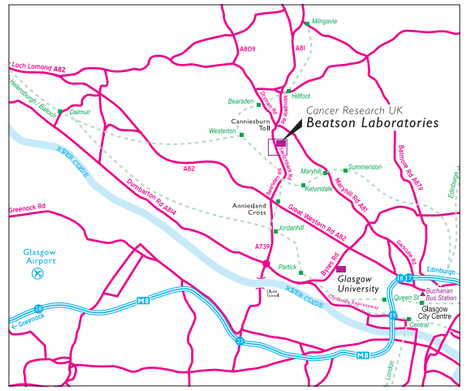
The Beatson Institute for Cancer Research is a registered charity in Scotland (SC006106) and registered as a company limited by guarantee in Scotland (84170).
Registered address: Cancer Research UK Beatson Institute,
Garscube Estate,
Switchback Road, Bearsden,
Glasgow G61 1BD

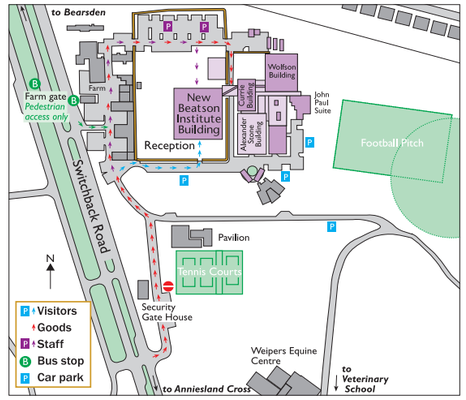
Tel +44(0) 141 330 3953
www.beatson.gla.ac.uk

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

ISSN 1479-0378

Edited by: Katharina Schraut
Jacqueline Beesley

CANCER RESEARCH UK
BEATSON INSTITUTE

Garscube Estate
Switchback Road
Bearsden
Glasgow
G61 1BD
Telephone +44(0) 141 330 3953

www.beatson.gla.ac.uk