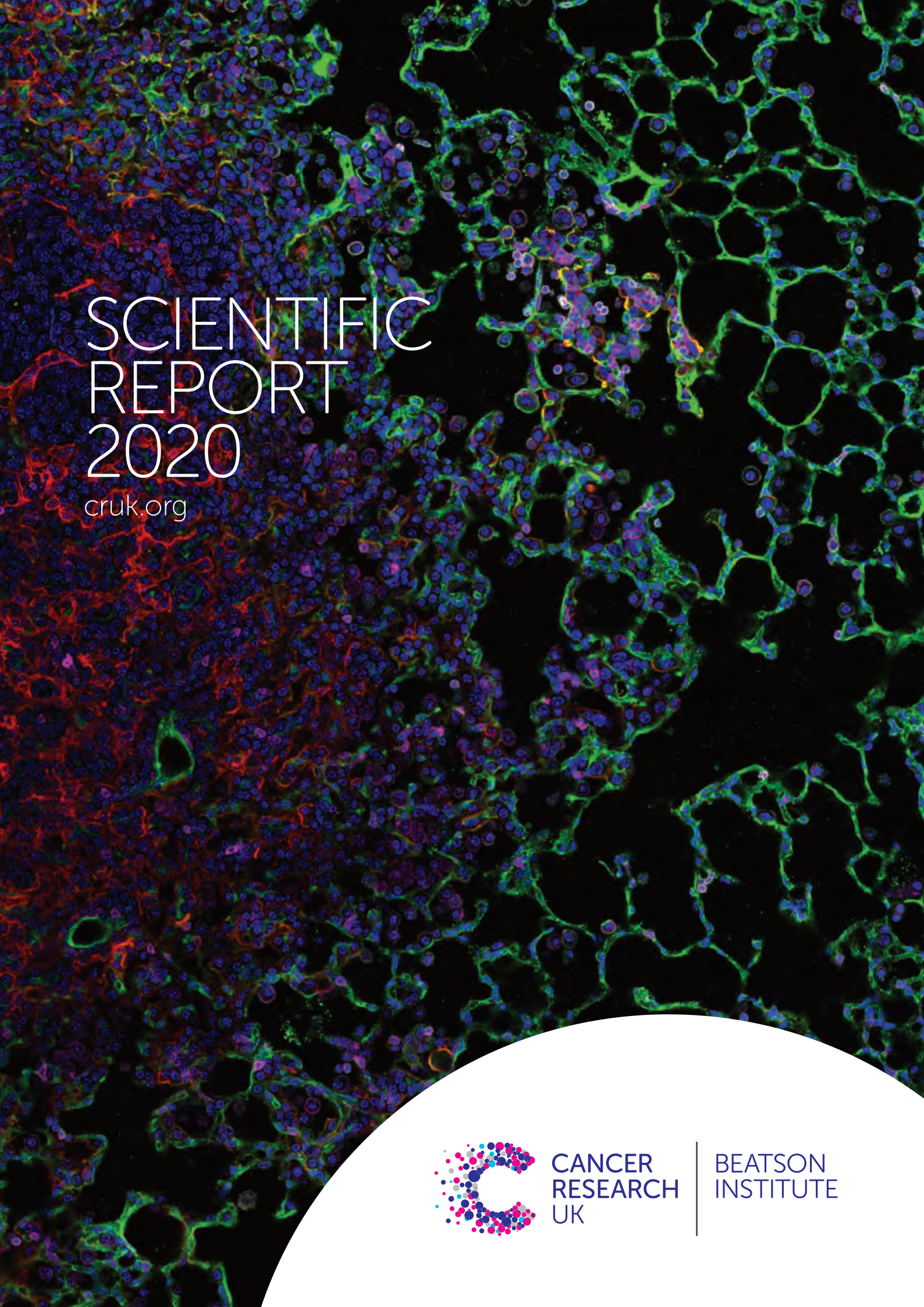

SCIENTIFIC REPORT 2020

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COVER IMAGE

Metastatic lung slice taken from an endpoint model of pancreatic ductal adenocarcinoma. Blue is the nuclei; Green is the blood vessels of the lung; Magenta is the immune cells; Red is the adhesion molecules on the blood vessels.

Photo by Marco de Donatis

SCIENTIFIC REPORT 2020

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

Driven by our mission to maintain our lead in discovery cancer research and to maximise clinical translation of our findings for the benefit of cancer patients, we focus on two main areas of cancer research at the Beatson: **energetic stress/cancer metabolism** and **microenvironment/metastasis**.

We are also building a third area aimed at understanding the **biology of early disease** with a view to developing scientifically-informed cancer prevention approaches. Despite the problems of COVID-19, and the impact that this has had on our funding, we have continued to make considerable advances in our strategic focus areas. These advances are highlighted by the number of ground-breaking papers that we have published this year and of which we can be extremely proud. Furthermore, in 2020 the Beatson Institute was extremely fortunate to receive a significant legacy from Ms **Annie McNab** and this has enabled us to maintain our lead in discovery and translational research whilst supporting certain critical research areas which have been brought into sharp focus by the pressures of COVID-19. Foremost amongst these is the need to support the young talented scientists and clinicians that are so important to the future of cancer research at the Beatson and further afield.

1) Supporting early career researchers. One of the consequences of COVID-19 is that early career researchers have experienced delays to their experimental work, publications, funding applications and progression to postdoctoral or independent positions. We believe it is important for us to ensure the next generation of cancer researchers is supported throughout this period, while also continuing to support excellence. We have therefore decided to use some of our McNab legacy to fund our PhD students and postdocs for additional years to conduct experimental work that is key to our strategy and to complete the submission and revision of important publications. We have also appointed a number of 'McNab Fellows' to further our work in targeting metastasis and these include: Dr **Colin Steele**, Dr **Fieke Froehling**, Dr **Seth Coffelt** and Dr **Kristina Kirschner**.

Group leader Dave Bryant, pictured with husband Zachary and their dog Lucy, encouraged people to support Cancer Research UK's Race for Life.

2) Maintaining our lead in cancer models. Sophisticated cancer models are central to our strategy, and despite the expense of genetically engineered mouse (GEM) models, we will continue to support these. Moreover, we believe that increases in mouse costs (leading to corresponding reductions in mouse numbers) poses a significant risk to cancer research in the UK. Therefore, it is critical that the Beatson maintains its leadership in the advance and application of mouse models of cancer. This key strength has been reinforced by my appointment this year as Director of the MRC's National Mouse Genetics Network. Furthermore, we are keen to sufficiently invest in liver cancer and mesothelioma, which are emerging areas we have recently developed as priorities. Taken together, these research programmes necessitate greater investment in disease-positioning to support development and characterisation of our models, as well as sequencing—including single cell sequencing—of key human samples.

It is of critical importance that we also build our capacity for exploiting human organoid approaches, and we have resolved to use part of the McNab legacy to develop these approaches to study how KRAS-driven colorectal cancers instruct the microenvironment of metastatic target organs (such as the liver, lung and bone) thus enabling us to identify the metabolic vulnerabilities of metastases. This work will be led Professor **Ross Cagan** (the University of Glasgow's recently appointed Regius Chair of Precision Oncology, formerly Mount Sinai, New York, USA). Ross has close connections with the Beatson and is utilising *Drosophila* to investigate cancer therapeutics and metastasis, particularly in colorectal cancer, making him an excellent fit for us. He will bring considerable strength to cancer research in Scotland and the rest of the UK. Importantly, this programme of human organoid-based research will be closely benchmarked against our GEM models of cancer.

Grant McGregor, Lynn McGarry and Natasha Malik from the Beatson Institute who helped set up the Lighthouse Lab in Glasgow.

3) Tumour mapping infrastructure. One of our key aims is to generate detailed spatial maps of human cancer tissue, and to overlap these with our complex mouse models. This is essential to ensure that the key biological processes we study in our preclinical models (i.e. energetic stress/metabolism and microenvironment/metastatic niche) reflect disease in patients, and that we accurately understand how these processes are modulated by therapy. Furthermore, use of state-of-the-art imaging in our mouse models and in human tissue will allow us to make critical new insights into the biology and potential vulnerabilities of the metastatic niche and tumour microenvironment. To drive this, we have appointed Professor **John Le Quesne** (from University of Leicester) to the University of Glasgow's Mazumdar Shaw Chair and Professor of Molecular Pathology, and invested in cutting-edge equipment for spatial transcriptomics, multiplexing immunofluorescence and high-resolution microscopy. John is a thoracic pathologist working on both mesothelioma and lung cancer, and one of the lead pathologists for CRUK's landmark TRACERx study to track small cell lung cancer evolution. Importantly, John's research focuses on how translational control can dictate proliferation and metastatic phenotypes, which fits well with our energetic stress strategy. John and his team will be housed in the Beatson, and we will be providing key resources and space so that this technology is adopted optimally throughout the Institute and used on key disease cohorts. In addition, we are keen to prioritise mass spectrometry approaches to image the metabolome and proteome of tumours and metastatic niches *in situ*, thus capitalising on our successful spearheading of these novel techniques within the Rosetta Grand Challenge consortium.

We said farewell to two of our junior group leaders in 2020 and wish them the very best in their new, exciting roles; Dr **Shehab Ismail** secured a professorship at the University of Leuven in Belgium, while Dr **Alexei Vazquez** took up a post at the German Aerospace Centre. One of our senior postdocs, Dr **Rene-Filip Jackstadt** also became an independent group leader at the DKFZ in Germany. The Beatson Drug Discovery Unit (DDU) scored 'Forefront' at its QQR held in March, which was a very positive endorsement of the work of Dr **Heather McKinnon**, Dr **Justin Bower** and their team, and our overall strategic approach to drug discovery. A KRAS agreement with Novartis was also signed at the start of the year.

Despite the challenges this year, several group leaders were awarded project grants (Professor **Martin Bushell**/BBSRC, Dr **Leo Carlin**/Breast Cancer Now, Professor **Kevin Ryan**/Kay Kendall Leukaemia Fund) or PhD studentship funding (Professor **Laura Machesky**/Medical Research Scotland, Professor **Danny Huang**/BBSRC AZ Industrial CASE, Professor **Gareth Inman**/British Skin Foundation), while Dr **David Bryant** was successful in his UKRI Future Leaders Fellowship application. One of our postdocs, Dr **Victoria Wang**, was also awarded a Wellcome Trust Fellowship, and we established several important collaborative agreements with industry: BMS, Aligos, Boehringer Ingelheim, AZ, RedX and Sityx.

Finally, in addition to research achievements, it is important to recognise other activities our staff and students participated in this year which are key to the ethos of our Institute. As part of efforts to combat the pandemic, the University of Glasgow has been hosting one of the Lighthouse Labs for COVID-19 testing at the QEUH campus. The Beatson Institute played its part in getting the Lighthouse lab up-and-running by donating PCR machines and vital reagents, and 18 staff members from a range of Institute teams volunteered their time and expertise to the lab: **Joanna Birch, Simona Buracco, Billy Clark, Sarah Edwards, Yasmin El Maghloob, Esther Garcia Gonzalez, Lynn McGarry, Grant McGregor, Sarah Palmer, Peggy Paschke, Nikki Paul, Karen Pickering, Christina Schoeherr, Andrew Shaw, Peter Thomason, Joe Waldron, David Stevenson and Natasha Malik**.

In addition, a number of our clinical research fellows suspended their PhD studies to return to work in the NHS full-time; the DDU helped a chemist from Strathclyde, Andrew Jamieson, to develop anti-COVID-19 peptides, by performing binding assays with their SPR machine; and Dave Bryant promoted CRUK's Race For Life At Home campaign in Scotland.

BEATSON STARS 2020

Many people at the Institute really stepped up in 2020 during what were very difficult times, often taking on new or additional roles to support others, reduce the impact of COVID-19 and ensure that our important research continued. These are the Beatson's 'stars'; individuals who work tirelessly, often behind the scenes, to keep a research institute like ours functioning at its very best. Hopefully, we have managed to recognise all of this year's stars but apologies if we have missed anyone, it certainly wasn't intended. We are very grateful to the individuals and teams listed here and highlighted elsewhere throughout the report.

Anna Kilbey in Seth Coffelt's group who worked in the Lighthouse Lab for several weeks before returning to the Beatson and quickly making up for lost time. Anna helped restart several research projects, including some for people who had caring responsibilities. Seth mentions how 'her optimism, unshakable motivation, dependability and steadiness have been a real inspiration for the whole team'.

Catherine Winchester from the Research Management team who like many others spent 2020 working from home but continued to offer a very high level of support to our researchers submitting grant applications and manuscripts.

Our HR team – Elaine Marshall and Selina Mungall - who throughout have worked to support our recruitment efforts, negotiating their way through Brexit and the constantly changing immigration requirements, and also set up and managed the furlough scheme, which so far has realised £1M in savings for the Institute. Angela Stuart, Head of HR, feels their efforts should be recognised as they have been 'stellar'.

Colin Nixon who kept Histology running like clockwork even in the midst of a huge rush of samples needing to be processed and in particular, Gemma Thomson in his team who not only helped in the restructured service during lockdown, but also patiently supported many researchers who needed to scan and analyse their slides – providing training and help setting up bespoke algorithms. With researchers working from home demand for this went through the roof, which meant Gemma was constantly on call. Her support was invaluable as it meant researchers were able to continue to analyse and publish their data.

All of the staff in the Biological Services Unit who have been described as 'amazing' for the essential role they played this year – firstly, preparing as we went into lockdown, when there was so much uncertainty, and then coming in every day throughout lockdown, often at antisocial hours to service the BSU and support our vital and world-leading *in vivo* research. Also, the many users of the facility who worked so hard coming in on rotas to support one another


Anna Kilbey


Catherine Winchester


Elaine Marshall


Gemma Thomson


Heather Spence


Peter Thomason


Ruhi Deshmukh


Yachuan Yu

and keep our mouse models going during this difficult time, including Karen Blyth, Rachel Ridgway and Ed Roberts who deserve a special mention.

Karen Thomas in Lab Management who took on a new, additional role in Stores in 2020 to provide maternity cover and maintain our supply lines at a time when there were many issues to be resolved as a result of both COVID and Brexit. Also, the Stores team - Emma Walker, George Monteith, Michael McTaggart – without whom our supplies would have quickly run out and James Dyball in Lab Management who maintained and serviced some vital equipment.

All members of the Drug Discovery team who over the last year showed commitment and flexibility in dealing with operational changes against the backdrop of the integration of the Drug Discovery Unit with Cancer Research UK. They have been hugely proactive and professional throughout.

Scott Kelso, Head of Lab Operations, who has been outstanding in steering us through the pandemic and ensuring our work has continued safely and in compliance with the regulations.

Heather Spence in Laura Machesky's group who worked hard to keep the lab and one of the Institute's busiest tissue culture facilities going and COVID-compliant while pushing forward with her research and remotely training several PhD students. Laura comments how 'she has constantly had a positive and proactive attitude toward making sure things are working well in a very challenging environment'.

Peter Thomason who not only kept everything running in Robert Insall's group but also helped cover for someone in the BAIR who needed to shield so that researchers could still access and use our microscopes. In addition, he worked for several months at the Lighthouse Lab.

Ruhi Deshmukh from Saverio Tardito's group whose endless availability and willingness to help allowed the rest of the group to carry on, at least to some extent, with their research projects. Ruhi took on a broad range of tasks, from cell culture to mass spec to imaging, and several times worked late so others could work during more 'normal' time slots.

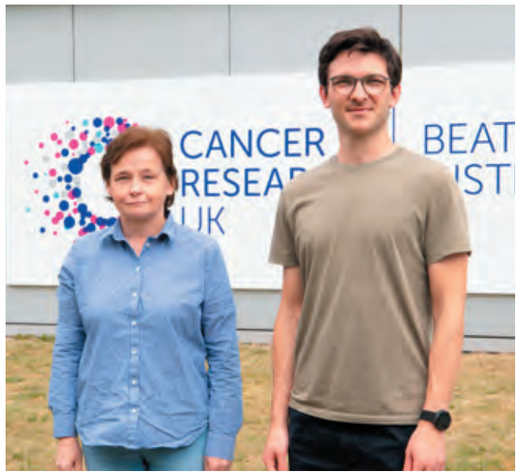
Yachuan Yu from Julia Cordero's group whose commitment during the last year has been amazing - working weekends, out of hours and helping multiple researchers with their projects, even during a rare visit to his family. Thanks to his unwavering support the group has been able to complete and publish two pieces of work. Julia is not sure what she would have done without him when things were so difficult.


Scott Kelso

Karen Thomas, James Dyball and Emma Walker

Peter McHardy and Iain White in IT who took on the major task of rapidly switching us all to using VPN, Zoom, etc. so that we could work from home and continue to be productive. Kevin Ryan comments that 'they have been 5-star'.

Tracy Shields and her team of Lab Aides - Linda Scott, Kirsty McPherson, Jonny Sawers, Euan Murray, Elizabeth Cheetham, Dilhani Kahawela and Connor Gilbey - who adapted their work patterns so that they could still come in and ensure our essential work was being serviced properly. Scott Kelso, Head of Lab Operations, says they have 'coped admirably throughout'.



As well as managing their own very demanding workloads, Lynn McGarry and John Mackey in the BAIR supported many others this year. Lynn was instrumental in setting up workflows in the Lighthouse Lab and worked there for a stint of many weeks, initially every day including weekends without a break, while John did a considerable amount of flow cytometry for others so that papers could be completed.

Alistair Wilson and his Facilities team, especially Andy Hosie and Mark Deegan who were relatively new to the Beatson when COVID hit, who kept the building running smoothly throughout and dealt with several contractors who needed to visit.

RESEARCH HIGHLIGHTS

COVID-19 had a significant impact on our research activity last year but our researchers adapted and continued to be productive. They published and contributed to 133 research papers in 2020 (this is compared to 104 in 2019). Projects are also still moving forward in the lab and yielding exciting data, particularly some of the larger team science ones. Here are some of the highlights in our key research themes from 2020:

Energetic Stress / Cancer Metabolism

One of our priorities is to understand energetic stress and metabolism *in vivo* to allow us to target metabolic vulnerabilities. This year, our researchers made key mechanistic insights that may allow us to target protein synthesis or protein turnover in the future. By manipulating amino acid transport, they now have strategies for targeting protein synthesis in colorectal cancer following metabolic rewiring (Najumudeen *et al.* Nature Genetics 2021). Importantly, they also showed that SLC7A5, a glutamine transporter, is critical for colorectal tumourigenesis in models of both early and late-stage, metastatic disease. In addition, using structural biology approaches, Danny Huang's group described a mechanism where ubiquitin itself becomes a target for post-translational modification and identified novel targets that could be inhibited through protein turnover (Ahmed *et al.* Sciences Advances 2020). As part of our translational control initiative the Bushell, Le Quesne and Sansom groups have published an exciting novel combination of MNK/MTOR inhibition for KRAS mutant cancers (Knight *et al.* Cancer Discovery 2020). We are now working with Richard Wilson (University of Glasgow) and Sarah Blagden (University of Oxford) to secure funding and develop trials in metastatic KRAS mutant colorectal cancer.

Biology of Early Disease

We continue to use our model systems to understand the early events of carcinogenesis. Interlinking the biology of early disease with energetic stress, researchers this year showed that mitochondria mutations are associated with tumour initiation in colorectal cancer and may induce a targetable state (Smith *et al.* Nature Cancer 2020). Furthermore, the Gammage group re-analysed the mutational spectrum of the mitochondria genome and found that mitochondrial mutations are functional drivers in colorectal cancer, suggesting a key role in tumour initiation (Gorelick *et al.* Nature Metabolism 2021). We have also generated a number of exciting, novel models of early disease (Flanagan *et al.* Nature 2021, Leach *et al.* Nature Communications 2021), which will form the basis of an early detection programme application (colon, liver, lung/mesothelioma) and be associated with the development of the MRC National Mouse Genetics Network.

Microenvironment/Metastasis

For us to be able to stop recurrence and target metastasis, we need to understand the significance of targeting different cells in the tumour microenvironment. This year, our researchers described novel mechanisms of how pancreatic tumours evade Natural Killer cells (Muthalagu *et al.* Cancer Discovery 2020). This involves the suppression of the type I IFN pathway by mutant KRAS and deregulated MYC. Bringing together two key research themes, Laura Machesky's group identified metabolic determinants for pancreatic cancer metastasis (Papalazarou *et al.* Nature Metabolism 2020). They used metabolomics to reveal that the creatine-phosphagen ATP-recycling system is a major mechanosensitive target. Importantly, this study straddles our microenvironment and metabolic themes, and together with publications from 2018-2019 and a body of ongoing work in the Machesky, Norman, Zanivan, Bushell and Sansom labs, helps consolidate our position as the world's leading centre for studying the role of metabolism in metastasis and the microenvironment. Continuing this focus on the pancreas, researchers also modelled key concepts of cell migration that are conserved from *Dictyostelium* to pancreatic cancer tumour cells (Tweedy *et al.* Science 2020). They showed how cells navigate complex environments such as man-made labyrinths by following and leaving chemical 'breadcrumbs', allowing pursuing cells to instantly find the shortest path and to avoid dead ends. Following work showing the clear efficacy of CXCR2 (neutrophil) and checkpoint inhibition in hepatocellular carcinoma models, a CRUK supported HCC trial CUBIC opened this year.

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

This image shows the dynamics of the endocytic network, here labelled by the early endosomal marker RAB5A. Each shade of yellow represents a position in time for each vesicle. *Image by Anh Le*

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 is 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 PPARG 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 (PPARG, which encodes a ligand-activated transcription factor), as a promoter of metastatic prostate cancer. PPARG is a critical regulator of fatty acid and glucose metabolism, influencing lipid uptake and adipogenesis. In our model, upregulation of PPARG was associated with an activation of lipid signalling pathways, including upregulation of lipid synthesis enzymes (fatty acid synthase (FASN), acetyl-CoA carboxylase (ACC) and ATP citrate lyase (ACLY)), resulting in aggressive prostate cancer.

As a proof of principle, we were able to demonstrate that inhibition of PPARG suppressed tumour growth *in vivo*, with downregulation of the lipid synthesis programme. We showed that elevated levels of PPARG strongly correlate with elevation of FASN in human prostate cancer and that high levels of PPARG/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 PPARG/FASN and PTEN levels to identify patients with aggressive prostate cancer who may respond favourably to PPARG/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 this lipid synthesis phenotype may be driven through alterations in mitochondrial function and AKT3 activations (manuscript in preparation).

In addition to our knowledge, we are the first to demonstrate the strength of the Sleeping Beauty transposon model system in successfully determining low-frequency somatic mutations that may drive prostate tumourigenesis. 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.

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.* 2020).

Publications listed on page 96

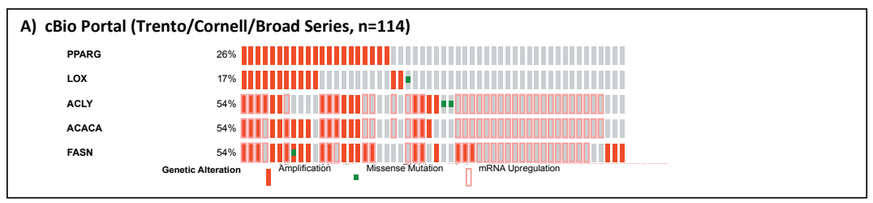


Figure 1
Data from cBio portal (www.cbioportal.org) demonstrating *PPARG* gene amplification or its upregulated mRNA expression in 26% of clinical castrate-resistant prostate cancer specimens, with upregulation of one or more of the lipid synthesis genes *(FASN, ACC, ACLY)*

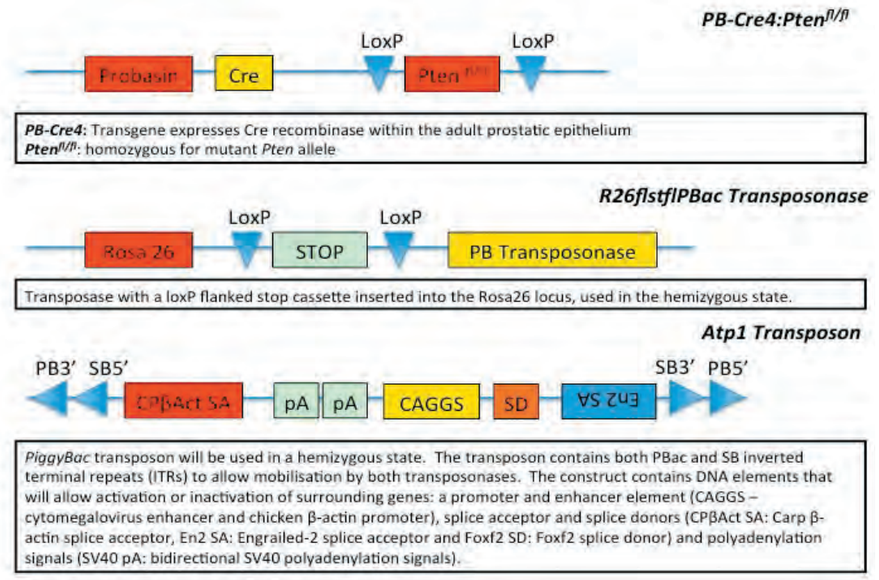
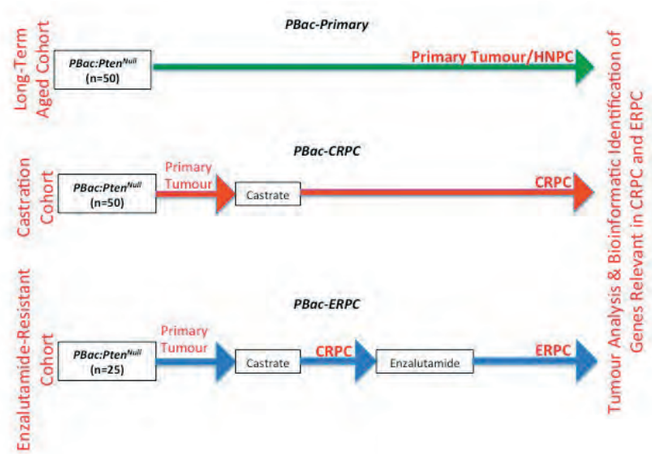
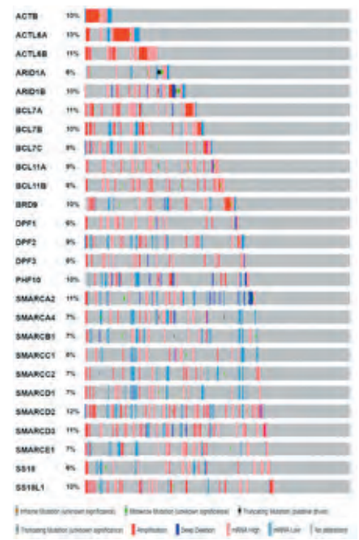


Figure 2
Genetic modifications of the PiggyBac mice.

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

Figure 4
Mutations in the BAF complex in metastatic prostate cancer





LIVER DISEASE AND REGENERATION

Group Leader
Tom Bird

Honorary Consultant Hepatologist and Wellcome Intermediate Clinical Fellow

Research Scientists
Stephanie May
Miryam Mueller[1]

Senior Scientific Officer
Ee Hong Tan[2]

Clinical Research Fellow
Tom Drake[3]

Graduate Students
Anastasia Georgakopoulou[4]
Christos Kiourtis

[1]Wellcome Trust
[2]CRUK HUNTER Accelerator/AstraZeneca
[3]University of Glasgow Paterson Endowment Fund
[4]CRUK HUNTER Accelerator

Liver cancer is the fourth most common cause of cancer-related death worldwide and is increasing at alarming rates, trebling in the UK in the last 25 years. Working at the interface between clinical care in the NHS and the development of preclinical models to study liver biology, the focus of my group is to understand dysregulated liver regeneration during cancer development with the aim of developing therapy to improve outcomes for patients 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 been identified in HCC. Understanding why in only a fraction of instances these mutations will lead to cancer formation is central to precision prevention strategies for liver cancer development and can 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 to study how hepatocytes escape the normal controls governing regulated regeneration. The GEMMs are designed using the genetic blueprint of different human HCCs and aim to identify novel therapeutic targets. The long-term 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.

Mechanisms controlling hepatocyte proliferation

Many pathways control liver proliferation and are dysregulated in HCC. One exemplar is the Wnt/β-catenin signalling pathway, which is crucial for establishing and maintaining the liver. Activation of the Wnt pathway is sufficient to cause hepatocytes to divide and the liver to grow. Nonetheless, this activation is itself insufficient to lead to tumour formation. A reason for this disconnection between hyper-proliferation and cancer is that upon pathway activation, anti-proliferative pathways are also engaged, preventing perpetual growth. These pathways also engage the immune system and can promote the clearance of potentially malignant cells.

We are investigating numerous signalling pathways in HCC, and how they might be controlled therapeutically. The process of preventing proliferation may result in a state of permanent cell cycle arrest known as senescence. Senescence leaves many of the functional abilities of the hepatocyte preserved but renders them incapable of participating in regeneration. In severe liver injury we described how senescence occurs in response to injury and activates immune cells (Fig. 1). Ongoing work suggests senescent hepatocytes are dramatically altered in their mechanisms of protein production and their interaction with their environment.

Transformation of regenerative hepatocytes into malignancy

We have developed a number of models of HCC utilising combinations of targeted cancer drivers in a clonal population of hepatocytes based upon the genetic changes most frequently occurring in human HCCs to create avatar-like models of human cancers (Fig. 2). We are then able track the expansion of the altered hepatocyte clones as they progress rapidly from single cells into large nodules and within months into HCC. These tumours model human disease well, including spread (metastasis) to other organs and responses to therapies. Using the CRUK Beatson Institute's advanced facilities, we are able to track and characterise tumours as they develop using a combination of preclinical imaging and molecular analysis. We study the evolution of tumours as they grow from a single transformed cell with a distinct phenotype to a tumour with a different phenotype. Our aim is to map the evolution of the tumours and test therapies aimed at preventing tumour initiation, expansion and metastasis. We are dissecting these models in collaboration with the CRUK HUNTER Consortium (https://research.ncl.ac.uk/hunter/). This consortium's aim is to create a network for HCC biomedical research and develop innovative HCC therapies through improved understanding of immune interactions with this cancer.

Ongoing work targeting cancer is examining combinations of therapies to target growth and senescence in HCC. As β-catenin mutations drive proliferation and are emerging also as a resistance pathway to immune checkpoint anti-cancer 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 can inhibit successful immune checkpoint anti-cancer therapy in preclinical models of HCC, and we aim to translate this with a clinical trial shortly. Using a pipeline of drug identification, we are examining repurposing existing anti-cancer therapies for subtype-specific treatment in HCC. We have shown that different types of GEMM respond differently to therapy and that therapies identified in this way can be highly effective in the GEMMs at both prolonging survival and eradicating tumours.

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 rescue therapies for those detected with late-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. We hope to provide a rationale for inclusion of these biomarkers in routine NHS practise to facilitate the early treatment and cure of HCC. We are collaborating with experts in public health and statistics to gather and analyse additional data collected from across Scotland and have already shown that applying novel statistical analysis of dynamic changes in serum biomarkers for individual patients can detect HCC in its early forms and make even the most advanced screening tests more accurate. With the integration of additional clinical variables and other biomarkers, we aim to improve the accuracy of this approach and move towards clinical trials.

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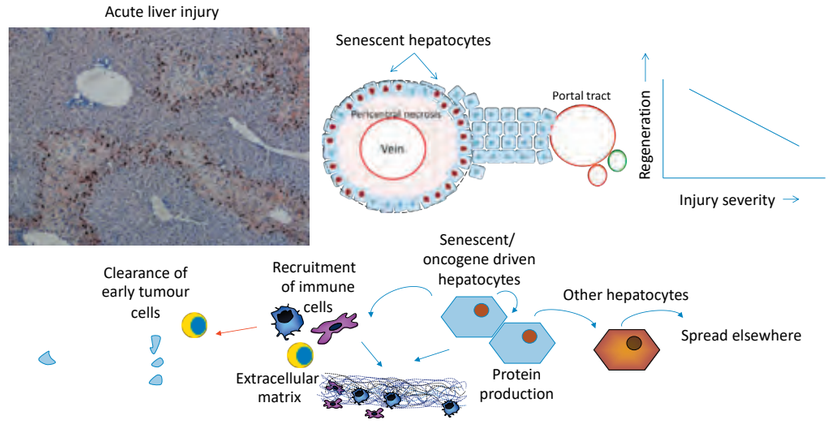

Figure 1
Damage/oncogene-induced senescence in the liver.
Following acute insult (e.g. paracetamol toxicity) senescence develops in hepatocytes, normally the regenerative cell of the liver. Instead of local regeneration, hepatocellular proliferation is activated elsewhere. However, when injury becomes increasingly severe liver regeneration reduces progressively. The senescent cells are able to affect their environment and influence tissue resident populations, in addition to stimulating cell recruitment and further cell senescence. (Adapted from Bird *et al.* Science Translational Medicine 2018). Senescent cells are growth arrested, acting as a break on cancer development. They are also targets for immune clearance to prevent cancer formation. Some oncogenes influence this immune interaction and are targets for anti-cancer therapies.

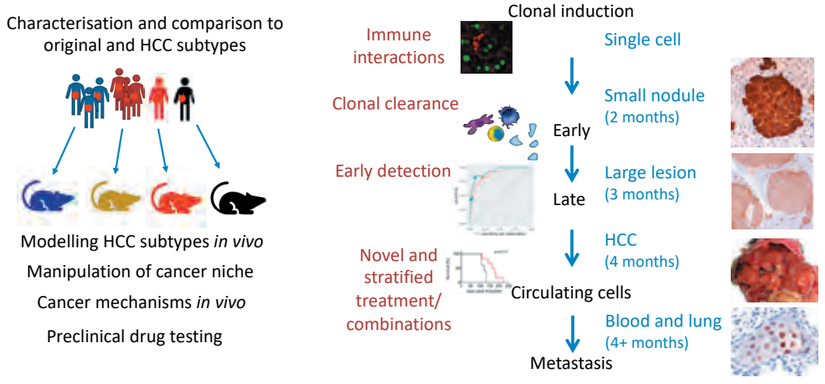

Figure 2
Human HCCs can be grouped into different functional and genetic subclasses.
We are mimicking the genetic alterations in human HCC subclasses using *in vivo* models in the mouse. Our strategy is to induce clonal hepatocytes with these targeted genetic alterations and then follow the clones as they develop into metastatic HCC. With this information and the ability to manipulate these cells and their environment separately, 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.

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IN VIVO CANCER BIOLOGY

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Our group 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 for patient benefit. 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

The *RUNX* genes are key transcription factors which play important roles in mammalian development, being for example indispensable for normal haematopoiesis and osteogenesis. However, these proteins, along with their obligate DNA binding partner CBFβ, are also implicated in various types of cancer, most notably in haematological malignancies but also in epithelial cancers. Indeed, *RUNX1* and *CBFβ* are among the most recurring genetic anomalies in breast cancer, with around 13% of all breast cancers exhibiting alterations in this gene family. Intriguingly, both loss and gain of RUNX function have been linked with different subtypes of breast cancer (Riggio & Blyth, *FEBS J.*, 2017; Rooney et al, *Adv Exp Med Biol.*, 2017). Our group have therefore been exploring this enigmatic role of the RUNX/CBFβ complex using *in vivo* models of breast cancer.

Loss of *Runx1* in the *MMTV-PyMT* mouse model results in early tumour onset. However, there is no difference in overall survival, and indeed there is selection against cells having lost RUNX1 at later stages in the disease progression, suggesting this model may exhibit both the tumour suppressor and pro-oncogenic features of RUNX1 function. Deletion of *Runx1* in an oncogenic β-catenin setting also significantly accelerates disease onset, and this phenotype is even more pronounced if both *Runx1* and *Runx2* are deleted. Loss of RUNX function with activated β-catenin elicits an abnormal expansion of a stem/progenitor population with a skewed enrichment of basal-like colonies in organoid culture, potentially expanding a population of cells exquisitely sensitive to the transforming properties of WNT signalling. Furthermore, by modulating *Runx1* in a murine epithelial cell line, we have conclusively shown that RUNX1 regulates mammary stemness, whereby loss of *Runx1* potentiates mammosphere capability (Figure 1A). Complementary to this, overexpressing *Runx1* seems to constrain the ability of the cells to form mammospheres (Figure 1B). It is notable these effects only happen in 3D cultures, whereas modulating *Runx1* does not affect mammary cell proliferation when cultured in 2D, thus providing rationale for the importance of investigating these genes in a physiologically relevant model.

Given that CBFβ plays a crucial role in the regulation of RUNX proteins, it is exciting that loss of *CBFβ in vivo* phenocopies the combined loss of *Runx1* and *Runx2* with accelerated disease onset in the same β-catenin driven mouse model of breast cancer. Whether CBFβ also regulates mammary stemness is currently being investigated. We are also profiling the transcriptional signature of RUNX/CBFβ deleted mammary tumours to unravel the mechanism/vulnerabilities of RUNX pathway alteration in cancer.

MCL-1 is a clinically actionable vulnerability in breast cancer

Around 11,500 women die each year in the UK from breast cancer and new treatments are required to help prevent these deaths. MCL-1 is a protein best known for its role in cancers of the blood, but we have found that high MCL-1 predicts poor prognosis in breast cancer (Campbell *et al, Cell Death Disease*, 2018). This is particularly relevant in triple negative breast cancer where treatment resistance and disease recurrence remain a major challenge. Using mouse models of breast cancer, we have found that MCL-1 is required for both tumour development and for maintenance of established tumours (Figure 2A).

Many functions for MCL-1 have been reported. In collaboration with Professor Stephen Tait, we have found that it is the anti-apoptotic function of MCL-1 that is key in breast cancer. This is important because a new class of drugs specifically targeting MCL-1 anti-apoptotic function, known as BH3-mimetics, have been developed and are in clinical trials for haematopoietic malignancies. In our experiments, targeting MCL-1 pharmaceutically, or genetically, in human breast cancer cell lines and in mouse models of breast cancer restricted growth and induced tumour regression *in vivo*. This highlights the potential for BH3 mimetic drugs targeting MCL-1 to be used to treat breast cancer.

We have also found that breast cancer stem cells, the cells thought to be responsible for metastasis and treatment resistance, are particularly dependent on MCL-1 and treatment with BH3-mimetics targeting MCL-1 effectively kills these cells (Figure 2). We believe that targeting MCL-1 could be particularly important in the context of advanced disease, treatment resistance and recurrence, and that targeting MCL-1 could offer a new therapeutic axis in breast cancer.

Challenges and Achievements

It has been an unusual and challenging year! But we are incredibly proud of the lab achievements under the circumstances, not least how everyone adapted to home working, lab meetings over Zoom, online seminars and courses, and carrying out essential research under COVID-secure conditions when access to the labs was permitted. We were delighted to publish our study describing a novel role for *RUNX1* and *RUNX2* as drivers of renal cell carcinoma and how these genes correlate with poor clinical outcome (Rooney *et al*, Cancer Research, 2020). Congratulations also go to our student Narisa Phinichkusolchit, who spent most of lockdown writing her PhD thesis '*ROCK1-mediated apoptotic blebbing in genetic models of tissue homeostasis and tumourigenesis*', which she successfully defended in November. It was very disappointing that we couldn't celebrate Narisa's success, due to COVID regulations, and while we will miss Narisa we wish her well in her new position in London. Meanwhile, we welcomed PhD student Matthew Winder to the group in October. Matthew will be further investigating the role of MCL-1 in breast cancer stem cells.

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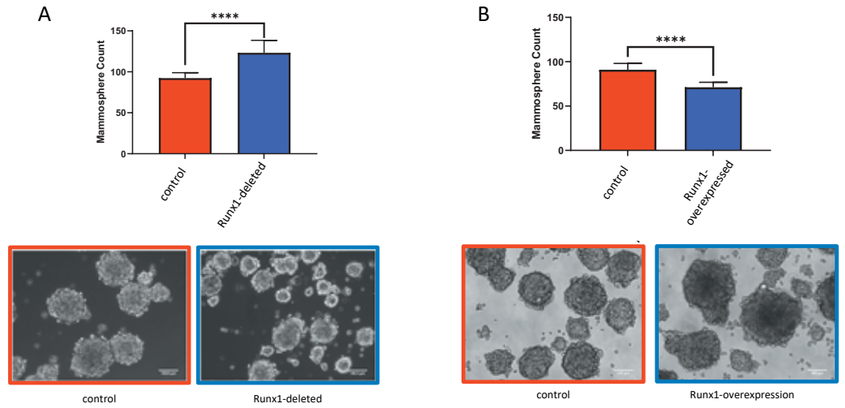

Figure 1
Runx1 regulates stemness in mammary cells. A) CRISPR/Cas9-based genome editing was used to delete *Runx1* in HC11 mammary cells. *Runx1*-deleted cells formed significantly more mammospheres than control cells in a 7-day assay, as shown in the graph and representative images. B) HC11 mammary epithelial cells with overexpression of *Runx1* resulted in significantly less mammospheres being formed compared to control cells.

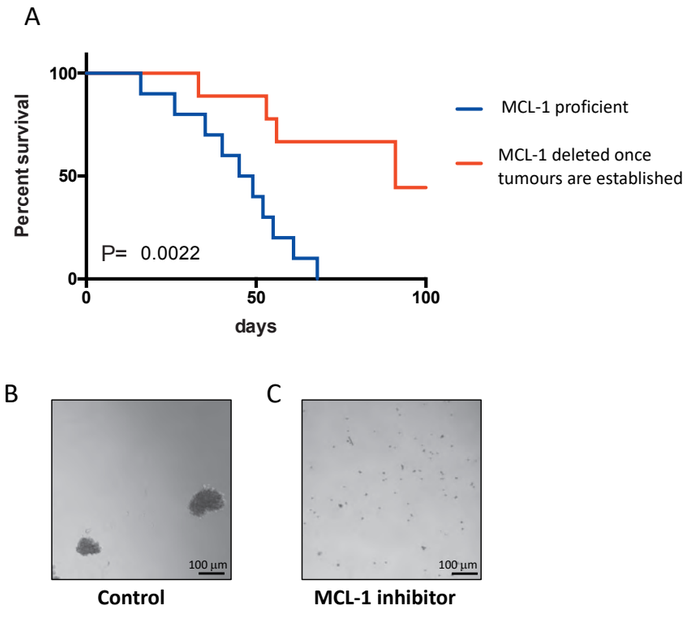

Figure 2
A. Genetic deletion of MCL-1 in mammary tumours prolongs survival. Tumour-related survival of mice whose tumours were allowed to grow to 5mm prior to *in situ* deletion of MCL-1 (red line, n=9 mice) or control (blue line, n=10 mice). Median survival increased from 47 to 91 days upon MCL-1 deletion with 4/9 mice showing complete tumour regression and long-term tumour-free survival. B-C. Tumoursphere assay reveals requirement for MCL-1 in breast cancer stem cells. Plating of breast cancer stem cells in non-adherent culture conditions gives rise to tumoursphere formation (**B**). Addition of a BH3-mimetic drug to target MCL-1 inhibits tumoursphere formation (**C**).

EPITHELIAL POLARITY

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

Our group studies the gain and loss of collective cell polarity and invasion in tumours. Our research is focused on two intersecting streams: 1) understanding the molecules that regulate collective cell polarity, and 2) developing the computational image analysis tools that allow us to dissect cell polarity.

Developing tools for collective 3D invasion analysis

Traditionally, cell movement has been studied using single cells grown on glass or plastic. However, 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.

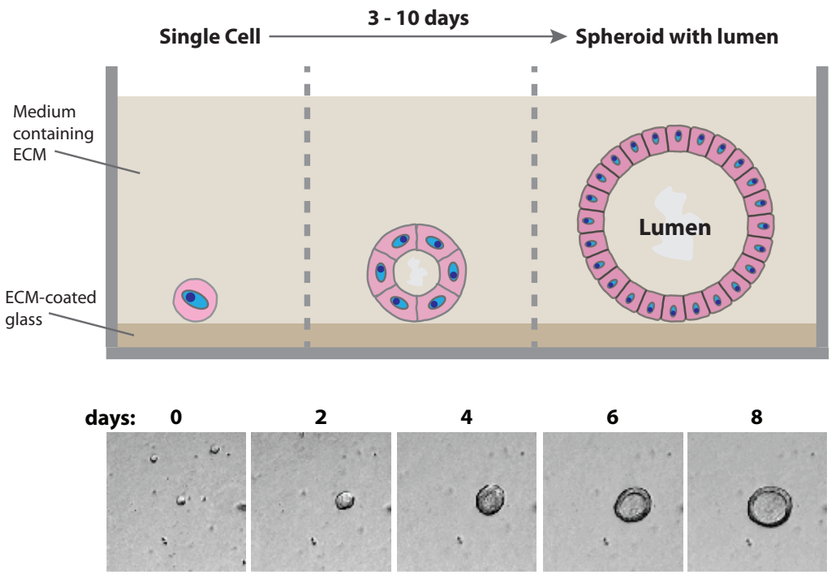

Figure 1
By culturing cells on glass-bottomed chambers coated with extracellular matrix (ECM), we direct the self-assembly of single cells into multicellular spheroid structures with a single, central lumen. This process occurs over 10 days, allowing us to study the dynamics of tissue formation.

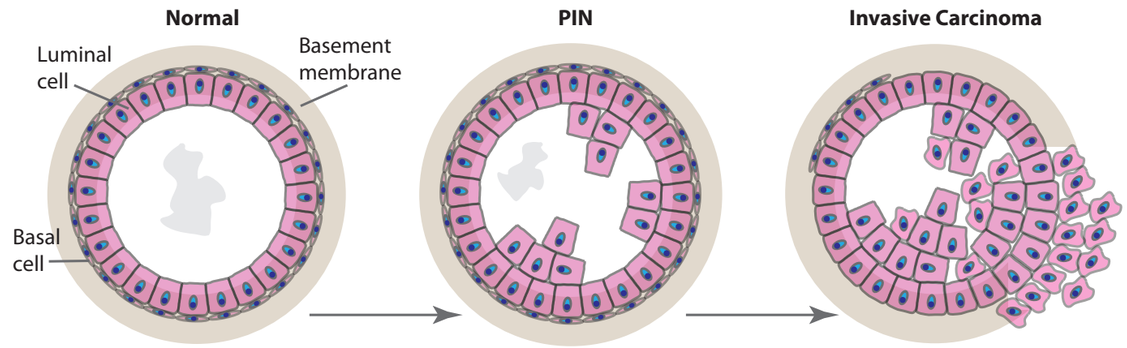

Figure 2
3D cultures of cells to form cysts (also called spheroids or organoids) also allows us to model the loss of normal tissue architecture that occurs in cancer. For example, the progressive disrupted organisation of Normal, to Prostatic Intraepithelial Neoplasia (PIN), to Invasive Carcinoma typifies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

In collaboration with the Blyth, Leung and Zanivan groups, we are interrogating their function in metastasis. We find that many ARFome family members assumed as redundant have highly divergent and sometimes opposing roles in invasion, and we show that there is specificity of signalling between family members. We have identified a key ARF GTPase module based around the IQSEC1 GEF protein that controls 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 group, 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.

Publications listed on page 99

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RNA AND TRANSLATIONAL CONTROL IN CANCER

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[5]CRUK SMERP award
[6]MRC Toxicology Unit
[7]CRUK core funded

The dysregulation of protein synthesis is an emerging hallmark of cancer, with altered translation being essential for the induction of oncogenic gene programmes. Tumour cells require enhanced production of proteins that drive cellular growth and division, while the stromal compartment is mainly engaged in producing extracellular proteins needed to create the microenvironment essential to support the growth of the tumour. These distinct programmes of gene expression drive tumour growth and create the supportive environment in which it flourishes. Our research is directed at understanding how certain core components of the translation machinery can selectively increase the rate of translation of key mRNAs encoding oncogenic proteins.

Understanding mechanistically how eIF4A1 drives tumorigenesis

RNA helicases are critical in sculpting and rearranging mRNA secondary structure within cells. As such, these enzymes play pivotal roles in every stage of the mRNA lifecycle. One such critical RNA helicase is eIF4A1, which is responsible for the rate-limiting initial step of mRNA translation. While all mRNAs require eIF4A1 for their translation, it is now clear that oncogenic mRNAs are more dependent on this protein for their translation, through the unwinding of RNA structures within their 5'UTR. Importantly eIF4A1 activity is frequently increased in tumour cells and as such a number of companies are developing drugs which target it for the treatment of cancers. Our recent work has uncovered unforeseen mechanistic insights on how this helicase functions to specifically promote oncogenic gene expression. We find that efficient unwinding of RNA secondary structure requires eIF4A1 to act in a multimeric form, composed of a loading subunit and one or more unwinding subunits (Fig 1a), which we've shown occurs both *in vitro* (Fig 1b) and in cells (Fig 1c). Moreover, the rate of unwinding is dependent on the sequence of the single stranded region of RNA bound by the loading subunit of eIF4A1, and highest levels of unwinding occur when this region is composed entirely of purines (Fig 1d). Our RNA structure profiling data shows increased secondary structure roughly 30-50nt downstream of 10nt stretches of purines in cells following eIF4A inhibition (Fig 1e) and *in vitro* translation assays show that adding a purine-rich sequence 30nt upstream of a hairpin was able to alleviate its inhibitory effect on translation (Fig 1f). Our data suggest that oncogenic mRNA translation is being driven by eIF4A1 through its role in unwinding RNA structure as a multimeric complex during scanning and that this is distinct from its role during ribosome recruitment.

Oncogenic gene programmes, codon usage and modulated tRNA expression

The degeneracy of the genetic triplet code means that multiple codons encode the same amino acid. It was long believed that in complex organisms the redundancy within the code had no functional consequences. However, the passive nature of decoding has been challenged and it is now clear that synonymous codon usage affects the expression of proteins through altering the rate of translation elongation and the mRNAs half-life. Critically, the expression of synonymous tRNAs is profoundly different in proliferating cells compared to differentiated cells and in tumours compared to normal tissues (Gingold *et al.* Cell 2014). Moreover, the anticodon signature of tRNAs in cancer cells specifically matches the codon composition of mRNAs required for cell proliferation. These observations suggest that genes required for proliferation are stabilised at the mRNA level and highly expressed in neoplastic clones due to the embedded codon usage and the levels of corresponding tRNAs. Using a combination of RNA-seq and tRNA-seq we show that in human fibroblasts, mRNAs with A/U ending codons are more highly expressed in proliferative conditions (fed) compared to when serum starved (Fig 2A).

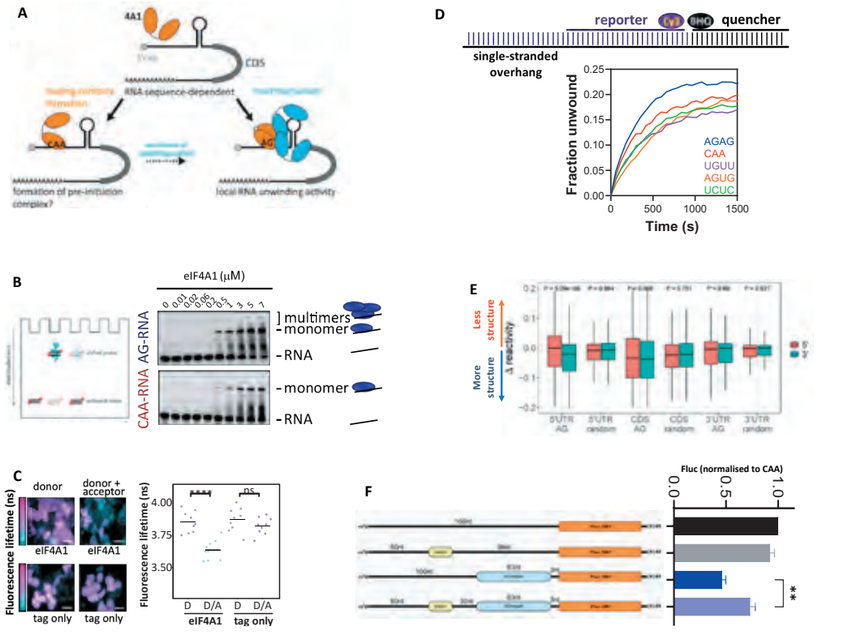

Figure 1
AG motifs stimulate the unwinding activity of eIF4A1 by inducing multimeric formation. (A) The following data support the model in which eIF4A can form multimeric complexes which are composed of a loading subunit and one or more unwinding subunits. (B) Electrophoretic mobility shift assay shows that eIF4A1 oligomerises on an AG but not CAA RNA substrate. (C) Fluorescence lifetime imaging with mCitrine and mTurquiose-tagged eIF4A1 show eIF4A1 oligomerisation also occurs in cells. (D) RNA unwinding assays show the helicase activity of eIF4A1 is highest with an AG-rich overhang. (E) RNA structure profiling following eIF4A inhibition in MCF7 cells with hippuristanol shows greater increased structure roughly 30-50nt downstream of AG rich motifs, but not random motifs from the same 5'UTRs or within the CDS or 3'UTR. (F) *in vitro* translation assays show that placing an AG-motif 30nt upstream of a hairpin, alleviates the inhibitory effect of the hairpin structure on reporter translation.

Indeed the tRNA profile matches this, with tRNAs containing A or U at the wobble position of the anticodon being more highly expressed in fed vs starved cells (Fig 2B). Recent data from yeast has shown that the CCR4-NOT component NOT5 is able to sense non-optimal codons, by binding to the E-site of the ribosome, when the A-site is unoccupied (Buschauer *et al.* Science 2020). Our data supports a model in which the CCR4-NOT complex is involved in sensing codon optimality in humans, as we observe that upon CNOT1 knockdown, mRNAs that are enriched for G/C-ending codons are most stabilised in human cells (Fig 2C). This is therefore consistent with a role of the CCR4-NOT complex being involved in the preferential degradation of mRNAs based on their codon usage.

What remains unknown is how the stoichiometry of the tRNA pool, and the proportion of tRNAs charged with amino acids, relates to the synonymous codon changes in different cellular states and tumour tissues. We are currently conducting tRNA sequencing in proliferative and quiescent cells as well as different tumour models in the institute to address these questions. These approaches will then be used to determine how these processes define and dictate the different gene expression programmes within the tumour environment and whether either specific tRNAs or amino acids could be possible candidates as therapeutic vulnerabilities.

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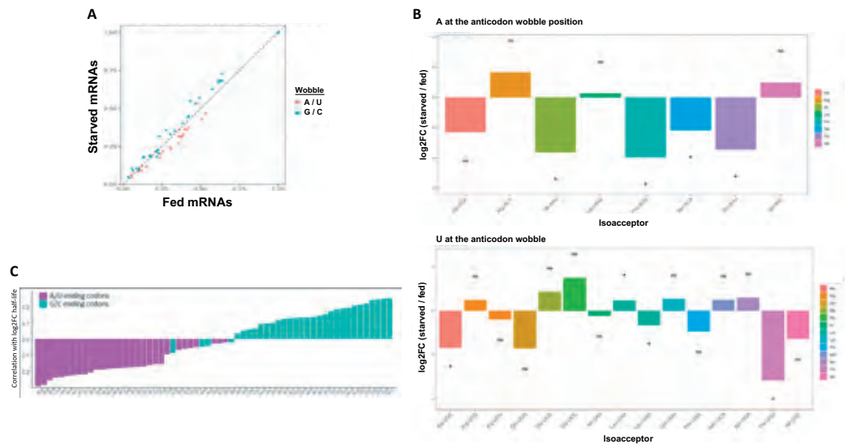

Figure 2
tRNA expression levels are altered between cellular states and determine codon optimality which is sensed by the CCR4-NOT complex. (A) The frequency of synonymous codons within mRNAs overexpressed in either fed or starved conditions in BJ-5TA fibroblasts. (B) log2FCs of all tRNAs with either an A or U at the anticodon wobble position between fed and starved conditions in BJ-5TA fibroblasts. In general these tRNAs have a negative log2FC, meaning they are more highly expressed in fed conditions. (C) 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 are coloured in magenta and codons ending in G/C are coloured in cyan.

LEUKOCYTE DYNAMICS

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The immune system can exert both anti- and pro-tumour activity; therefore, understanding the role of immune cells in the cancer microenvironment is of critical importance. Our lab uses cutting-edge light microscopy and other techniques to investigate the 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 they are exquisitely sensitive to their microenvironment, a feature previously thought to only apply to other myeloid cells. In addition to potent effector mechanisms, including phagocytosis, degranulation and the recently described process of NETosis, neutrophils can contribute to the inflammatory milieu in a number of ways. Neutrophils can produce and consume chemokines, cytokines and growth factors and can modify the extracellular matrix. Additionally, the accumulation of apoptotic neutrophils and their subsequent clearance is thought to directly contribute to anti-inflammatory programmes at the end of acute inflammatory responses. Taken together, these features mean neutrophils have the potential to both antagonise and promote tumours depending on context, 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.

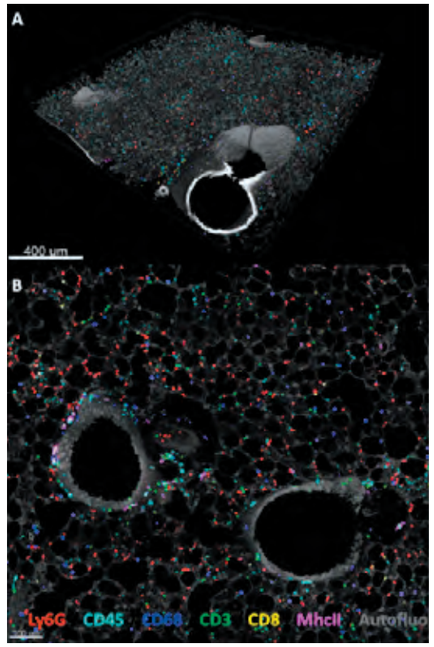

Figure 1
Imaging the (pre-) metastatic niche in the lung. **A.** rotated and **B.** top down projections of 3D multiplexed immunofluorescence to identify immune cells in the lung from a mouse bearing a primary mammary tumour.

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. Recently we have extended a cleared or live 3/4D tissue spectral unmixing workflow (see example image from Fred Fercoq in collaboration with the Roberts, Blyth and Coffelt groups) to image 6+ cellular markers *in situ*. We are actively applying this technique, which preserves important localisation data lost in many other techniques, to better understand the "who, when and where" of the immune (pre-)metastatic niche in the lung and liver.

As you would expect this has been a challenging year for our group. However, the team and our colleagues both in the Institute and further afield have shown great resilience and have made the most of any and all resources available to continue to make progress in addressing our central question: How do the immunological mechanisms that regulate neutrophil retention in the lung and liver (including their site of developmental origin) influence cancer, metastasis and immunotherapy?

Publications listed on page 100

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BIOLOGY OF THERAPEUTICS

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Translating therapeutics from the bench to the bedside has proven a challenge. Focusing on cancer and rare genetic diseases, my laboratory explores the 'biology of therapeutics': why do some therapies make the successful leap from pre-clinical to clinical while others fail? We use Drosophila as our lead tool to explore these questions, focusing on developing genetically complex models and using these to develop lead therapeutics including a fly-to-bedside clinical trial.

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 an experimental fly-to-bedside clinical trial 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 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.

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 this past year 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 'personalized 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.

Triple negative breast cancer: Our approach—capture a patient's tumour details with complex fly 'avatar' lines—has promise for a broad palette of cancer types. One class of cancers that is particularly challenging to model are those characterised by copy number variation (CNV). Chromosomal duplications and deletions can alter hundreds of genes, and identifying the primary cancer drivers in each region can be challenging. In a report we published this year, we took on this challenge for triple-negative breast cancer (TNBC), the breast cancer type with the highest mortality rate and for which we have few downstream treatments. As a result, fewer than 40% of women with metastatic TNBC survive five years.

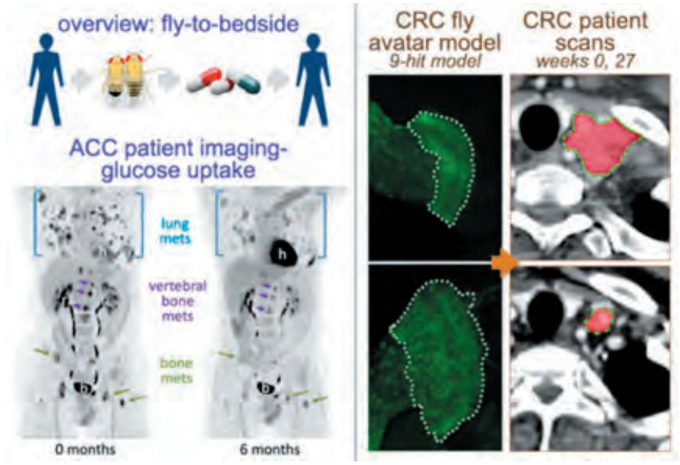


Figure 1
Our fly-to-bedside, which led to successful treatment of adenoid cystic and colorectal cancer patients.

To model TNBC in flies, we first performed a computational analysis, identifying the most common CNVs in TNBC, then using fly genetics to create a database of functional TNBC drivers. Using this database to create a set of fly TNBC transgenic lines, we demonstrated how the additional mutations promoted resistance to the chemotherapeutic drug fluorouracil, mirroring outcomes in patients. This database of fly lines provides a new tool in the search for more effective TNBC treatments.

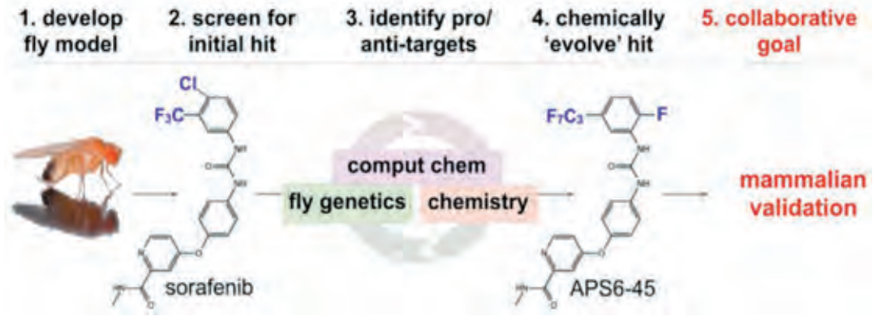
RASopathies: Rasopathies are a family of rare Mendelian diseases characterised by mutations that activate RAS pathway signaling. There are currently no treatments approved for RASopathies, a common situation for inherited diseases. Further, accruing sufficient Rasopathy patients for clinical trials is challenging and, ideally, a trial would accept a broad cross-section of Rasopathy patients.

As we recently reported, we have developed 14 Drosophila models that express human RASopathy isoforms of *PTPN11, KRAS, HRAS, BRAF,* and *RAF1* (Figure 2). 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 signaling 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 a drug company along with our growing database of RASopathy fly lines on promising therapeutic leads that do work broadly and hold promise for future 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 optimize efficacy and minimize liabilities including toxicity. Polypharmacology is challenging, and several laboratories including my own are working to bridge this chemistry gap. We recently reported a DREAM Challenge in which we challenged the computational chemistry community to develop innovative software to predict polypharmacology.

We have also developed a 'drug evolution' platform designed to attack disease networks through 'rational polypharmacology'. 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.

Figure 2
Platform to 'tune' therapeutic leads.



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IMMUNE CELLS AND METASTASIS

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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. We also study the role of immune cells during anti-cancer therapy response in ovarian cancer models.

At the beginning of 2020, we were excited to have Rob Wiesheu start as a PhD student after we secured funding from Breast Cancer Now. Soon after, the COVID-19 pandemic forced a major disruption to our work and dramatically slowed our progress. Our experimental work was limited, and we started working from home. Despite the upheaval, our lab accomplished many things in 2020. We were proud that two of our lab members made a huge contribution to controlling the pandemic by volunteering at the Lighthouse COVID-19 testing facility. Our lab wrote and contributed to six scientific papers. Mark Lawrence was featured in a media article on the impact of lockdown on his PhD studies (https://www.glasgowlive.co.uk/news/glasgow-news/glasgow-lives-lockdown-mark-25-18475727?utm_source=twitter.com&utm_medium=social&utm_campaign=sharebar). Rob Wiesheu won 1st place for his virtual presentation on anti-tumour γδ T cells at the British Society for Immunology London Translational Immunology meeting. Most importantly, we welcomed two new babies into the group.

Breast cancer

The first study from our lab was published in *Molecular Oncology*, describing the role of macrophage-stimulating protein (MSP) and its receptor, RON, in triple negative breast cancer mouse models. We showed that targeting the interaction between MSP and RON could delay breast cancer progression.

In previous years, we had 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 regulated IL-17A expression, while the function of ICOS remained unknown (Figure 1). Excitingly, mammary tumours regulated expression of another co-inhibitory molecule on lung γδ T cells, which is 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. We are testing different methods to increase their killing ability to use in adoptive cell transfer experiments to target primary and secondary tumours.

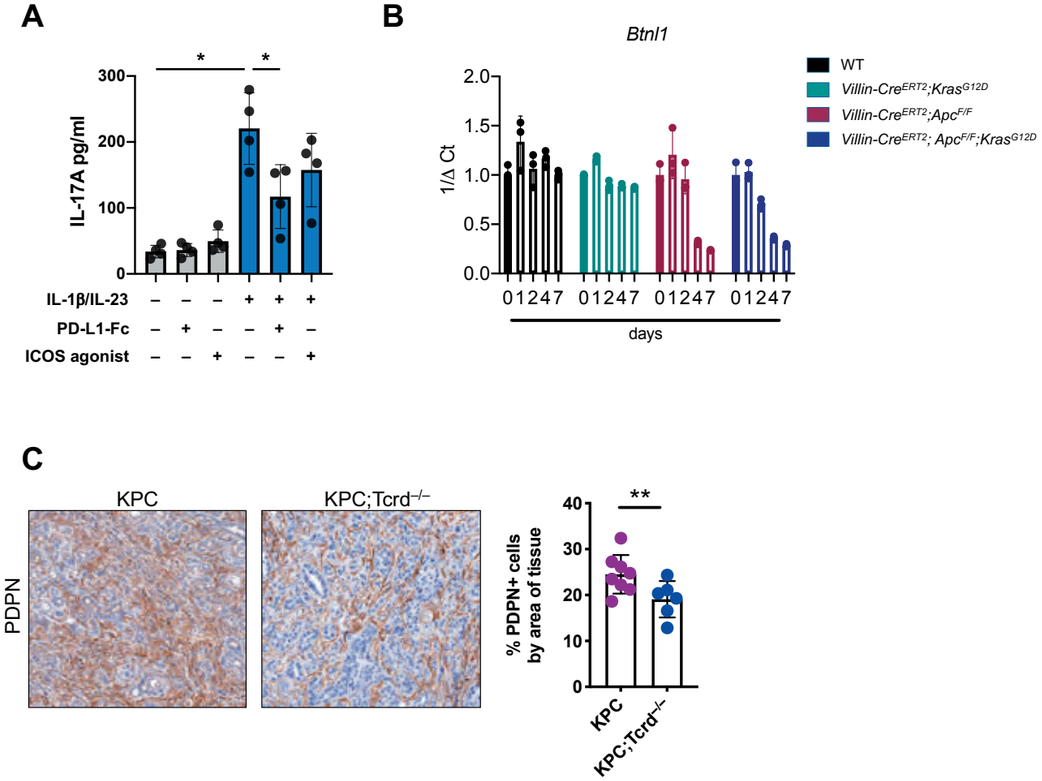

Figure 1
γδ T cells in mammary, pancreatic and colon cancers.
(A) γδ T cells were isolated from lung of wild-type mice and treated with IL-1b/IL-23. PD-L1-Fc fusion protein and ICOS agonistic antibodies were coated onto wells and used to stimulate PD-1 and ICOS receptors, respectively. ELISA was used to measure IL-17A. This experiment showed that triggering PD-1 (but not ICOS) on γδ T cells reduces IL-17A expression. (B) Organoids derived from the indicated genotype were treated with tamoxifen on Day 0 to induce Cre expression. mRNA was generated from organoids on days indicated. Expression of *Btnl1* was measured by qPCR. (C) Pancreatic tumour sections from KPC and KPC;*Tcrd*−/− mice were stained for expression of podoplanin (PDPN) by immunohistochemistry. The proportion of positive stain was quantified by HALO image analysis and the data are represented graphically. Each dot represents one mouse. **p < 0.01, Mann-Whitney U test.

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 T cell receptor chain and their role in cancer progression. We have found that these cells counteracted intestinal adenoma formation and killed transformed enterocytes in mice. When tumours developed, however, these cells were largely excluded from the tumour microenvironment. We have found that Butyrophilin-like 1 (BTNL1), a molecule expressed on gut epithelial cells required for survival of Vγ7 cells, was absent from tumours in the bowel. This observation has led to an examination into the mechanism of BTNL1 loss. We have found that deletion of the tumour suppressor *Apc* induced the down-regulation of *Btnl1* mRNA using organoids derived from our mouse models. This down-regulation of *Btnl1* was accompanied by decreased expression by gut-specific transcription factors. 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 drove 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 were reduced in pancreatic tumours from γδ T cell-deficient mice, indicating that γδ T cells regulated these cells in some way to support metastasis. Currently, we are investigating the mechanisms by which this occurs.

Ovarian cancer

PARP inhibitors are powerful drugs that induce synthetic lethality in ovarian cancer cells. We are studying how immune cells participate in the response to PARP inhibitor treatment in mouse models. We have focused our attention on macrophages, since the combination of PARP inhibitor treatment together with macrophage depletion reduced survival in mouse models. These data indicated that macrophages mediated some of the anti-tumour effects of PARP inhibitors. We are trying to understand how macrophages contribute to the efficacy of PARP inhibitors.

Publications listed on page 100

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MITOCHONDRIAL ONCOGENETICS

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Mutations of mitochondrial DNA 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 are limited in number, 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), that mtDNA mutational status is unaffected by nuclear DNA mutation burden or microsatellite stable (MSS) / microsatellite unstable (MSI) state (Figure 1b,c), that recurrent hotspots define the patterning of severe mtDNA mutations (Figure 1d) and 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. Armed with this information, we will now seek to create models of disease-relevant mtDNA mutations for further study.

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, in a cancer-specific fashion, commonly demonstrate significant 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 near-complete lack 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 102

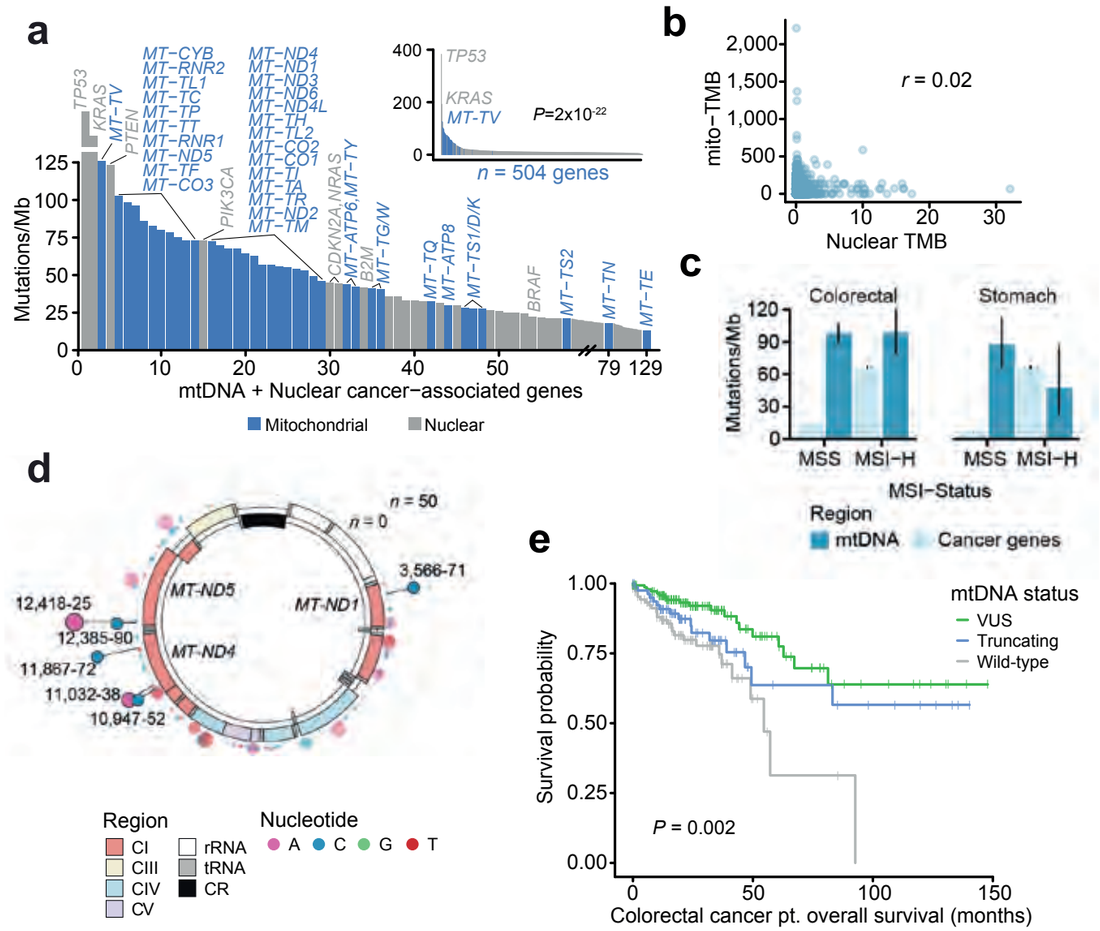

Figure 1

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

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UBIQUITIN SIGNALLING

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

Ubiquitin conjugation cascade

Covalent attachment of Ub involves three key enzymes, namely E1, E2 and E3 (Figure 1). E1 adenylates Ub's C-terminus in the presence of Mg^{2+} and ATP, followed by formation of a covalent thioester intermediate with Ub. E1 then recruits an E2 and transfers the thioesterified Ub to the E2's catalytic cysteine, forming an E2~Ub thioester intermediate (~ indicates the thioester bond). E3 generally consists of an E2-binding module (HECT, RING, RBR or U-box domain) and a protein–protein interaction domain that can recruit the substrate directly or indirectly. With this configuration, E3 recruits E2~Ub and the substrate to promote Ub transfer from the E2 to a lysine side chain on the substrate. In humans, there are ~600 RING E3s, and we are interested in uncovering their regulation and function and to explore the Ub system for cancer therapeutics.

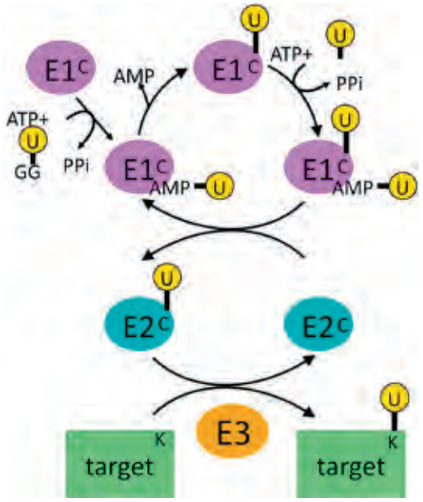
Deregulation in CBL ubiquitin ligase

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. We investigated the mechanism by which CBL mutants exert oncogenesis and showed that CBL mutants inactivate E3 activity thereby functioning as adaptor to recruit other proteins such as CIN85 to elicit oncogenic signalling. Mechanistically, CBL mutants bind to receptor tyrosine kinases such as EGFR, which leads to phosphorylation of CBL mutants' C-terminal tyrosines. Phosphorylated tyrosines induce conformational changes that enable CBL mutant-CIN85 interaction. CBL mutants cannot ubiquitinate CIN85, leading to deregulated CBL-CIN85 signalling which alters transcriptome landscape that in turn up-regulates PI3K-AKT signalling cascade to drive oncogenesis.

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 the 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 cannot 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, 24, 578-587). In collaboration with Prof. Karen Vousden's group at the Francis Crick Institute, we showed that expression of MDM2 E3-inactive mutant is tolerated in adult mice. Despite high levels of p53, the 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 regain homeostasis. These studies support the view that inhibitors that target MDM2 E3 activity could activate p53 in tumours with reduced on-target toxicities.

Figure 1
Enzymatic cascade for Ub modifications



It remains unclear how p53 is rapidly activated upon DNA damage. MDM2 is phosphorylated near its C-terminal region, notably Ser429, which is adjacent to the RING domain after DNA damage. We showed that Ser429 phosphorylation enhances MDM2's E3 activity. A crystal structure of pSer429–MDM2 RING domain bound to E2¬~Ub revealed that pSer429 functions by stabilising Ub to keep E2~Ub in the active conformation to enhance the activity (Figure 2A). Upon DNA damage, Ser429 phosphorylation enhanced MDM2 autoubiquitination and degradation in cells explaining the rapid p53 activation. We also described a strategy for preparation of MDM2 RING domain for structural analyses to enable rapid development MDM2 RING inhibitor.

Insights into Deltex ubiquitin ligases

Deltex (DTX) family E3s share a highly conserved C-terminal RING domain followed by a Deltex C-terminal domain (DTC). DTXs have been linked to developmental processes involving Notch signalling and histone ubiquitination during DNA damage repair. However, their functions remain largely unknown. We discovered that DTX E3s harbour dual activities and catalyse both ubiquitination of ADP-ribosylated substrate and ADP-ribosylation of Ub. We showed that DTX's C-terminal DTC domain harbour a conserved pocket that binds both ADP-ribose (ADPr) and NAD^+ (Figure 2B). The DTC domains also bind poly-ADP-ribosylated substrates in cells. The proximity of RING and DTC domain enables the DTX to catalyse the ubiquitination of ADP-ribosylated substrates. Interestingly, when DTC domain is bound to NAD^+, it catalyses Ub ADP-ribosylation. Mechanistically, the flexible linker connecting the RING and DTC domain enables the juxtaposition of the RING domain bound E2~Ub and the DTC-bound NAD^+; the reaction proceeds when Ub is released from E2, leading to ADPr modification at the C-terminal Gly76 of Ub. ADPr-modified Ub is a new post-translational code with unknown function, and we are investigating this exciting crosstalk. Moreover, we have shown that DTXs display different interactomes and are investigating their cellular functions.

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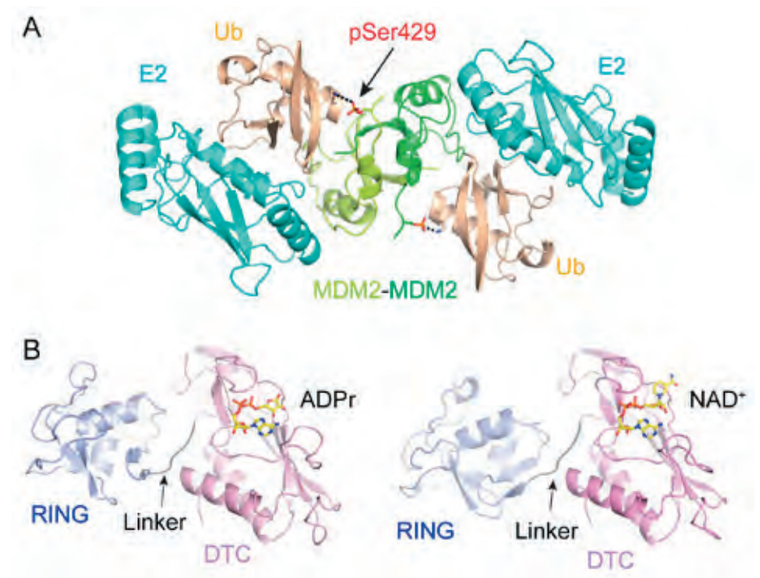
Figure 2
Structure and mechanism of RING E3s
A) Crystal structure of pSer429-MDM2 RING homodimer bound to E2–Ub. B) Crystal structures of DTX RING-DTC domain bound to ADPr (left) and NAD^+ (right).



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GROWTH FACTOR SIGNALLING AND SQUAMOUS CANCERS

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The transforming growth factor beta (TGFβ) superfamily comprises approximately forty related dimeric polypeptide cytokines, including the bone morphogenetic proteins (BMPs), the growth and differentiation factors (GDFs), activin, nodal and the TGFβs (TGFβ1, TGFβ2, TGFβ3). 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 cutaneous squamous cell carcinoma

TGFβ exerts its biological 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, accumulate in the nucleus and regulate expression of hundreds of target genes. In collaboration with Owen Sansom's group, we have previously shown that both TGFBR1 and TGFBR2 are mutationally inactivated in ~ 30% of human cutaneous squamous cell carcinomas (cSCC) and that combined deletion of TGFBR1 coupled with activation of the MAPK pathway is sufficient to drive rapid invasive cSCC formation from the Lgr5+ve hair follicle bulge stem cells in the mouse (Cammareri *et al.*, Nat Commun. 2016; 7: 12493). We are currently investigating how driver gene combinations act in concert with loss of TGFβ signalling to influence cSCC progression both *in vitro* and *in vivo*. As well as possessing potent tumour suppressor activity, members of the TGFβ superfamily can also act as potent tumour promoters. Our analysis of sporadic cSCC indicates that TGFβ signalling is maintained in ~70% of tumours, and we are investigating if this may drive tumour progression and represent a potential therapeutic target.

cSCC is a significant life-threatening complication for patients who suffer from recessive dystrophic epidermolysis bullosa (RDEB), a skin blistering disease caused by germline mutations in collagen VII, the anchoring fibril component which is responsible for maintaining normal dermal–epidermal junctional architecture in the skin (Figure 1). Unlike in sporadic cSCC, RDEB SCC tumours do not contain inactivating mutations in TGFβ receptors (Cho et al., Sci Transl Med. 2018; 10. pii: eaai7795), and our studies indicate that they exhibit elevated canonical TGFβ signalling activity. We are investigating the potential tumour-promoting role of TGFβ signalling in RDEB cSCC in collaboration with Dr Andrew South (Thomas Jefferson University,

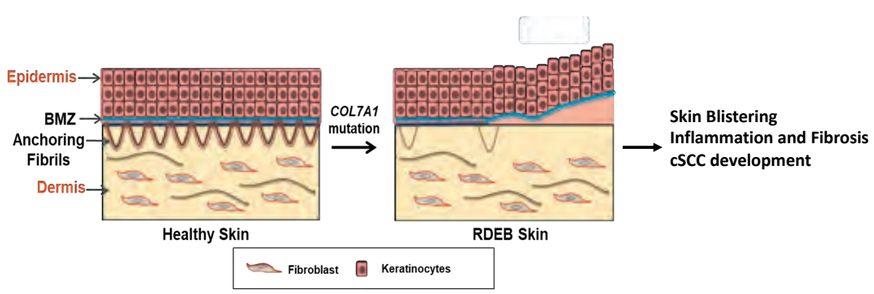

Figure 1. Loss of type VII collagen promotes skin blistering in RDEB patients. RDEB patients have loss-of-function mutations in the collagen VII gene, which results in separation of the epidermis from the underlying dermis as a result of mild friction/trauma. This results in severe blistering and wounding of the skin, leading to inflammation, fibrosis and cSCC development.

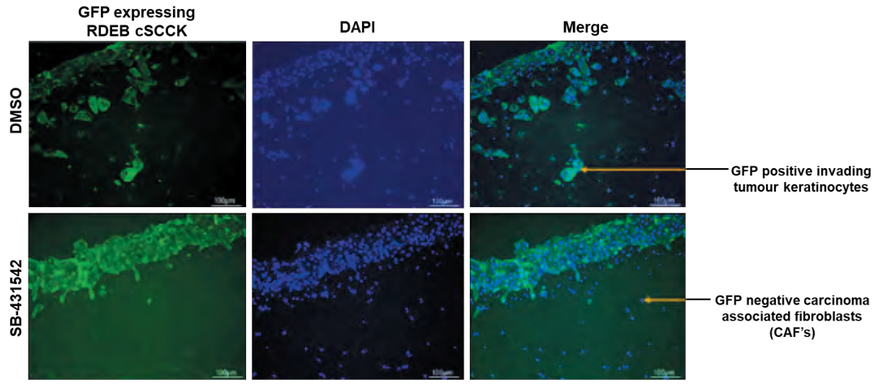

Figure 2
Organotypic assays indicate endogenous TGFβ signalling promotes invasion of RDEB cSCC tumour cells.
3D organotypic assays using RDEB cancer-associated fibroblasts embedded in type 1 collagen-matrigel gels forming a dermal component to test the invasive potential of GFP-positive RDEB cSCC tumour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

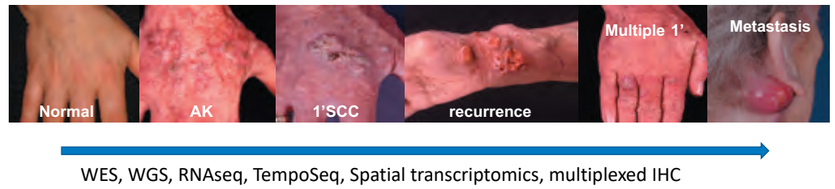

Figure 3
Disease progression of human cSCC.
Images illustrating disease progression(Courtesy of Professor Charlotte Proby, University of Dundee).
We are performing molecular profiling of human disease progression using next-generation sequencing approaches, immunohistochemistry and spatial transcriptomics.

Philadelphia, Pennsylvania). Our studies so far indicate that whilst stimulation with exogenous TGFβ ligand can inhibit proliferation of all RDEB cSCC patient-derived cell lines (PDCLs) they also exhibit heterogenous TGFβ addiction to endogenous TGFβ signalling. Inhibition of endogenous TGFβ signalling can markedly inhibit the proliferation, clonogenicity, migration and invasion in organtotypic culture (Figure 2) of the majority of but not all RDEB PDCLs. 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 currently exceeds that of all other cancers combined and is increasing year on year in our ageing population. In the case of squamous cell carcinoma, development of primary tumours may be preceded by the development of pre-malignant actinic keratosis (Figure 3). In contrast to most other epithelial malignancies, more than a third of patients develop multiple primary cSCC. This is especially true in immunosuppressed individuals, with evidence in organ transplant recipients of a more than 100-fold increased risk of developing 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., Acta Derm Venereol. 2016: 96, 3-16,). 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) and Peter Bailey (University of Glasgow) we are embarking on a detailed molecular characterisation of cSCC disease progression using a variety of state-of-the-art next-generation sequencing approaches coupled with spatial analysis of protein and RNA expression. Our initial whole-exome sequencing analysis of primary tumours confirmed the high mutational load of cSCC, with tumours exhibiting an average of 50 mutations per megabase of DNA. (Inman et al., Nat Commun. 2018 Sep 10;9(1):3667). We are now analysing whole-genome and bulk RNA-seq profiles of human and murine cSCC samples derived from genetically engineered mouse models (in collaboration with Owen Sansom). 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 actionable susceptibilities for future therapeutic intervention.

Squamous tumours from other primary sites such as the head and neck, oesophagus, lung and the squamous subtype of pancreatic ductal adenocarcinoma (PDAC) share many common molecular features with cSCC, with prominent dysregulation of TGFβ superfamily signalling. We are assembling panels of PDCLs from these tumour types, and in collaboration with Jen Morton, Peter Bailey and Claire Paterson (NHS Greater Glasgow and Clyde) we are investigating mechanisms of therapy resistance and disease progression in HNSCC and PDAC both *in vitro* and *in vivo* with an initial focus on TGFβ superfamily signalling.

Publications listed on page 102

CELL MIGRATION AND CHEMOTAXIS

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The cell migration & 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 supervised 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 main 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 it. 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.

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 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.

Publications listed on page 103

DEEP PHENOTYPING OF SOLID TUMOURS

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Solid tumours are complex assemblages of malignant cells, inflammatory cells, fibroblasts, blood vessels and other tissue types, and tumours 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 what ought to be an extremely hostile environment, we must understand this microscopic environment at a cellular level, and visualise the competing cellular strategies of malignant cells and their genomically normal hosts. 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 established numerous highly multiplexed IF/ISH staining assays on two new Roche Ventana autostainer platforms and have started to 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. Then we acquire high-resolution images, with separate layers for each marker of interest. These images are then converted into quantitative data, typically single-cell quantitative measures and/or cellular phenotypes. These spatial and quantitative cell data are used as the substrate for classical or more advanced modelling techniques intended to answer biological questions about tumour function.

Key projects:

1) Translational control in tumour cells

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

2) Tumour immunophenotyping

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

To achieve this, we are applying highly multiplexed panels of markers to identify tumour and immune cell phenotypes, and to link the presence and relative spatial distribution of these cells to patient outcomes. We intend to apply

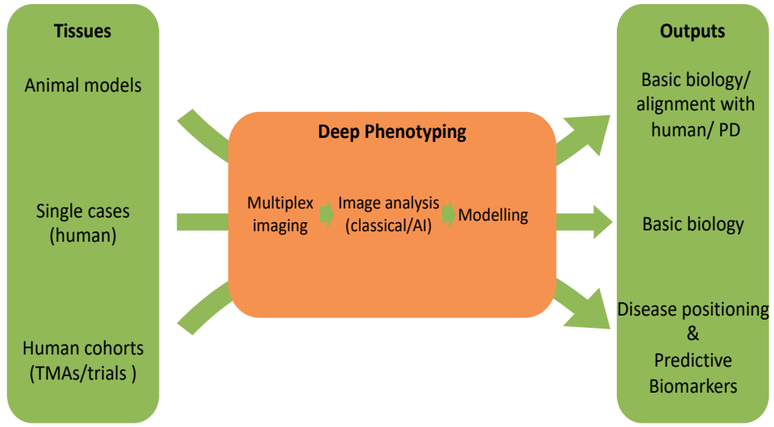

Figure 1
Workflow schematic of deep phenotyping methods. The basic pipeline (centre) is applied to a range of tissue types to achieve answers to diverse scientific questions.

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, and 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.

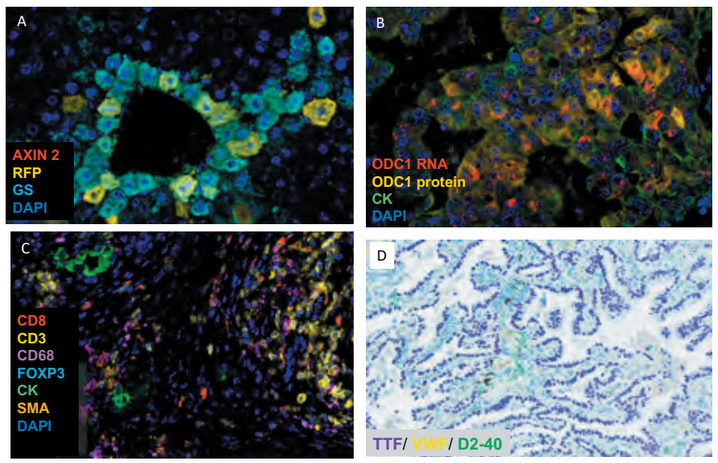

Figure 2
Example multiplex images. **A)** Spectrally unmixed co-ISH IHC of AXIN 2 mRNA with IF markers for red fluorescent protein and glutamine synthase in transgenic mouse liver. **B)** Spectrally unmixed co-ISH IHC of ODC1 mRNA with IF markers for ODC1 protein and cytokeratin in archival human lung tumour tissue. **C)** Spectrally unmixed multiplex staining for T-cell phenotypes and macrophages in archival human colorectal carcinoma tissue. **D)** four-colour chromogenic staining for human lung adenocarcinoma cell nuclei (TTF-1), capillaries (VWF) and lymphatics (D2-40) with haemotoxylin counterstain.

PROSTATE CANCER BIOLOGY

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Prostate cancer affects one in eight men in the developed world, and now accounts for more cancer related deaths in men than females dying of breast cancer. Despite improvement in patient survival with novel androgen receptor (AR) inhibitors and taxane chemotherapy, a significant proportion of patients with advanced disease still die within five years of diagnosis.

Our research aims to highlight novel molecular drivers of treatment resistance, thus identifying new therapeutic strategies for future development. In this report, we describe our recent findings from a novel panel of preclinical models that mimic treatment resistant prostate cancer - specifically castration resistance- and highlight key molecular players that allow tumours to evade androgen deprivation therapy.

Proteomic analysis of 2- and 3- dimensional *in vitro* cultures of treatment resistant human prostate cancer cell models

Despite the clinical success of AR-targeted therapies, reactivation of AR signalling remains the main driver of castration-resistant prostate cancer (CRPC) progression. Human androgen receptor expressing and hormone-dependent LNCaP prostate cancer cells were chronically exposed to multiple AR inhibitors (namely bicalutamide, apalutamide and enzalutamide) to generate CRPC cell models.

Combined proteomics and metabolomics analyses revealed a shared adaptive metabolic phenotype among the treatment resistant cells with perturbed glucose and fatty acid metabolism. In an AR-dependent manner, the observed metabolic rewiring ultimately led to a profound reorganisation of the cellular lipidome of resistant cells, marked by an accumulation of multiple classes of sphingolipids and polyunsaturated triglycerides.

To exploit this phenotype, we delineated a subset of proteins consistently associated with resistance to AR pathway inhibitors, and highlighted mitochondrial 2,4-dienoyl-CoA reductase (DECR1), an auxiliary enzyme of beta-oxidation, as a clinically relevant biomarker for CRPC. Mechanistically, we found that DECR1 participated in redox homeostasis to maintain a balance between saturated and unsaturated phospholipids. *DECR1* knockout induced ER stress and sensitised CRPC cells to ferroptosis. *In vivo*, *Decr1* deletion impaired lipid metabolism and reduced CRPC tumour growth, suggesting a potential value of exploring DECR1 as a target to overcome treatment resistance towards androgen receptor inhibitors.

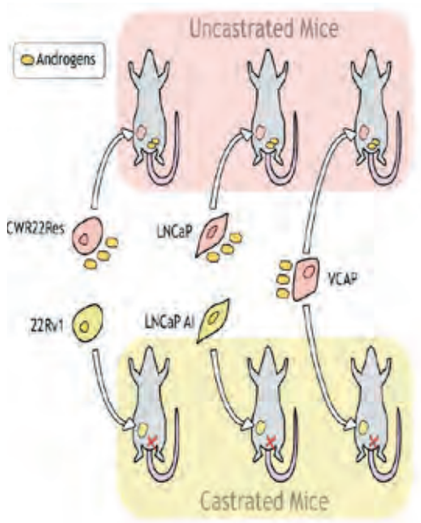
Proteomics analysis of matched isogenic hormone-naïve and castration-resistant prostate cancer orthotopic xenografts (orthografts)

To study androgen deprivation resistance, we developed three sets of CRCP orthograft models by injecting matched pairs of hormone-naïve and castration-resistant prostate cancer cells into the prostates of immuno-deficient mice. The isogenic pairs of hormone-naïve and castration-resistant cell lines were LNCaP/LNCaP AI and CWR22res/22rv1 respectively, while VCaP cells were able to grow as orthografts in both androgen-proficient and -deprived conditions.

To achieve patient-like CRPC conditions, orthotopic injections of prostate cancer cells were immediately followed by orchidectomy to mimic androgen deprivation therapy (Figure 1).

In-depth quantitative SILAC-based proteomic analysis revealed a complex response to hormone deprivation therapy, indicating distinct molecular mechanisms across different castration-resistant prostate cancer models. Comparing all three sets of hormone-naïve and castration-resistant orthografts, we noticed a shared theme of altered metabolism. Of note, differences in the underlying metabolic pathways involved signified the molecular heterogeneity among the three models of CRPC. Nonetheless, we identified Schlafen family member 5 (SLFN5) as a common target; its expression was consistently upregulated in all three CRPC prostate orthografts. In clinical tumours, SLFN5 expression was elevated in treatment resistant prostate biopsies, while SLFN5 deletion dramatically impaired the growth of CRPC tumours *in vivo*. Mechanistically, our data

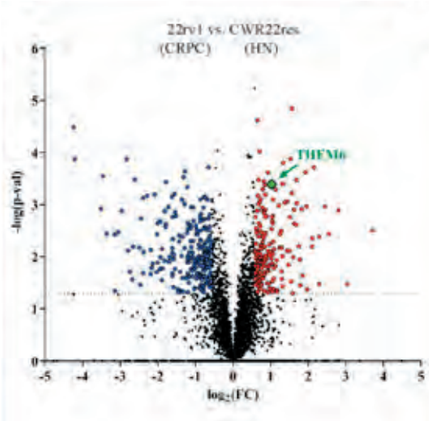
Figure 1
Schematic representation of the three sets of isogenic hormone-naïve and castration-resistant prostate cancer orthograft models



highlighted direct interaction between SLFN5 and the transcription factor ATF4, and that SLFN5 modulated the expression of several ATF4-target genes, including the amino acid transporter LAT1 (Large Amino Acid Transporter 1). Furthermore, we demonstrated that SLFN5 knockout in CRPC cells could alter amino acid metabolism through altering intracellular levels of amino acid substrates of LAT1, ultimately resulting in suppressed mTORC1 signalling in a LAT1-dependent manner. Our data, therefore, highlighted the AR/SLFN5/mTORC1 pathway as a potential therapeutic target.

In a more focused analysis of the isogenic pair of hormone-naïve CWR22 and derived castration-resistant 22rv1 orthografts, we identified upregulated thioesterase superfamily member 6 (THEM6) expression in treatment resistant 22rv1 tumours (Figure 2). THEM proteins (classified as Type II Acyl-CoA thioesterases) are characterised by the presence of an evolutionarily conserved "Hotdog" domain, which gives rise to their thioesterase enzymatic activity to deactivate fatty acyl-CoA thioesters and generate free fatty acids and CoA.

Figure 2
Volcano plot of the differentially modulated proteins in 22rv1 castration-resistant prostate cancer (CRPC) versus CWR22res hormone-naïve (HN) tumours. Red and blue dots represent the proteins that are significantly up- and down-regulated, respectively (p-value < 0.05, FC = 1.5). THEM6 protein is highlighted in green.



In patients, THEM6 expression correlated with progressive disease and was associated with poor patient survival. THEM6 deletion reduced *in vivo* tumour growth and re-sensitised castration-resistant orthograft to androgen deprivation therapy by means of surgical castration. THEM6 was located at the endoplasmic reticulum membrane and mechanistically, controlled lipid homeostasis by regulating intracellular levels of ether lipids. We found that THEM6 loss in CRPC cells significantly altered endoplasmic reticulum function, preventing lipid-mediated induction of ATF4 and reducing *de novo* sterol biosynthesis. Interestingly, *THEM6* and *cMYC* were often co-amplified in advanced prostate cancer. Our data suggested that THEM6 was required for the establishment of the MYC-induced stress response. Beyond prostate cancer, our research highlighted that loss of THEM6 expression significantly impaired tumorigenesis in the MYC-dependent subtype of triple negative breast cancer. Altogether, our results highlighted THEM6 as a novel component of the treatment-induced stress response and a promising target for the treatment of CRPC and MYC-driven cancer.

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.

We tested whether a network of genes similarly regulated by transcription factors (gene products that control the expression of target genes) were associated with patient outcome. We identified regulons (networks of genes similarly regulated) within 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 data from transcriptomic network analysis highlighted the value of future studies on JMJD6 mediated function in prostate cancer biology.

Concluding comment

In summary, the use of novel preclinical models that mimic clinically relevant treatment resistance in prostate cancer has identified multiple actionable genes as potential targets for therapy in castration-resistant prostate cancer.

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MOLECULAR IMAGING

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Our lab develops new ways to visualise cancer – we create novel molecular imaging agents targeting metabolic reprogramming, a hallmark of cancer, and use state-of-the-art imaging 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 and combination treatments.

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 subtype-specific susceptibilities that we can subsequently 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 develop a complementary panel of PET tracers and a better understanding of how imaging signatures relate to underlying molecular features of cancer, we could identify metabolic differences between or within patients and use this to stratify treatment.

Lung cancer has 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. Important questions remain, such as determining the clinical significance of metabolic heterogeneity in relation to cancer aggression, metastatic potential and therapy resistance.

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 appeared to demonstrate reciprocal metabolic phenotypes within the same mouse (Figure 1).

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

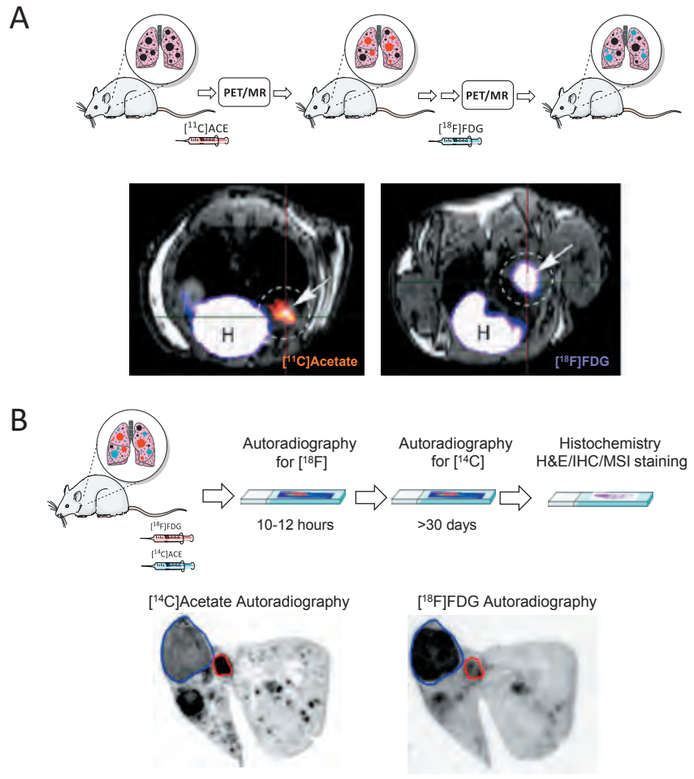

Figure 1
Multiplexed metabolic imaging of cancer heterogeneity in Kras$^{G12D/+}$, p53$^{-/-}$ driven non-small cell lung cancer.
(A) Dual radioisotope PET imaging with sequential [^{11}C] acetate and [^{18}F]FDG injections indicating respective tumour regions with elevated fatty acid synthesis and glucose uptake. (B) Dual radioisotope autoradiography of lung cancer sections highlighting FDG (blue) and acetate-avid (red) tumours.

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

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

Tomographic imaging of spontaneous tumourigenesis and treatment response

Genetically engineered mouse models of cancer (GEMMs) 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, developing radionuclide imaging of the sodium iodide symporter (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. We have taken two approaches to the gene delivery of NIS: somatic induction and germline transgenesis. Somatic induction has the advantage that the vector could 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 contained 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 was the first example of a radionuclide reporter gene used to monitor spontaneous tumour development in a live mouse. This was important, as unlike bioluminescent approaches, it enabled sensitive three-dimensional imaging of tumour development *in vivo* that was 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.

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MIGRATION, INVASION AND METASTASIS

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Metastasis and recurrence remain largely untreatable aspects of many solid cancers. We are interested in discovering how cells escape from tumours and how they succeed or fail to colonise in new sites. Adhesion, migration and the balance of mechanical forces all contribute to metastasis via cell signalling and feedback. We hypothesise that understanding the molecular conversation between cancer cells and their surroundings will reveal new avenues for combatting metastasis.

Solid tumours are frequently mechanically stiffer than the surrounding tissue, due to increased pressure, cell crowding, fluid accumulation and extracellular matrix (ECM) deposition. Tumours not only experience increased mechanical forces, but they show a force imbalance, thought to disrupt the normal tissue architecture and promote invasion and escape from the primary site (Figure 1). Pancreatic ductal adenocarcinoma (PDAC) presents with stiff tumours containing a fibrotic ECM and is highly metastatic. Vassilis Papalazarou, a former PhD student co-supervised by Prof. Manuel Salmeron-Sanchez in the School of Engineering and Centre for the Cellular Microenvironment, asked how mechanical stiffness of the ECM affects PDAC cells, using bioengineered matrix substrates of controlled stiffness. To our surprise, cells showed a strong link between metabolic flux and ECM stiffness, with soft matrix promoting glycolysis and stiff ECM promoting mitochondrial oxidative phosphorylation. We went on to dissect connections between mitochondria morphology, cellular position and metabolic flux. Vassilis discovered that mitochondria are recruited into invasive pseudopodia of PDAC cells, where they provide ATP to power actin dynamics and invasion. He further found, together with Dr Oliver Maddocks, that PDAC cells also upregulated their phosphocreatine shuttle, a major ATP recycling pathway, in response to ECM stiffness. This provided a new link between mechanosensing, ATP production and ATP recycling. We are continuing to explore how the phosphocreatine shuttle is regulated by Yap/Taz signalling, which impacts on expression of the enzyme creatine kinase B (CKB) and how ATP recycling powers cell migration and invasion.

We have further revealed important functions of the major actin nucleation promoting complex, the Scar/WAVE complex, and its target, Arp2/3 complex, in migration and invasion and in melanoma progression. Postdoctoral researcher Jamie Whitelaw explored new connections between the actin nucleating functions of Scar/WAVE and cell-matrix adhesions. Former postdoctoral Researcher Karthic Swaminathan explored the role of the NckAP1 subunit of the Scar/WAVE complex in the melanocyte lineage of mice predisposed to melanoma driven by $BRaf^{V600E}$ and a loss of the tumour suppressor PTEN. These mice develop melanomas when NckAP1 is present, but NckAP1 deletion severely impairs melanoma development and progression, with tumours showing very slow growth and enhanced fibrosis and immune infiltration. Our study implicated NckAP1 and the Scar/WAVE complex in cell cycle progression, likely as a key downstream target of Rac1, a key activator of the Scar/WAVE complex. We also showed that the Arp2/3 complex is crucial for migration of mouse embryo melanoblasts in the skin. Loss of the Arp3 subunit caused a failure of melanoblasts to expand in the dermis and reduced migration and mechanical stability, demonstrating the importance of Arp2/3 as a driver of migration *in vivo*.

The liver is a major site of metastasis of many solid cancers, and liver metastasis is a major cause of death from cancers, including PDAC. Postdoctoral researcher James Drew and PhD student Elaine Wing-See Ma pioneered a new model for liver metastasis *in vitro*, using human induced pluripotent stem cell (iPSC) derived mini-liver spheres. Prof. David Hay's lab at the Centre for Regenerative Medicine in Edinburgh have developed mini-liver spheres from human iPSCs to model healthy and diseased liver function. Together with Hay's group, we explored the capability of metastatic PDAC to colonise the liver spheres and to interact with the hepatocytes, stellate cells and endothelial cells present in these mini-liver spheres (Figure 2). We propose that mini liver spheres have the potential to be developed into a powerful new model for cancer metastasis *in vitro* that could be exploited to search for new therapies.

Looking ahead, we (PhD students Sonia Rolo and Elaine Wing See Ma, and postdoctoral researcher James Drew) continue to explore the liver metastatic niche and ask how crosstalk between disseminated tumour cells and their environment controls their tendency to lie dormant or to grow into a new metastasis. We are also continuing to unravel how creatine kinase B controls ATP recycling via the phosphocreatine shuttle (Anh Le, postdoctoral researcher) to power invasive migration and link mechanosensing with metabolism.

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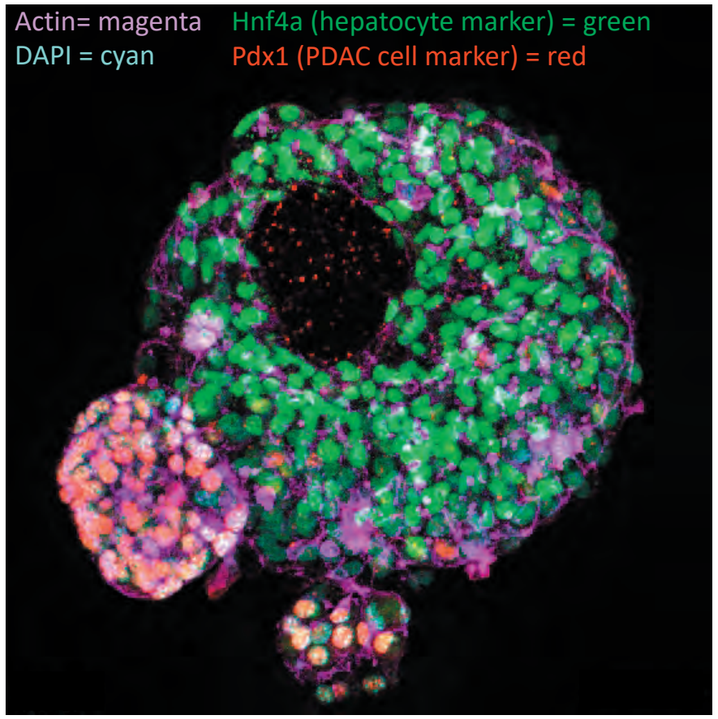

Figure 2
Mini liver sphere with attached PDAC mini metastasis

Image credit: James Drew

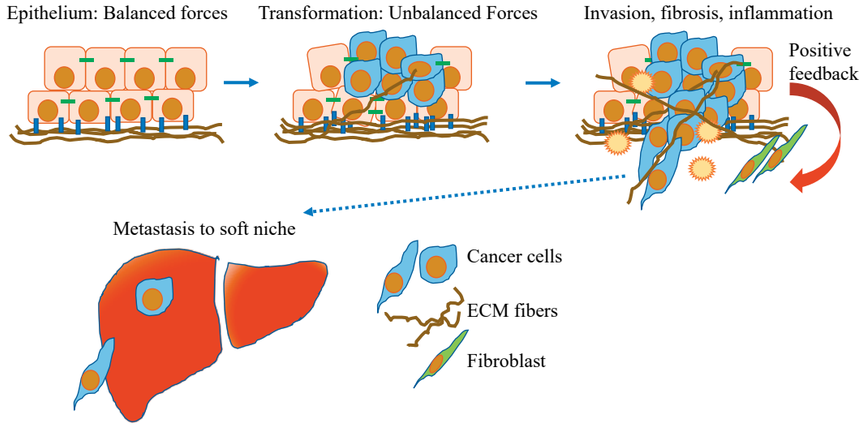

Figure 1
Forces in the primary tumour driving metastasis

COMPUTATIONAL BIOLOGY

Group Leader
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Research Scientist
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.

The group was established in 2019, and over the last year we have continued to develop our research programme. 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.

Rapid advances in technology have made it possible to generate simultaneous measurements across the same cell and tissue samples. These can describe a diversity of changes in genome structure and organisation, mRNA expression and protein levels. These present a computational challenge not only in terms of the mathematical models required to properly integrate and analyse these complex multi-omics datasets, but also in the mapping of these data into clinical datasets arising from, for example, tumour RNA-seq. We are particularly interested in strategies that support the joint analysis of single-cell and bulk sequencing datasets.

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.

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PRECISION-PANC PRECLINICAL LAB

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The aim of our research is to better understand how pancreatic cancer develops and progresses, and to use this knowledge to identify and test new clinically relevant therapies and combinations. To do this, we study mouse models of pancreatic cancer driven by the same genetic lesions we observe in human tumours. Importantly, the tumours that develop mimic human tumours, not just in genetic make-up but also in terms of the dense fibrotic and immunosuppressive stroma, making them ideal models in which to perform preclinical trials of more targeted and personalised therapies.

Pancreatic cancer kills around 340,000 people every year and is predicted to be the second most common cause of cancer death within the decade. Pancreatic ductal adenocarcinoma, the most common and aggressive form of the disease, is almost always driven by activating mutations in *KRAS*, accompanied by mutations in tumour suppressor genes, most commonly *TP53*, *SMAD4* and *CDKN2A*. However, there are other mutations found in subsets of patient tumours that might confer sensitivity to specific targeted therapies if the biological consequences of those mutations were better understood (Biankin et al. (2012). *Nature* 491, 399–405). For that reason, part of our work involves modelling mutations in the genes that are mutated in smaller subsets of human pancreatic cancer. Another feature characteristic of PDAC is the dense fibrotic stromal microenvironment that surrounds and supports the tumour cells and can account for up to 90% of the tumour volume in the human disease. 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

To model further recurring mutations in patients which may be actionable, we developed a model of Kdm6a deletion. KDM6A is a histone demethylase; patients with pancreatic tumours bearing mutant *KDM6A* or low mRNA expression exhibit poor survival. We found that deletion of one or two copies of *Kdm6a* in either KPC or KC mice resulted in a dramatic acceleration of tumourigenesis with a median survival of less than four weeks in KPC *Kdm6a*$^{fl/fl}$ mice (Figure 1a). We did not observe changes in the overall level of H3K27 methylation, but we could observe transcriptomic changes in pancreatic cell identity markers, developmental signalling pathways, and most intriguingly, downregulation of proliferation and deregulated cell cycle control. We found that this was associated with downregulation of *KrasG12D* expression (Figure 1b) and hypothesise that loss of KDM6A during tumour initiation may allow a reduction in Kras signalling in PanINs that allows them to circumvent senescence and rapidly progress to PDAC.

We have also developed and characterised several other models bearing deletion of genes

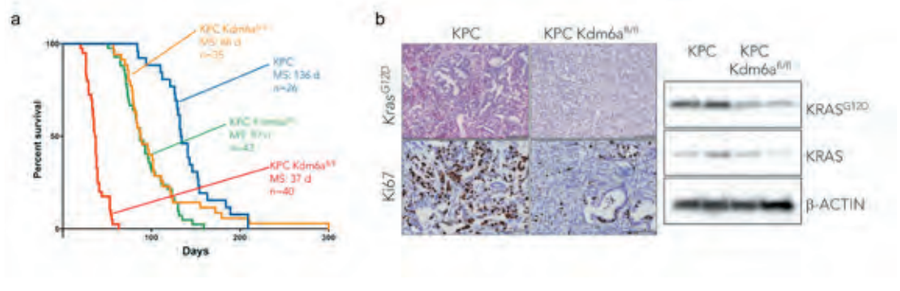

Figure 1
a) Kaplan¬–Meier survival curve of KPC mice of the Kdm6a genotype indicated, aged until clinical endpoint. b) Representative images of RNAscope for *Kras*^{G12D} and IHC for Ki67, alongside immunoblotting for KRAS and KRASG12D (β-actin as loading control) in KPC and KPC *Kdm6a*$^{fl/fl}$ tumours.

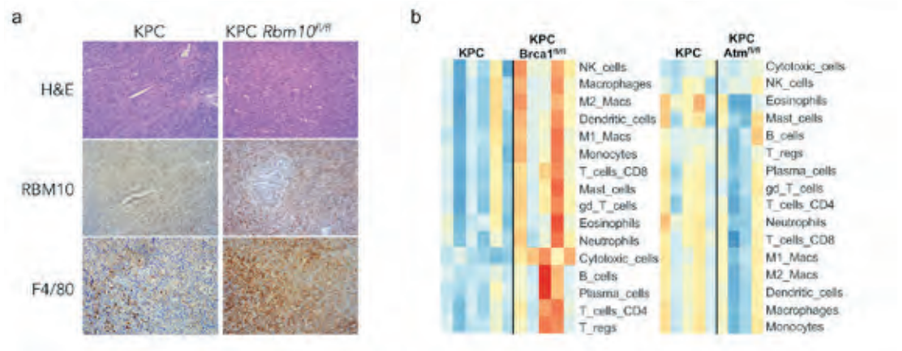

Figure 2
a) Representative images of H&E staining and IHC for RBM10 and the macrophage marker, F4/80, in KPC and KPC *Rbm10*$^{fl/fl}$ tumours showing increase in infiltration of RBM10-expressing macrophages. b) RNAseq analysis shows marked differences in the immune microenvironment of KPC- based models of DDR deficiency.

that are lost or mutated in subsets of patients, for example, the DDR repair genes *ATM* and *BRCA1*, and the mRNA processing genes *RBM10*, *SF3B1* and *U2AF1*, which together are mutated in around 15% of pancreatic cancer patients (Biankin et al. (2012). *Nature* 491, 399–405). The spliceosome, which removes introns from pre-mRNA, is a complex of small nuclear RNAs and small nuclear ribonucleoproteins (snRNPs). Specific sequence elements in pre-mRNAs are recognised and the spliceosome catalyses the removal of introns and the ligation of the flanking exons. RBM10 is an RNA binding protein which regulates alternative splicing (Seiler et al. (2018) *Cell Reports* 23, 282–96), and loss or low expression correlates with poor survival in pancreatic cancer patients. We find that loss of RBM10 can accelerate tumourigenesis in the KPC mouse model, and intriguingly, loss of RBM10 coincides with a massive influx of RBM10*hi* macrophages (Figure 2a). We are currently investigating these cells as we, and others, have shown that macrophages are pro-tumourigenic and immune-suppressive in PDAC (Candido et al. (2018) *Cell Reports* 23, 1448-60). On the other hand, loss of U2AF1, a protein required for the binding of U2 snRNP to the pre-mRNA branch site (Obeng et al. (2019) *Cancer Discovery* 9, 1493-510), had no obvious effect on tumour progression. Interestingly, deletion of SF3B1, a component of the U2 snRNP (Obeng et al. (2019) *Cancer Discovery* 9, 1493-510) was not tolerated during pancreatic development, and surviving mice always retained some expression of the protein. Together, these data highlight how mutations in related genes can lead to profoundly different phenotypes. Similarly, investigation of pancreatic tumours bearing mutations in the DNA damage repair genes *Atm* and *Brca1* uncovered a substantial difference in the immune landscape of KPC *Brca1*$^{fl/fl}$ mice compared with KPC *Atm*$^{fl/fl}$ mice (Figure 2b), and only in these mice did we observe enhanced cytotoxic T cell infiltration, suggesting that DDR-deficient tumours are not a homogenous group, at least in terms of immune infiltration.

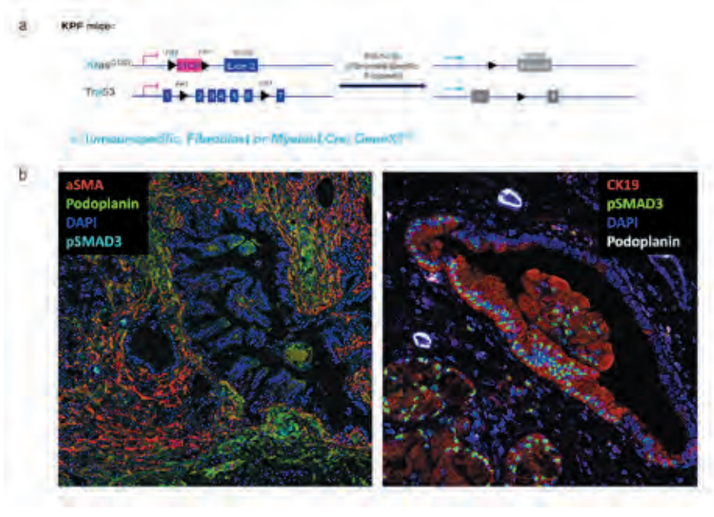

Figure 3
a) Schematic of dual recombinase strategy. b) co-IF for the markers indicated highlights significant heterogeneity of TGFβ signalling (indicated by pSMAD3 staining) in pancreatic cancer and its microenvironment.

Targeting stromal signalling

Each component of the pancreatic cancer stroma plays an important role in pancreatic cancer progression, able to influence tumour cell proliferation, survival, metabolism, migration, immune surveillance and response to chemotherapy. We are currently using preclinical models to better understand the pancreatic cancer microenvironment, how it becomes so immuno-suppressive, and the signals we can target for therapeutic effect. In order to interrogate communication between cells in the microenvironment, we have been working with new models using alternative promoters to drive Cre expression in subsets of pancreatic cells, as well as 'next-generation' dual recombinase systems (Schonhuber et al. (2014) *Nature Medicine* 20, 1340-7). These models rely on the alternate Flp-FRT recombinase system to activate or delete genes in the pancreas and allow genetic manipulation in distinct cellular components within tumours, for example cancer-associated fibroblasts and immune cells (Figure 3a). In particular, we are working to understand the role of TGFβ signalling networks in tumour - microenvironment communication. We have uncovered significant heterogeneity in signalling across different cellular compartments and between different tumours (Figure 3b). These novel approaches will allow us to investigate the importance of this crosstalk during pancreatic tumour progression.

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ONCOGENE-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.

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[7]Merck Pharmaceuticals
[8]Scottish Government Chief Scientist Office
[9]Medical Research Council Doctoral Training Programme
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We use genetically engineered mouse models, primarily of lung cancer and mesothelioma, to understand how developing tumours cope with such conflicting cues in their natural environment. Our overarching hypothesis is that such 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. MYC overexpression may arise from focal or broad chromosomal amplification, gene translocation, enhanced mRNA and protein stability or indeed increased signalling through upstream regulatory factors such as Ras, Notch or ß-catenin. In a number of *in vivo* settings, MYC overexpression is sufficient to initiate or exacerbate tumourigenesis and moreover is typically required to sustain the cancerous phenotype. A successful therapeutic strategy that exploits MYC overexpression would likely have a tremendous impact on human health. In order to facilitate investigation of physiologically relevant levels of deregulated MYC expression in any tissue, we have generated and characterised Rosa26-lsl-MYCDM mice and deposited them with Jaxmice for unrestricted distribution to the broader scientific community.

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, effectively 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 an unexpected requirement for signal transduction through the ERBB receptor tyrosine kinase network for both establishment and maintenance of KRAS mutant lung cancer. Our data suggest that KRAS-driven tumours actively seek ways to amplify signalling through the RAS pathway in order to sustain the tumour phenotype. As there are presently no clinically proven small molecule 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 the very large number of 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 group has teamed up with respiratory physician Kevin Blyth to build an international network of clinicians and researchers with the common goal of improving patient outcomes for this dreadful disease. We have developed a new mouse model of mesothelioma that will enable us to investigate the interplay between asbestos-driven chronic inflammation and the major recurring mutations that are commonly found in human mesothelioma. Significantly, intrapleural injection of asbestos dramatically accelerates onset and severity of mesothelioma in our mice, even after homozygous deletion of three major tumour suppressor genes, indicating that chronic inflammation continues to contribute to mesothelioma beyond the acquisition of rate-limiting mutations. This startling observation suggests that patients may benefit from interventions that aim to reduce inflammation, in addition to those directly targeting the tumour population.

Major developments in 2020

Oncogene cooperation in evasion of anti-tumour immunity

Our major publication of 2020 revisited the mechanism of oncogenic cooperation of MYC and KRAS in the context of pancreatic cancer. We showed that acute activation of MYC or KRAS in otherwise wild-type fibroblasts elicits largely overlapping transcriptional responses, with genes regulated by MYC comprising a large subset of KRAS-regulated genes. The combination of simultaneous activation of MYC and KRAS increased the potency of gene regulation, extending and deepening the transcriptional responses induced by either alone. Although perhaps not surprising to a core of investigators that follow MYC biology closely, our data contradict a long-standing dogma of MYC and KRAS complementarity which predicted largely non-overlapping transcriptional impacts of these two oncogenes. From pathway analysis, 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 and presentation forefront in these transcriptional responses. Importantly, we showed similar suppression of the Type I Interferon cascade during MYC-accelerated progression of pancreatic ductal adenocarcinoma (PDAC) and, moreover, identified repressive transcriptional complexes comprising MYC and MIZ1 binding directly to multiple key regulators of Type I Interferons in PDAC. Genetic suppression of MYC or MIZ1 restored Interferon signalling, enabling PDAC tumours to elicit CXCL13 production in nearby macrophages and thereby recruit anti-tumour effector immune cells to limit tumour progression and extend survival. These exciting observations were published in the June issue of Cancer Discovery.

New Initiatives

Despite the profound and severely negative impact of COVID-19 on our research activities, we initiated and continue to prosecute an active drug discovery programme involving the Institute's Drug Discovery Unit, CRUK Translational Discovery Labs and Merck pharmaceuticals, financially supported by the latter. This collaboration saw postdoctoral scientist George Skalka join our team.

We also started work on the CRUK Accelerator PREDICT-Meso, with postdoctoral scientist Katarina Gyuraszova continuing development and characterisation of our suite of GE mouse models of mesothelioma, greatly assisted by Pooyeh Farahmand and latterly Jennifer Doig. This exciting initiative embeds the group within a large international consortium focussed on a better understanding of progression from benign asbestos-associated pleural inflammation to Malignant Pleural Mesothelioma.

Career Progression

I gained promotion to Professor of Lung Cancer & Mesothelioma within the Institute of Cancer Sciences, University of Glasgow.

Sarah Laing submitted and successfully defended her thesis on development of an immunogenic mouse model of Lung Cancer and subsequently continued in my group as maternity cover for Jennifer Crowe. Indeed, the group welcomed two newborns to the world in 2020 with congratulations to Jennifer & her partner and to Katarina & Björn.

A major highlight of the year was the recruitment of Nathiya Muthalagu to an independent faculty position at the Indian Institute of Technology, Madras, marking the conclusion of an immensely successful postdoctoral tenure in my group. The group collectively wishes Nathiya every success in her future career.

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INTEGRIN CELL BIOLOGY

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Determining how certain mutations can drive carcinoma growth and progression, and how tumour cells with particular mutational landscapes may be targeted therapeutically, is of key importance. However, it is now clear that a tumour's ability to engender alterations to the extracellular matrix, both locally and within organs which are distant from the primary tumour, influences tumour growth and metastatic spread. We are, therefore, focusing on describing the molecular and cellular mechanisms through which tumours influence extracellular matrix organisation. We are investigating how some of the mutations and metabolic alterations which occur in cancers lead to the release of factors (exosomes and metabolites) which influence extracellular matrix deposition. Furthermore, we are studying the molecular details of how a cell's protein synthesis machinery may be reprogrammed to promote synthesis of extracellular matrix proteins which favour tumour initiation, growth and metastasis.

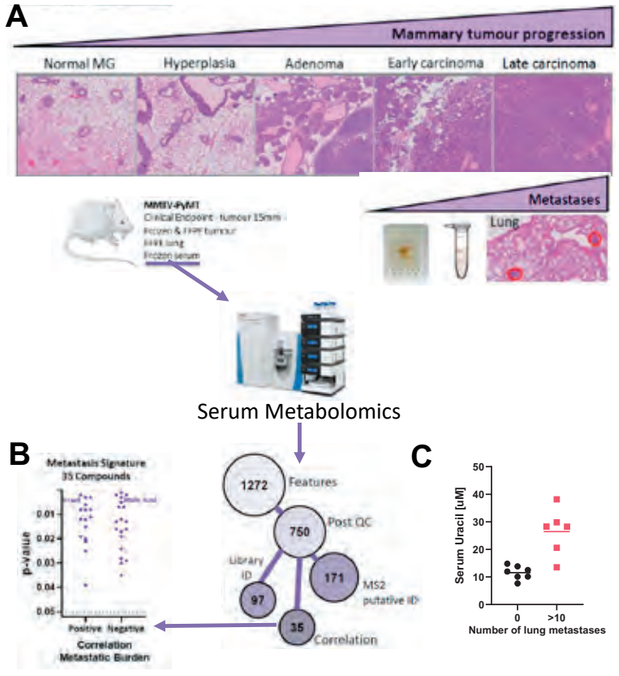

Figure 1
The circulating metabolic landscape of metastasis in mammary cancer
(A) The MMTV-PyMT mouse spontaneously develops mammary cancer which metastasises to the lungs.
(B) The level of metastasis varies from mouse to mouse, and we have used this variation to identify serum metabolites which correlate with the amount of lung metastasis. Indeed, 35 metabolites correlate significantly with metastasis.
(C) Principal amongst the circulating metabolites which correlate with lung metastasis is the pyrimidine uracil. The concentration of circulating uracil is increased by 3-fold in mice with high levels of lung metastasis by comparison with animals which were metastasis free.

Our laboratory is dedicated to furthering our understanding of how primary tumours influence the extracellular microenvironment of primary tumours and of metastatic target organs by addressing two interlinked research aims:

1. To establish how the metabolic re-wiring, which occurs in primary tumours to enable them to grow, influences metastasis. We are particularly interested in how tumours influence metabolite levels in the circulation and how these changes promote metastatic seeding.

2. To investigate how exosomes and other extracellular vesicles released by primary tumours influence the surrounding microenvironment, and that of other organs, to favour invasive spread of tumour cells and metastasis.

By addressing these aims, we will be able to determine how factors released by primary tumours can prime metastatic niches and thus influence the likelihood of disease recurrence following excision of primary tumours. This will facilitate the prediction of metastasis, and the stratification of therapies aimed at preventing post-surgical recurrence.

Metabolism & metastasis: Understanding the role of uracil in priming metastatic niches and influencing invasive cell behaviour

In collaboration with Karen Blyth, Owen Sansom and David Sumpton

To characterise the metabolic landscape of metastasis, we profiled non-polar metabolites in the serum of mice with varying levels of metastatic disease. Initially, we deployed the MMTV-PyMT mouse model of metastatic mammary cancer. We collected serum from a cohort of mice with mammary cancer, and then looked for specific metabolites which tracked with the number of metastases in the lungs (Figure 1A). This indicated that there are 35 circulating metabolites which correlate with metastasis, and notable amongst these is the pyrimidine uracil (Figure 1B). We also used genetically engineered mouse models of pancreatic adenocarcinoma (PDAC), comparing a model which metastasises efficiently to the liver and lung (mutant p53–driven KPC tumours), with one in which tumours appear with similar penetrance, but do not metastasise (p53 loss–driven KP^{fl}C tumours). This approach also identified uracil as a metabolite that was significantly increased in the serum of metastatic versus non-metastatic PDAC. Upon quantification, we found that the average concentration of serum uracil was 10μM in tumour-bearing mice with no lung metastases, whereas in mice with >10 lung metastases, this increased to 30μM (Figure 1C). To determine whether this change in circulating uracil concentration could influence the tumour microenvironment and/or the metastatic niche, we cultured mouse embryonic fibroblasts (MEFs) in the presence of 10μM or 30μM uracil and characterised the extracellular matrix (ECM) consequently deposited. Whilst uracil levels did not influence cell proliferation, the ECM generated by MEFs in the presence of 30μM uracil contained significantly increased levels of fibronectin in comparison with that deposited in the presence of 10μM uracil. Furthermore, increased recycling of active-conformation integrin α5β1, the primary fibronectin receptor of the cell, was also observed in MEFs treated with 30μM uracil. Consistent with the established role of fibronectin in cell adhesion and migration, the ECM deposited in the presence of 30μM uracil promoted the invasive behaviour of tumour cells. Having established that extracellular uracil influences ECM deposition, we proceeded to determine whether this might be owing to altered fibroblast metabolism. Indeed, MEFs were able to uptake exogenous uracil and this influenced their metabolism. We are currently investigating the mechanistic links between altered tumour cell metabolism and ECM deposition, with a specific interest in understanding how uracil may influence the pre-metastatic niche and promote metastasis.

Glioblastoma infiltration: Understanding how exosomes released from gliomas influence astrocyte function to pave the way for cancer spread throughout the brain.

In collaboration with Anthony Chalmers and Joanna Birch

Our previous studies indicating that exosomes released from mutant p53–expressing PDAC can prime metastatic niches led us to investigate whether extracellular vesicles also influence the progression of another highly invasive cancer, glioblastoma multiforme (GBM). Indeed, GBM spreads rapidly through the brain, and it is this infiltrative behaviour which renders the disease so difficult to treat. We have found that mutant p53–expressing GBM release exosomes which influence the brain microenvironment in a way which favours the subsequent migration and infiltration of these GBM cells. We have characterised a mechanism through which expression of the mutant p53 oncogene in GBM cells influences the sorting of a sialomucin – podocalyxin – into exosomes. These podocalyxin-containing exosomes then instruct the principal non-neuronal cells of the brain, the astrocytes, to deposit ECM which is highly enriched in hyaluronic acid. This hyaluronic acid–rich ECM then supports GBM cell invasion and migration. Thus, mutant p53s, by influencing the podocalyxin content of exosomes, allow GBM cells to pave the way for their own invasive migration through the brain. We are currently investigating how strategies for reducing hyaluronic acid content of the brain ECM may reduce GBM spread and render these tumours more amenable to therapy.

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IMMUNE PRIMING AND THE TUMOUR MICROENVIRONMENT

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Ryan Devlin
Youhani Samarakoon
Amy Shergold

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.

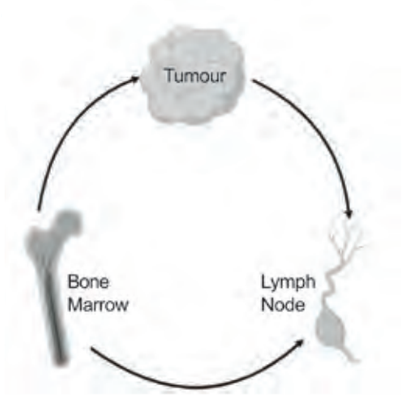

Figure 1
The DC lifecycle
DC precursors develop in the bone marrow and migrate to the tumour and the lymph node. Once within the tumour they sample proteins from the microenvironment and then mature and migrate to the lymph node. There the DC which migrated straight to the lymph node and those which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

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. We have also generated a novel strain of influenza virus allowing us to compare antigen traffic in the viral setting and have found that tumour-derived DC handle antigen distinctly from those under homeostatic conditions or indeed in response to viral challenge.

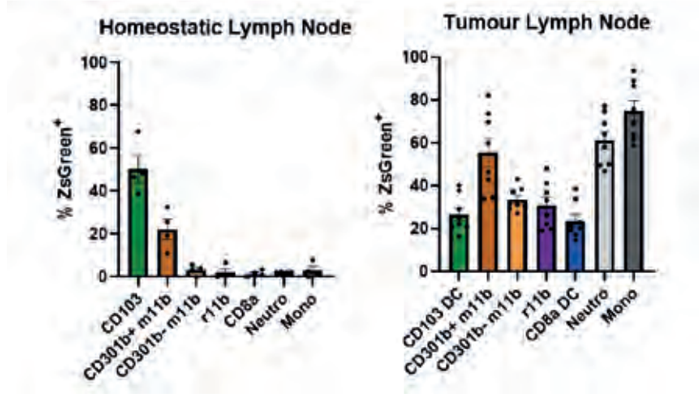

Figure 2
Tumour antigen is handled uniquely
ZsGreen expressed within the lung is carried to the lymph node by migratory DC, but the protein remains restricted to the migratory DC. When the same protein is expressed in a tumour, the protein is carried to the lymph node by migratory DC in a similar fashion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

DC functionality within the lymph node

Finally, once the antigen has been trafficked to the lymph node, in order to drive effective anti-tumour immune responses, the lymph node must be highly organised, facilitating numerous specific cell–cell interactions. During tumour development the draining lymph node has been shown to be disorganised, and it has been proposed that several of these critical cell–cell interactions are disrupted. We have, however, demonstrated that the tumour-draining lymph node is capable of supporting robust immune responses, suggesting the problem is with the tumour-derived DC rather than with the node as a whole. In order to study how these cells interact differentially in the tumour setting, we have developed a protocol allowing us to stain the entire lymph node and to identify the location of critical cellular subsets within the 3D environment of the lymph node (Figure 3). We have also developed complementary approaches to allow identification of even more cell types within the lymph node microenvironment and are now building systems to allow robust analysis of tissue organisation. We aim to use these approaches to identify organisational defects which occur in the context of tumour development which affect the quality of the anti-tumour immune response.

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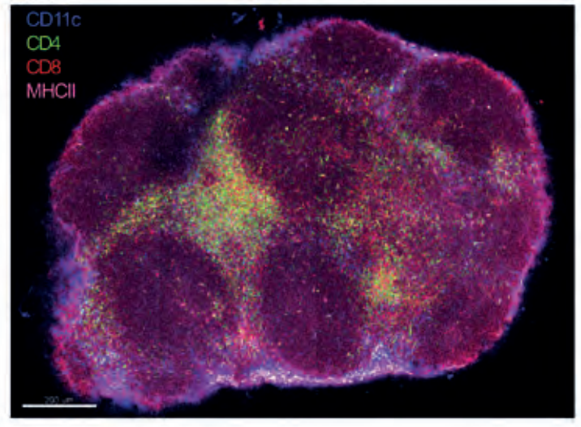

Figure 3
Lymph node organisation
A whole cleared lymph node stained for T cell, B cell and DC markers shows the organisation of a lung tumour-draining lymph node.

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TUMOUR CELL DEATH AND AUTOPHAGY

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

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

Autophagy in cancer

For several decades, the preservation of genomic integrity has been considered the central mechanism that protects us from cancer. While maintaining genomic fidelity is undoubtedly critically important, it must be remembered that a mutation in DNA usually only has an effect if it causes a mutation or dysregulation of an RNA or protein. As a result, because cancer can originate from a single errant cell, the preservation of RNA and protein integrity are also extremely important in protecting us against cancer.

There are two main systems for the removal and degradation of damaged or misfolded proteins: the ubiquitin–proteasome system and the lysosome. The lysosome degrades the bulk of the cell's mass and is responsible for the degradation of all cellular constituents, including organelles.

There are several ways in which cargoes are delivered to the lysosome for degradation, but perhaps the best recognised of these is a group of processes termed autophagy (literally, self-eating). There are three main types of autophagy: macroautophagy, microautophagy and chaperone-mediated autophagy. Macroautophagy is the most extensively studied and the main focus of research in our lab.

The process of macroautophagy begins with the formation of a double-membraned structure that encapsulates cargo destined for degradation as it grows to form a ball-like organelle called an autophagosome. Fusion events can then occur with endosomes and multivesicular bodies, but ultimately fusion occurs with a lysosome to form a new organelle termed an autolysosome within which degradation occurs by lysosomal hydrolases (Figure 1).

Macroautophagy can be stimulated by a variety of internal and external cues and both maintains cellular integrity and helps the cell adapt to numerous forms of stress, including starvation, hypoxia and oxidative damage. As a result, macroautophagy has a fundamental role in cancer. However, the role played by macroautophagy appears to be specific to cell type, context and tumour stage. It is widely considered, however, that in normal cells and in the early stages of tumour development,

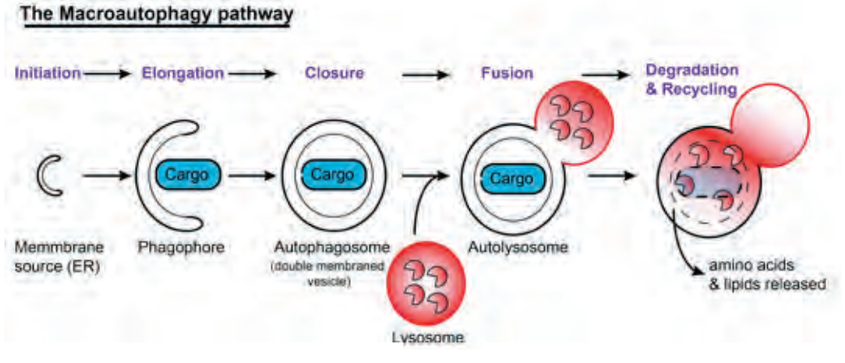

Figure 1
The pathway of macroautophagy.
The process of macroautophagy occurs in the cytoplasm of the cell and proceeds through various stages to encapsulate cargos destined for degration. Ultimately fusion occurs with a lysosome that provides the hydrolases required for cargo degradation. The breakdown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

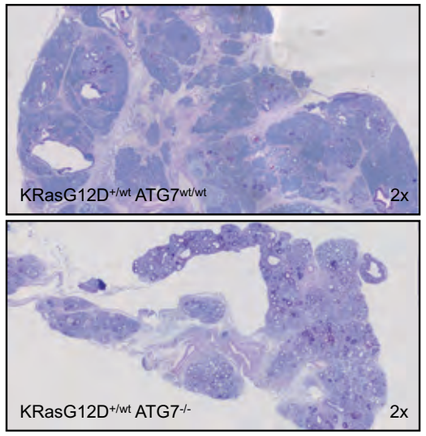

Figure 2

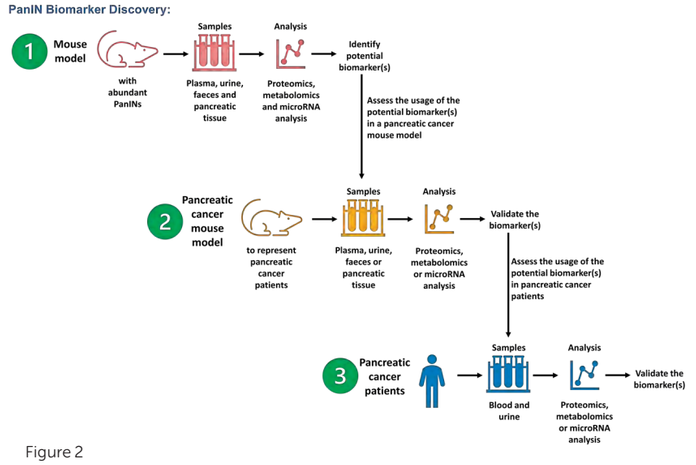

Figure 2

Figure 2
Mice expressing mutant Ras and lacking Atg7 in their pancreata have exacerbated PanIN formation.
Mice expressing mutant Ras and lacking Atg7 in their pancreata exhibit exacerbated PanIN formation when compared to mice which express mutant Ras, but are wild-type for Atg7. PanINs are detected with an Alcian blue/PAS stain. Data shown is taken from Rosenfeldt et al. (2013) *Nature* 504: 296–300

Figure 3
A strategy to search for liquid biopsy biomarkers of the precursors of pancreatic ductal adenocarcinoma.
Our plan to identify liquid biopsy biomarker(s) to detect PanINs involves identification and then two triage stages, firstly in mice expressing low levels of PanINs and secondly in samples from individuals with PanINs.

macroautophagy is tumour suppressive, whereas in fully developed cancers, macroautophagy is tumour promoting. This has made autophagy an attractive target for cancer therapy.

In order to target macroautophagy for cancer therapy, it is imperative that we understand when the process is contributing to the maintenance of cancer rather than acting in tumour suppression, and answering this question has been a major goal of our lab in recent years.

The role of autophagy in melanoma

In our previous work we showed that the role of macroautophagy in pancreatic cancer was determined by the tumour suppressor protein p53, with macroautophagy being oncogenic in the presence of p53 and tumour suppressive in the absence of p53 (Rosenfeldt et al. *Nature*, 2013; 504: 296–300). We have been exploring to see if similar 'switch proteins' like p53 exist in other tumour types and we have recently focused on melanoma, a highly malignant form of skin cancer. To do this we utilised a previously described mouse model of melanoma that is driven by an oncogenic mutation in *B-Raf*, the signature mutation driving a significant percentage of human melanomas. These mice were crossed to animals containing genetically designed alleles for an essential autophagy gene (*Atg7*) and the tumour suppressor *Pten*, which can be deleted by a recombinase that is selectively expressed in melanocytes (the cell of origin of melanoma). These studies revealed that in melanoma driven solely by mutant B-Raf, autophagy plays a tumour suppressive role, as its deletion results in acceleration of the disease. In contrast, in animals where melanoma is driven by mutant B-Raf and loss of one allele of *Pten*, the loss of autophagy has no impact on disease progression. We speculate that these findings may relate to the roles of autophagy and Pten in senescence, which is known to be a barrier to melanoma development. In the absence of either autophagy or Pten, the establishment/maintenance of senescence will be impaired, meaning that loss of macroautophagy will have no further effect in the context of Pten deficiency. Whatever the reason, these studies once again indicate that specific and definable molecular events can alter the role of macroautophagy in cancer in a way that determines that the potential for targeting this pathway for cancer therapy.

Searching for biomarkers for the detection of the pre-cancerous lesions associated with pancreatic ductal adenocarcinoma.

During our studies on the impact of p53 status in determining the role of autophagy in pancreatic cancer development, we observed that loss of autophagy in the pancreas of animals expressing mutant K-Ras resulted in a huge increase in the number of pancreatic intraepithelial neoplasia (PanIN), which are considered the precursor lesions leading to pancreatic ductal adenocarcinoma (Figure 2). As the earlier cancer is detected, the better the prognosis, and because pancreatic cancer is usually detected at an advanced stage, we considered that our mouse model with exacerbated PanIN formation could be utilised to identify a biomarker, which could be used to identify the presence of these precancerous lesions. This would then enable the identification of individuals who should be subject to enhanced screening for pancreatic cancer development.

As biopsy of the pancreas is an invasive procedure, its application is not feasible as a screening approach for a broad sector of the population. As a result, we decided to use our mouse model to hopefully identify a liquid biopsy biomarker in either blood or urine. Samples from animals with enhanced numbers of PanINs will be analysed by mass spectrometry (to identify potential protein and metabolic biomarkers) and by RNAseq (to identify potential RNA biomarkers). Biomarker(s) identified with this approach will then be triaged for detection in mice with a small number or PanINs (as is normal) and in material from patients with either PanIN formation alone or in the context of established pancreatic cancer (Figure 3).

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COLORECTAL CANCER AND WNT SIGNALLING

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Colorectal cancer (CRC) – the third most common cancer in the UK and the second leading cause of cancer mortality – is a heterogeneous disease comprising distinct molecular subgroups that differ in their histopathological features, prognosis, metastatic proclivity and response to therapy. Despite advances in the detection and treatment of early-stage disease, patients with advanced, recurrent or metastatic CRCs have few therapeutic options and a dismal prognosis. Utilising state-of-the-art preclinical models harbouring key driver mutations, our group is interrogating the molecular mechanisms underpinning CRC initiation, progression, metastasis and response to therapy. Our overarching goals are to identify early-stage diagnostic biomarkers and develop stage- and subtype-specific targeted therapies.

Deregulation of Wnt signalling is a hallmark of most CRCs, with loss-of-function mutations in the negative Wnt-regulator APC sufficient for adenoma formation. Progression to adenocarcinoma, however, requires the acquisition of additional compounding mutations such as in the *KRAS* proto-oncogene. Accordingly, approximately 35% of all human CRCs harbour concurrent oncogenic mutations in *KRAS* alongside APC deficiency, resulting in the constitutive activation of the pro-proliferative MAPK signalling cascade. In a background of *APC* loss, the acquisition of an oncogenic *KRAS* mutation not only fuels aberrant cell proliferation, but also confers intrinsic resistance to inhibitors of the downstream MAP-kinase MEK and the metabolic sensor mTOR, as well as intrinsic and acquired resistance to EGFR-targeted agents, thus posing a major therapeutic challenge. Furthermore, KRAS has been widely deemed undruggable as multiple attempts to directly target its oncogenic function have met with failure, underscoring the need to develop innovative therapeutic strategies for treatment-refractory *KRAS*-mutant CRCs. Using a suite of preclinical mouse and organoid models, we aim to delineate how oncogenic KRAS alters the molecular landscape of APC-deficient cells with a view to identifying actionable therapeutic vulnerabilities while also sparing normal intestinal homeostasis and Wnt-driven stem cells in bystander tissues.

To probe the mechanisms underlying the cooperation between *Apc* loss and oncogenic KRAS during adenocarcinoma progression, we crossed mice expressing a tamoxifen-inducible, intestine-specific Cre recombinase, under the control of the villin promoter (villinCre^{ER}), with mice harbouring conditional alleles of Apc (*Apc*^{fl}) either alone or in combination with oncogenic *Kras* (*Kras*^{G12D}). Using transcriptomic, translatomic and metabolomic profiling, we found a significant enrichment of pathways associated with mRNA translation and metabolism in *Apc*$^{fl/fl}$*Kras*$^{G12D/+}$ intestinal tissues, relative to *Apc*$^{fl/fl}$*Kras*$^{G12D+/+}$ counterparts, manifesting as elevated rates of cell proliferation and protein synthesis, and extensive metabolic reprogramming.

Targeting MNK/eIF4E translational output in Kras-mutant CRC (CRT/Celgene Translational Alliance)

We have previously shown that the loss of *Apc* alone is accompanied by a markedly increased rate in global mRNA translation that leads to elevated expression of the c-MYC oncoprotein, translational reprogramming and a hyperproliferative crypt-progenitor phenotype. Underpinning this phenotype is a dependence on mTOR signalling, which sensitises emergent and established APC-deficient adenomas to the mTOR-inhibitor rapamycin, conferring a marked survival benefit in early disease models. This approach has since shown efficacy in human patients with familial adenomatous polyposis that harbour germline *APC* defects.

Interrogating more advanced disease models, however, we found that concurrent oncogenic KRAS^{G12D} further augments the global mRNA translation capacity associated with *Apc* loss, rendering APC-deficient cells resistant to rapamycin. Mechanistically, oncogenic KRAS drives increased phosphorylation of the mRNA cap-binding protein eIF4E by the MAPK-interacting serine/threonine-protein kinases MNK1 and MNK2 (Figure 1A). This results in enhanced translation of a select subset of pro-oncogenic mRNAs, including transcripts encoding c-MYC, driving cell proliferation, oncogenic transformation and resistance to rapamycin (Figure 1B and 1C). Intestinal-specific deletion of MNK1/2 (Figure 1D) or treatment with the MNK1/2-inhibitor eFT508 (Figure 1E) restored sensitivity to rapamycin, curtailing tumour growth and extending survival of APC-deficient, *Kras*-mutant mice. Importantly, the MNK/eIF4E axis is not required for normal development and eFT508 is well-tolerated, lending hope for approximately 20% of poor-prognosis CRC patients whose tumours display elevated mTOR and MNK activity.

Oncogenic KRAS-driven metabolic reprogramming unveils novel therapeutic vulnerabilities ("Rosetta" CRUK Grand Challenge)

KRAS activation alone elicited a metabolic shift towards aerobic glycolysis (commonly known as the Warburg effect). Concomitant deletion of *Apc* and oncogenic activation of KRAS in the mouse intestinal epithelium additionally increased glutamine consumption through a pronounced upregulation of genes associated with glutamine transport and metabolism. 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 *Apc*$^{fl/fl}$*Kras*$^{G12D/+}$ intestinal tissues, relative to *Apc*$^{fl/fl}$*Kras*$^{G12D+/+}$ counterparts, and decreased channelling of isotope-labelled 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 neutral essential amino acids, such as leucine,

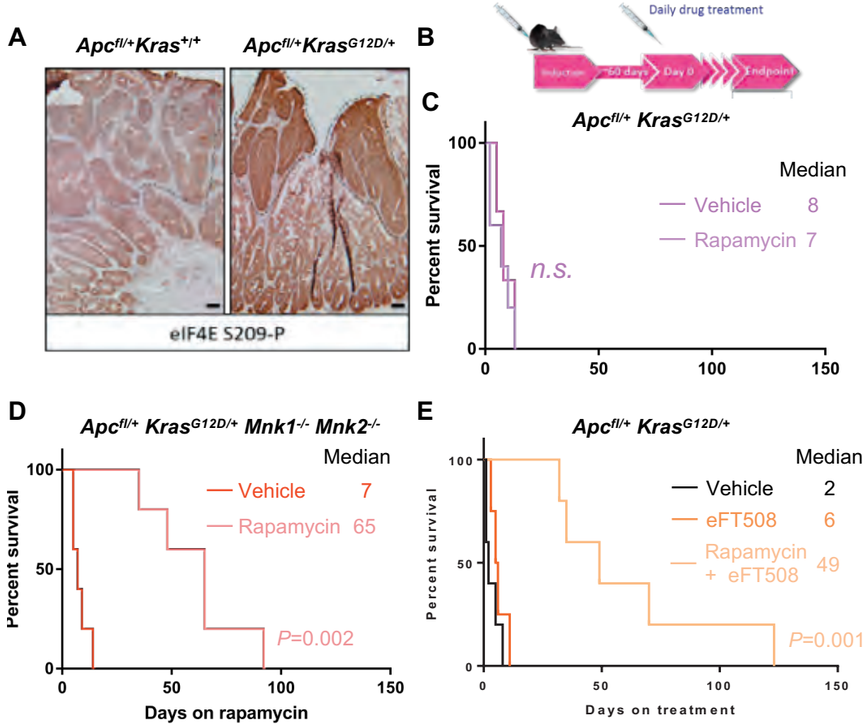

Figure 1
Oncogenic KRAS confers therapeutic resistance to rapamycin, which can be overcome by genetic or pharmacological targeting of MNK1/2.
(A) Oncogenic KRAS stimulates (MNK-mediated) eIF4E phosphorylation in colonic adenomas from *Apc*$^{fl/+}$*Kras*$^{G12D/+}$ mice relative to *Apc*$^{fl/+}$*Kras*$^{+/+}$ counterparts. Dashed lines denote adenoma boundaries. Scale bars, 50 µm. (B) Schematic of treatment timeline. Mice were aged until they developed symptoms of intestinal disease and, subsequently, treated with vehicle or the indicated drugs until endpoint. (C) Lack of survival benefit in *Apc*$^{fl/+}$*Kras*$^{G12D/+}$ mice treated with rapamycin versus vehicle. (D, E) Genetic (D) or pharmacological (E) targeting of MNK1/2, in combination with rapamycin treatment, significantly improves the survival of mice harbouring APC-deficient, *Kras*-mutant intestinal adenomas. Kaplan–Meier survival curves span the number of days on treatment.

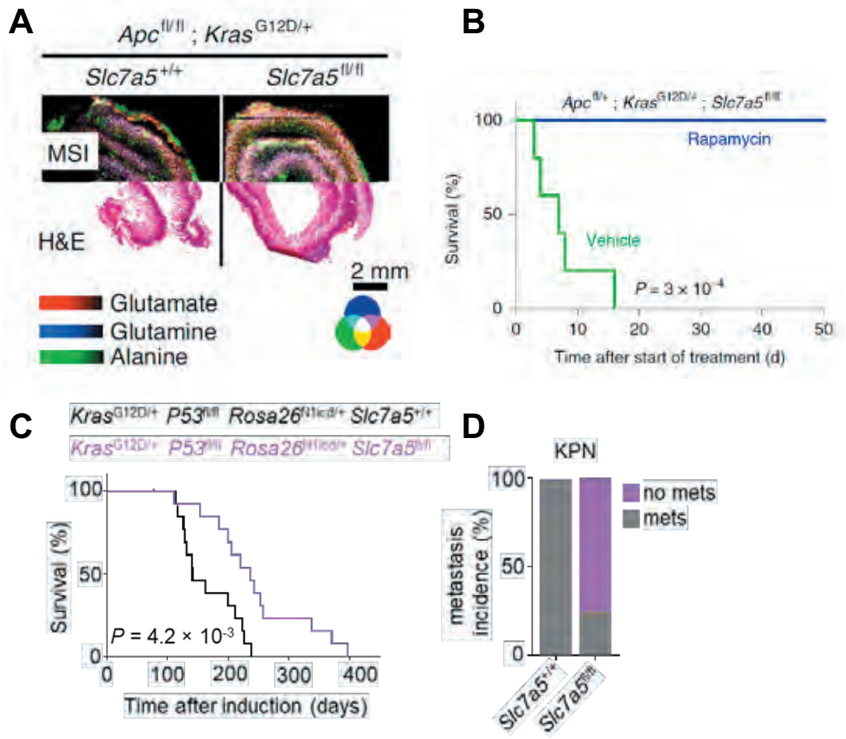

Figure 2
Targeting the glutamine antiporter SLC7A5 alters amino acid dynamics and extends survival of tumour-prone mice.
(A) Haematoxylin and eosin (H&E) staining (bottom) and ion intensity distribution of glutamine, glutamate and alanine (top) in intestinal tissues from *Apc*$^{fl/fl}$ *Kras*$^{G12D/+}$ *Slc7a5*$^{+/+}$ and *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) Kaplan–Meier survival curves for vehicle- and rapamycin-treated *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) Kaplan–Meier survival curves for *Kras*$^{G12D/+}$ *Trp53*$^{fl/fl}$ *Rosa26*$^{N1icd/+}$ *Slc7a5*$^{+/+}$ and *Kras*$^{G12D/+}$ *Trp53*$^{fl/fl}$ *Rosa26*$^{N1icd/+}$ *Slc7a5*$^{fl/fl}$ mice aged until clinical endpoint. (D) Incidence of metastasis (mets) in *Kras*$^{G12D/+}$ *Trp53*$^{fl/fl}$ *Rosa26*$^{N1icd/+}$ *Slc7a5*$^{+/+}$ (KPN Slc7a5$^{+/+}$) and *Kras*$^{G12D/+}$ *Trp53*$^{fl/fl}$ *Rosa26*$^{N1icd/+}$ *Slc7a5*$^{fl/fl}$ (KPN *Slc7a5*$^{fl/fl}$) mice aged until clinical endpoint.

isoleucine, histidine, and lysine, that stimulate mTOR signalling and fuel protein synthesis.

Targeted deletion of *Slc7a5* in the intestinal epithelium of *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 *Apc*$^{fl/fl}$ *Kras*$^{G12D/+}$ mice (Figure 2B). Deletion of Slc7a5 also compromised tumour formation and metastasis (Figure 2C and 2D) in our aggressive, metastasis-prone, KRAS-driven "KPN" model of CRC (see below). These 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 and underpinned by extensive metabolic reprogramming, but also for the highly aggressive CMS4 subtype.

Modelling and targeting late-stage CRCs

The lack of tractable – particularly metastatic and immunocompetent – preclinical models has hampered efforts to study late-stage CRCs. Having identified Notch-pathway enrichment in human metastatic disease, we engineered mice harbouring KRAS^{G12D} activation, TRP53 deficiency and constitutive activation of NOTCH1 signalling in the intestinal epithelium. These so-called
KPN mice develop highly invasive, poorly differentiated, serrated intestinal adenocarcinomas that readily metastasise to distant organs and molecularly recapitulate the poor-prognosis CMS4/CRIS-B subtypes of human CRC (Figure 3A and 3B). In this autochthonous metastasis-prone model, hyperactive NOTCH1 signalling drives the production of neutrophil chemoattractants – notably the CXCR2-ligand CXCL5 and TGFB2 – leading to the accumulation of neutrophils within the pre-metastatic niche while simultaneously repelling cytotoxic T-cells to facilitate immune evasion and metastasis (Figure 3C and 3D). Indeed, neutrophil infiltration correlates with poor survival in CRC patients with treatment-naive metastases, and human CMS4/CRIS-B tumours are enriched for a neutrophil gene expression signature. Moreover, elevated expression of Notch-pathway components and *TGFB2* correlates with poor patient survival. Targeting Ly6G^{+} neutrophil populations, using a small-molecule CXCR2-inhibitor, an ALK5-inhibitor, a TGFB ligand-trap, or anti-Ly6G antibodies, attracts cytotoxic T-cells to the pre-metastatic niche and abrogates metastasis without, intriguingly, impacting primary tumour burden [Jackstadt *et al.* Cancer Cell 2019, 36(3): 319–336.e7]. We will next assess how epithelial tumour cell-intrinsic signalling rewires the tumour microenvironment and identify key stromal determinants of immune evasion.

Using our suite of preclinical models, we are evaluating emerging therapeutics that target the altered translational dynamics and metabolite dependencies of transformed cells and partnering with leading industry innovators (CRT/Celgene Translational Alliance; Novartis) to accelerate the path from bench to bedside. Overall, these approaches will inform our understanding of CRC pathogenesis and metastatic competence, and provide a platform for the development of novel stage- and subtype-specific therapies.

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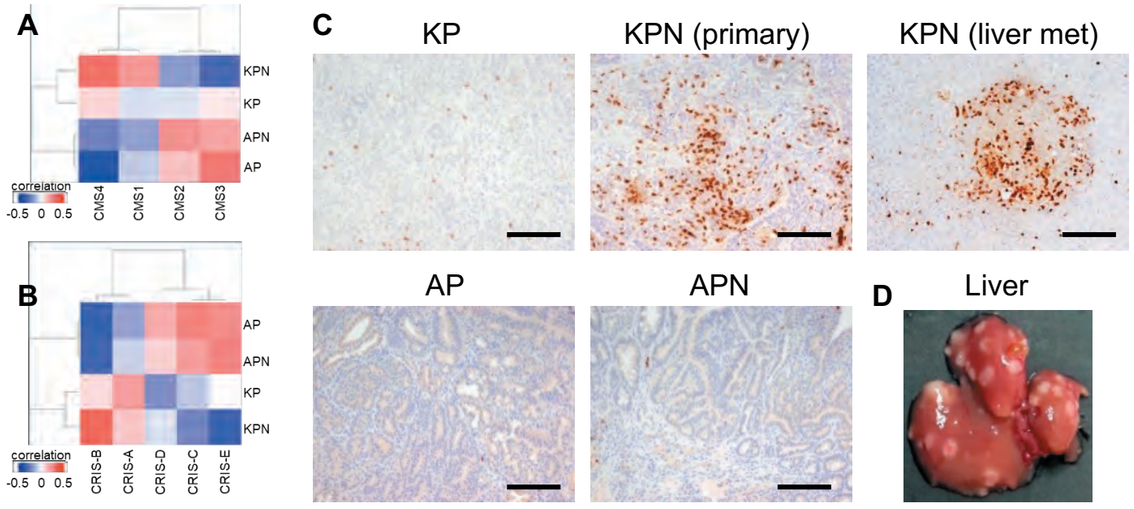

Figure 3
Epithelial NOTCH1 drives poor prognosis signatures in CRC and controls neutrophil recruitment to drive metastasis.
(A, B) Heatmaps showing expression correlation of our intestinal cancer models with: (A) the consensus molecular subtypes (CMS) of human CRC and (B) the CRC intrinsic subtypes (CRIS). Blue: lower correlation; Red: higher correlation. KP: villinCre^{ER} *Kras*$^{G12D/+}$ *Trp53*$^{fl/fl}$; KPN: villinCre^{ER} *Kras*$^{G12D/+}$ *Trp53*$^{fl/fl}$ *Rosa26*$^{N1icd/+}$; AP: villinCre^{ER} *Apc*$^{fl/+}$ *Trp53*$^{fl/fl}$; APN: villinCre^{ER} *Apc*$^{fl/+}$ *Trp53*$^{fl/fl}$ *Rosa26*$^{N1icd/+}$ [N1cd: intracellular domain of NOTCH1]. (C) Immunohistochemistry for the neutrophil marker Ly6G showing neutrophil infiltration of a primary tumour and metastasis from KPN mice not observed in non-metastatic tumour models. Scale bars, 100 µm. (D) Macroscopic liver metastases of KPN mice.

MITOCHONDRIA AND CANCER CELL DEATH

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

Mitochondria, cell death and cancer

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

Therapeutic targeting of BCL-2¬¬-regulated cell death in glioblastoma

Glioblastoma is an aggressive type of brain cancer with very poor prognosis. Mainstay current treatments entail surgery, radiotherapy and chemotherapy (temozolomide) and unfortunately provide limited long-term benefit. In collaboration with Prof. Anthony Chalmers (Institute of Cancer Sciences), we are investigating whether targeting pro-survival BCL-2 proteins (using BH3-mimetics) either alone, or combined with radiotherapy, may improve therapeutic outcome. Towards this goal, we apply various methods including patient-derived tumour cell lines, *in vivo* mouse models as well as *ex vivo* culture of primary tumour samples. We find that BH3-mimetics can cross the blood brain–barrier to reach effective concentrations. Most importantly, we are finding that glioblastoma often displays dependency on specific BCL-2 family members, including MCL-1 (Figure 1). Future work will determine the molecular basis for this survival dependency and address the efficacy of combining BH3-mimetics in combination with radiotherapy.

BCL-2 proteins, metabolism and cancer

BCL-2 proteins have been implicated in the regulation of metabolism, although exactly how they modulate metabolism remains elusive. Moreover, whether metabolic roles of BCL-2 proteins impinges on tumourigenesis is unclear. Applying BH3-mimetics as tool compounds, we have investigated how BCL-2 proteins regulate metabolism, with the aim of investigating whether this contributes to BCL-2 oncogenic effects and/or impinges on the efficacy of BCL-2-targeting BH3-mimetics. Our approach to this question has made use of metabolic flux analyses and mitochondrial activity assays. Interestingly, treatment of cells with the clinically approved BCL-2 inhibitor venetoclax (also called ABT-199) reduces basal oxygen consumption across a panel of cell lines and alters mitochondrial morphology (Figure 2). Importantly, these effects are independent of pro-apoptotic BAX and BAK, thus demonstrating that venetoclax suppresses oxygen consumption independently of the canonical apoptotic machinery. Moreover, we demonstrated that BCL-2-regulated metabolic effects appear solely through inhibition of cell death. Ongoing work aims to understand how venetoclax mediates these effects and define whether these affect its cell killing ability.

Publications listed on page 111

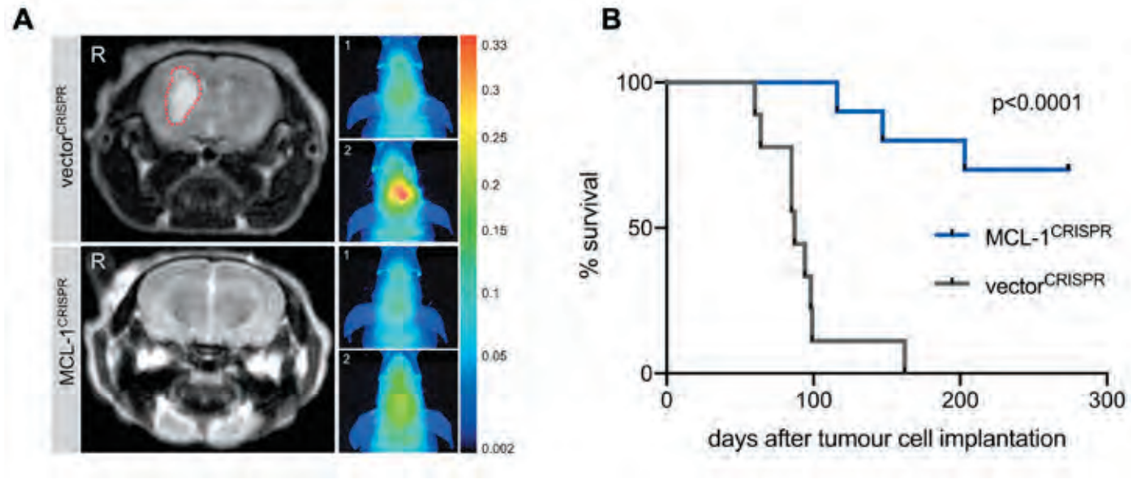

Figure 1
Anti-apoptotic MCL-1 supports glioblastoma growth
A) MCL-1 was deleted by CRISPR/Cas9 genome editing in patient-derived glioblastoma cells expressing iRFP. Following implantation in mice, brain tumour growth was measured by MRI or by iRFP analysis. Tumour cells lacking MCL-1 display strong inhibition in tumour growth
B) Survival plot showing that mice implanted with glioblastoma cells lacking MCL-1 have a significant survival advantage.

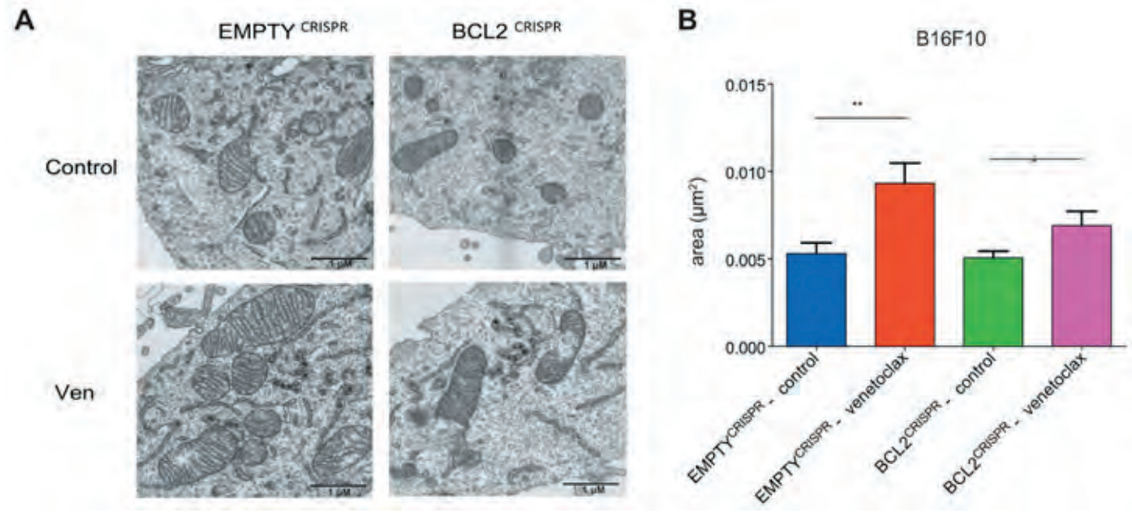

Figure 2
Bcl-2-targeting BH3-mimetic venetoclax alters mitochondrial morphology
A) Transmisison electron microscopy (TEM) images from B16F10 CRISPR-EMPTY and CRISPR-BCL2 cells after 24h of venetoclax (1µM) treatment. Scale bar=1µm.
B) A random selection of at least 25 mitochondria across different cells and fields was analysed for each condition from images in A).

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ONCOMETABOLISM

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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). 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 fixes 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 and sustained GS expression.

By means of HPLC–mass-spectrometry–based metabolomics and cell biology approaches, we are studying the carbon and nitrogen metabolism of liver tumours with high GS expression.

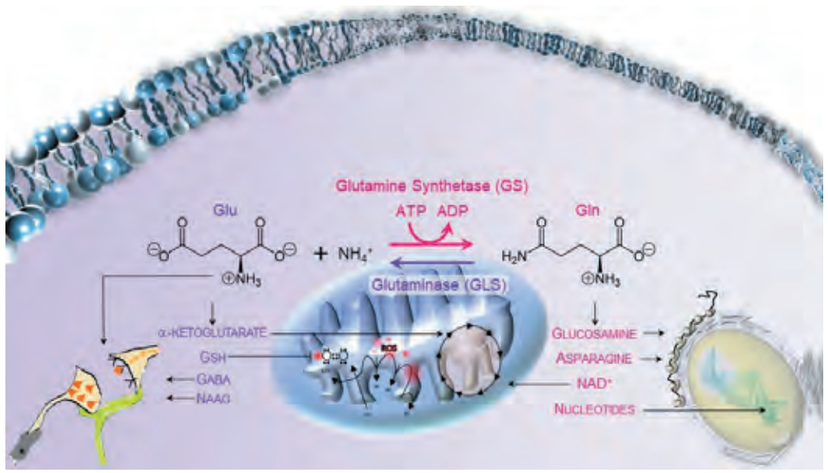

Figure 1
A schematic of the reactions catalysed by the enzymes Glutaminase and Glutamine Synthetase. These two enzymes catalyse opposed reactions essential to maintain glutamine and glutamate at homeostatic levels in cells, tissues and organisms. More than fifty metabolic reactions utilise or produce these two amino acids that are key for a multitude of cellular processes and tissue functions, some of which are selected by cancer cells, supporting their growth and survival.

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.

Identification of the 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, unavoidable collateral effects of these drugs 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 J *et al.* Sci Adv. 2019, Ackermann T, *et al.* Trends Cancer. 2019). 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 established primary human cells derived from different tissues.

In 2020, Plasmax™ became the first physiological medium to be commercially available (Ximbio.com). We are confident that 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.

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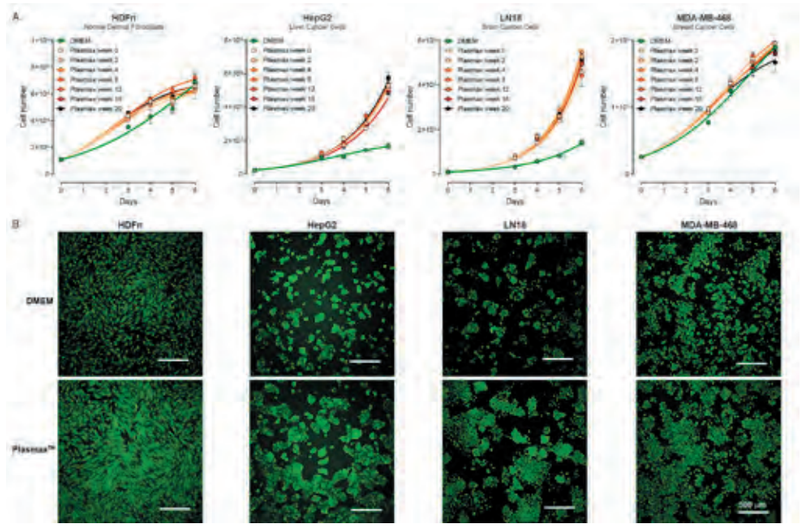

Figure 3
Plasmax™ sustains proliferations of normal and cancer cells comparably or better than DMEM even when stored at 4°C for up to 20 weeks.
(A) Normal Human Dermal Fibroblasts (HDFn), HepG2, LN18 and MD-MB-468 cells were seeded in DMEM in 24 wells plates, at 1x104, 2x104, 1x104, 2x104 cells/well 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.

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MATHEMATICAL MODELS OF METABOLISM

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Metabolism is essential for life, and its alterations are implicated in multiple human diseases. The transformation from a normal cell to a cancerous cell requires metabolic changes to fuel the high metabolic demand of cancer cells, including but not limited to cell proliferation and cell migration. Our group investigates cancer metabolism from an evolutionary point of view. We hypothesise that, given specific microenvironment conditions and metabolic constraints, there is an optimal mode of cell metabolism to achieve a metabolic objective. This metabolic mode will offer an evolutionary advantage and therefore will be selected for during the time course of cancer development. We are currently investigating the selective advantage of mitochondrial formate production and how this information can be used to reduce cancer-related mortality.

Excess formate production promotes cancer cell growth and proliferation

Formate is a precursor for the synthesis of nucleotides and it is essential for life. Deficiency in formate production is associated with embryonic lethality and can be rescued by formate supplementation. We discovered that cancer cells produce formate in amounts that exceed the demand for nucleotide synthesis. The excess formate is released from cancer cells, an observation we called formate overflow (Meiser et al., Sci Adv 2016, 2:e1601273). In collaboration with Karen Blyth's lab at the Institute, we have demonstrated the manifestation of formate overflow in mouse models of cancer (Meiser et al., Nat Commun 2018, 9:1368).

Our next challenge was to determine the selective advantage of excess formate production. Using a combination of theoretical modelling and metabolomics analysis, we discovered that formate induces a metabolic switch from low to high adenine nucleotide levels, increasing the rate of glycolysis and repressing the AMPK activity. *In vivo* data for mouse and human cancers confirms the association between increased formate production, nucleotide synthesis and energy metabolism.

Formate also increases the levels of orotate, a precursor of *de novo* pyrimidine synthesis. Since formate is not an orotate building block, we hypothesised it is an indirect effect. In collaboration with Sara Zanivan's group at the Institute, we combined phospho-proteome and metabolic profiling to interrogate the specific mechanism. We discovered that formate induces the kinase activity of the mechanistic target of rapamycin complex 1 (mTORC1). Treatment with the allosteric mTORC1 inhibitor rapamycin abrogates carbamoyl phosphate synthetase (CAD) phosphorylation and pyrimidine synthesis induced by formate. Furthermore, the formate-dependent induction of mTORC1 signalling and CAD phosphorylation is dependent on purine synthesis.

Our investigations indicate that formate is a potent regulator of cancer cell growth and proliferation (Figure 1). Excess formate production inhibits AMPK signalling, a pathway that represses growth. Excess formate production induces mTORC1 signalling, the master regulator of cell growth. The formate-dependent inhibition of AMPK and stimulation of mTORC1 signalling is mediated by purine synthesis. Therefore, the concomitant inhibition of serine catabolism to formate and purine synthesis is a potential therapy for cancer treatment. We are investigating this hypothesis in the context of leukaemia, in collaboration with Vignir Helgason's group at the Institute of Cancer Sciences, University of Glasgow.

Formaldehyde reacts with amino acids in cells

Formaldehyde is a highly reactive molecule and a known carcinogen. The cancer risk associated with environmental exposure to formaldehyde has been extensively studied. There is also an increased appreciation for the potentially harmful effects of formaldehyde generated by our endogenous metabolism. Formaldehyde is formed from demethylation reactions, the oxidative breakdown of folates and the metabolism of methanol, methylamine and adrenaline. Intracellular formaldehyde is turned over to formate dependent on the activity of mitochondrial aldehyde dehydrogenase (ALDH2) and/or cytosolic aldehyde dehydrogenase class 3 (ADH5). ALDH2 is expressed mostly in the liver and other visceral tissues, while ADH5 is ubiquitously expressed.

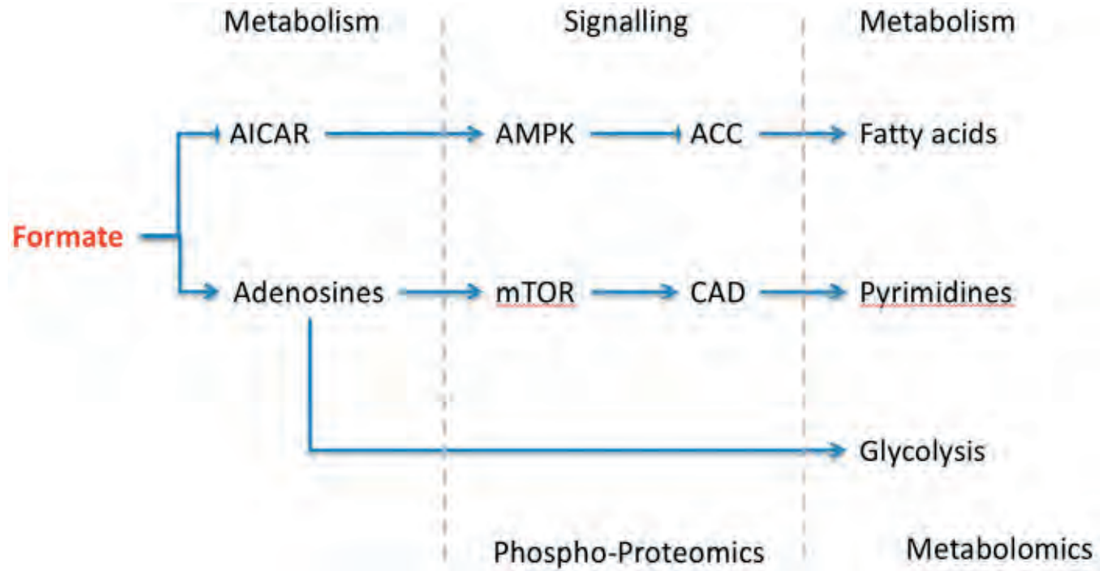

Figure 1
Formate-dependent metabolic switch.

Mice with *Adh5* deletion are healthy, suggesting the existence of other pathways of formaldehyde detoxification. To address this question, we have performed metabolomic analyses of cells exposed to formaldehyde. When mammalian cells are exposed to formaldehyde, the levels of the reaction products of formaldehyde with the amino acids cysteine and histidine – timonacic and spinacine – are increased. These reactions take place spontaneously and the formation of timonacic is reversible. The levels of timonacic are higher in the plasma of *Adh5*$^{-/-}$ mice relative to controls and they are further increased upon administration of methanol. We conclude that mammals possess pathways of cysteine- and histidine-dependent formaldehyde metabolism and that timonacic is a formaldehyde reservoir.

Publications listed on page 112

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TUMOUR MICROENVIRONMENT AND PROTEOMICS

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High grade serous ovarian cancer (HGSOC) and triple negative breast cancer (TNBC) have limited treatment options for the patients, because only few targeted therapies effectively kill cancer cells. However, cancer cells are embedded within a stroma populated by different cell types, which offer novel opportunities for therapy. Among them are cancer associated fibroblasts (CAFs), which our lab and other groups have shown to play major roles to support cancer progression. 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). Our research focuses on understanding the molecular mechanisms through which CAFs promote cancer; we envisage that targeting CAFs in combination with cancer cells is 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 is densely populated by CAFs (Figure 1), while CAFs have been 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.* Proteomics 2018). We aim to decipher how CAFs create a pro-tumorigenic and pro-metastatic microenvironment and how we can block this process; our overarching goal is to determine strategies to target CAFs for therapy.

We study how CAFs support cancer progression and the spread of metastases by directly influencing the behaviour of the cancer cells and of the tumour vasculature. In many solid tumours, the vasculature is responsible for the progression of the disease. Initially, tumours recruit blood vessels to obtain nutrients and oxygen to sustain the proliferation of the cancer cells. Later on, the tumour vasculature becomes leaky and provides a route for the cancer cells to escape and form distant metastases.

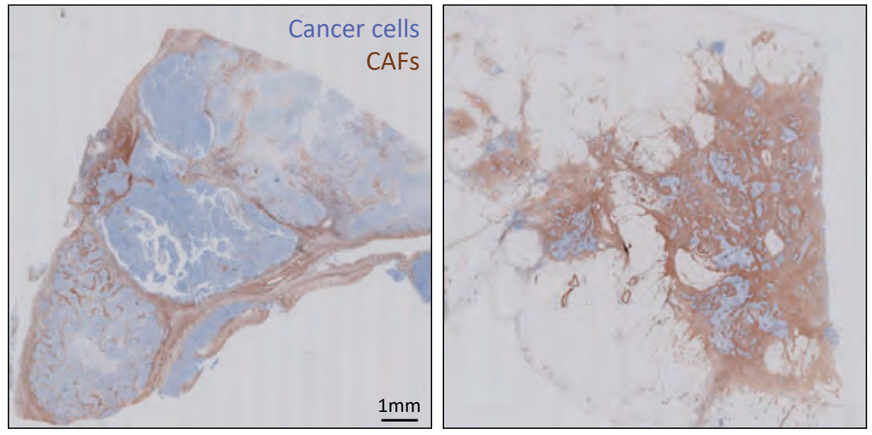
CAFs can originate from the normal fibroblasts resident at the site where the primary tumour develops. In the presence of cancer cells, normal fibroblasts become activated. This activation induces extensive reprogramming of gene expression and protein levels, such that CAFs become able to secrete a plethora of soluble factors and ECM components that actively support cancer progression. CAFs have also been shown to secrete EVs, whereby their cargos can support tumour progression by supporting cancer cell growth and invasion (Santi *et al.* Proteomics 2018).

To understand how to target CAFs in tumours, it is crucial that we understand how CAFs make the tumour microenvironment pro-tumorigenic and pro-metastatic, and what are the molecular mechanisms that sustain these CAF functions. Our major interests are the roles of cell metabolism and extracellular vesicles secreted by CAFs. As CAF models for our research, we mostly use CAFs that we isolate from tumour tissues that patients kindly donate for research purposes (Hernandez-Fernaud, Ruengeler *et al.* Nat Commun 2017, Kugeratski et al. Science Signaling 2019). Our group has strong expertise in mass spectrometry (MS)-based proteomics, and we integrate this innovative technology in our research to tackle the above questions and provide new levels of understanding of CAF biology.

CAF–tumour blood vessels interaction

Endothelial cells (ECs) are a key cellular component of the blood vessels; they line the inner layer of the vessel wall and regulate the functionality and growth of the vessel. In tumours, blood vessels are typically embedded within a CAF-rich stroma, such that ECs physically interact with CAFs or are exposed to the factors that they secrete. Our group has previously shown that CAFs secrete proteins that influence blood vessel growth and their functionality via altering endothelial cell behaviour (Hernandez-Fernaud, Ruengeler *et al.* Nat Commun 2017, Kugeratski et al. Sci Signal 2019). We have also shown that the ECM proteins secreted by CAFs play an active role in the metastatic dissemination by facilitating the binding of the cancer cells to the blood vessels (Reid *et al.* EMBO J 2017). Our ongoing work has found that CAFs influence EC behaviour also through the transfer of functional proteins mediated by EVs. We are currently investigating this process and its impacts on breast cancer progression.

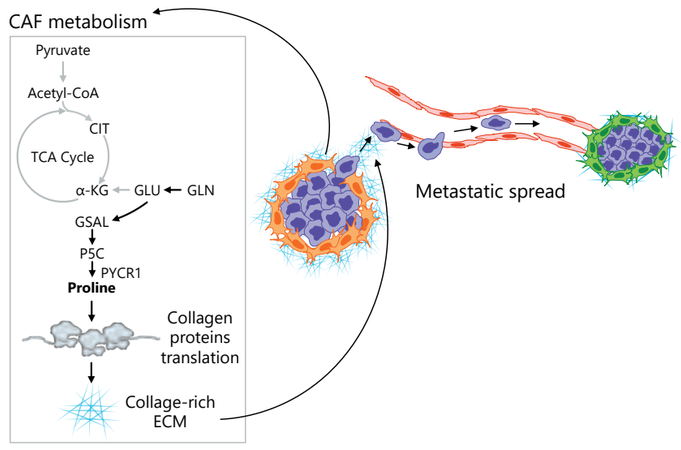
Figure 1
Immunohistochemistry staining for the CAF marker alpha-smooth muscle actin (αSMA) of sections of high grade serous ovarian tumours shows extensive CAF-rich regions of the tumour. In violet nuclear counterstain highlights tumour regions rich in cancer cells.



CAFs & metabolism

Metabolic alterations are a well-established hallmark of cancer. It has been known for a long time that the metabolism of the cancer cells plays crucial roles in promoting and supporting cancer. In the last few years, it has emerged that also the metabolism of stromal cells is an important regulator of cancer. Several works have shown that cancer cells can hijack CAF metabolism by inducing CAFs to secrete metabolites that are necessary for their own growth. Instead, we have found that CAF metabolism also supports hallmarks of CAFs important for tumour progression and metastatic dissemination. Exploiting state-of-the-art mass spectrometry technologies to measure the proteome and metabolome of CAFs and corresponding normal fibroblasts isolated from breast cancer patients, we have found that increased levels of the amino acid proline are key to support the production of pro-tumourigenic ECM by CAFs. In particular, increased proline availability is necessary for the production of collagens, which are highly abundant ECM components with an extremely high content of proline residues. Moreover, we showed that targeting proline synthesis via inhibiting PYCR1, an enzyme essential for the production of proline from glutamine, reduced tumour growth and strongly inhibited metastatic spread in *in vivo* models of breast cancer (Figure 2) (Kay *et al.* BioRxiv 2020). We are investigating further the potential of targeting proline metabolism in cancer.

Figure 2
CAF (orange cells) metabolism supports metastatic spread of the cancer cells (violet cells).



News

This year, Britt Sterken has joined our team as post-doctoral fellow funded by Breast Cancer Now and she works on how cell metabolism supports pro-tumourigenic and pro-metastatic hallmarks of CAFs in TNBC. Teresa Glauner has joined the group to do her PhD and she works on the role of metabolism in the generation of pro-tumourigenic extracellular matrix in HGSOC.

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DRUG DISCOVERY

DRUG DISCOVERY UNIT

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Kate Schraut

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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%).

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 101

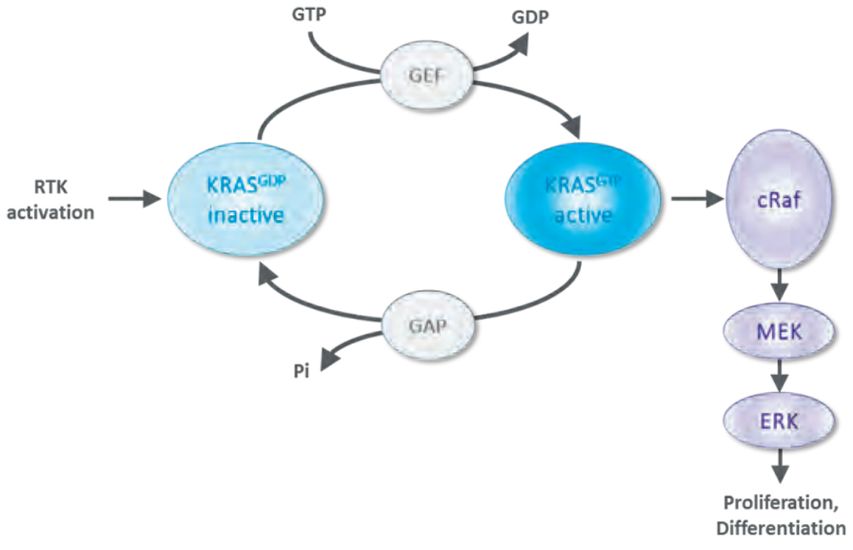

Figure 1
KRAS acts as a molecular switch, rapidly converting to an active state upon receptor tyrosine kinase (RTK) stimulation, by exchanging GDP for GTP via a guanine nucleotide exchange factor (GEF). Active KRAS binds to effectors such as cRaf and activates downstream signalling through the MEK/ERK 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.

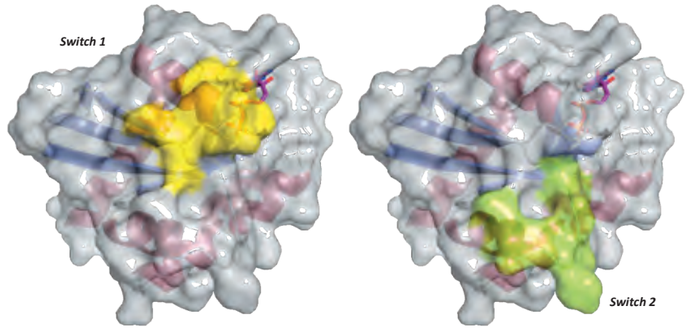

Figure 2
KRAS protein structure showing Switch 1 and Switch 2 which undergo conformational change upon activation to enable binding of effectors such as Raf1

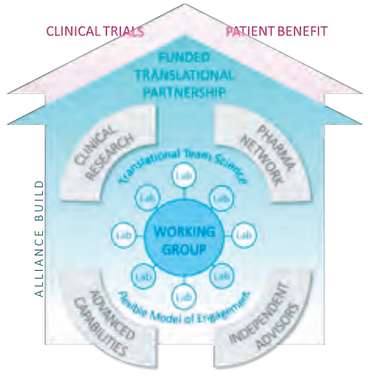

Figure 3
Working group model as a foundation for building translational partnerships that drive novel therapies into clinical trials for patient benefit.

ADVANCED TECHNOLOGIES

The image shows six COS-7 cells expressing LifeAct, a fluorescent reporter for actin filaments, demonstrating their intricate network of actin cytoskeleton. *Photo by Anh Le*

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BEATSON ADVANCED IMAGING RESOURCE

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Leo Carlin

Fellow of the Royal Microscopical Society (FRMS)

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Lynn McGarry
Ewan McGhee
Margaret O'Prey
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. These techniques allow us to 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 paper 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.

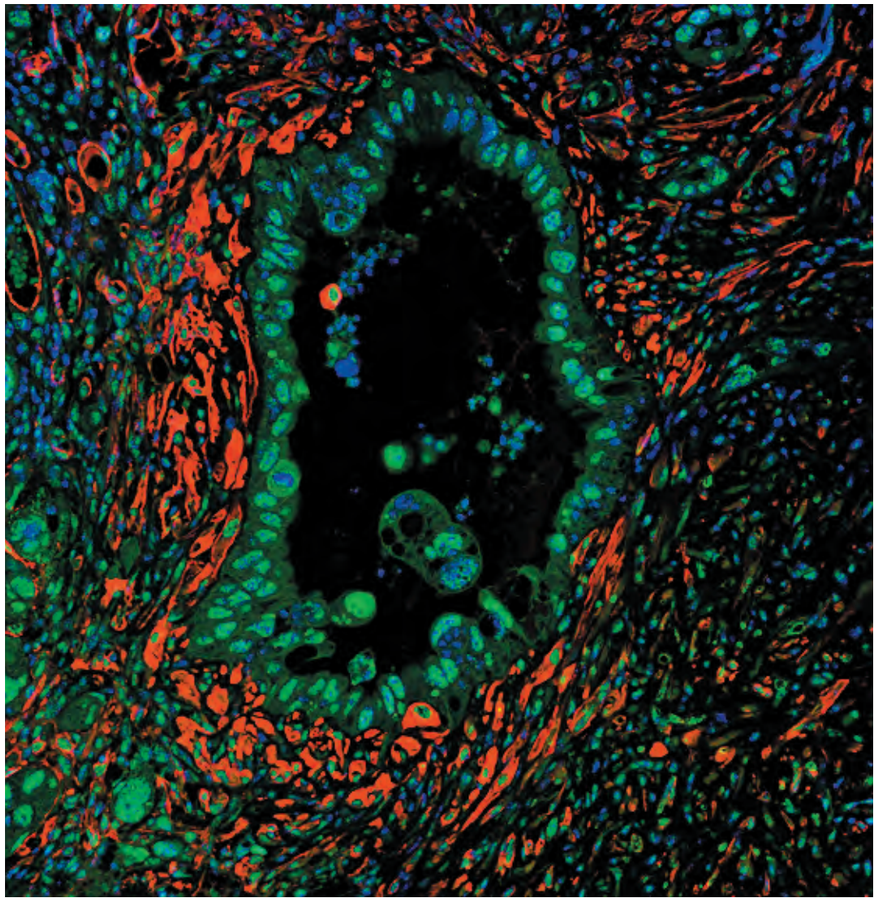
Imaging across different spatial and biological complexity scales

We now 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 technology 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) including label-free approaches (e.g. second harmonic generation to look at fibrillar collagen) by combining tissue clearing, multiphoton excitation and spectral imaging; Image cell behaviour over several days in tissue culture incubators; Address the physicochemical environment of probes, molecular activity and signal transduction pathways below the diffraction limit at different spatiotemporal scales using FLIM, FRET and super-resolution imaging; and address cell function in intact living organisms via advanced intravital microscopy. In this way, we hope to 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.

In addition to generously contributing their time to SARS-CoV2 testing at the Lighthouse Labs in Glasgow, the team have seriously innovated in the way they support our scientists. They have worked hard to develop and provide virtual training and support and also have taken on more experimental work on behalf of our researchers than ever before when training wasn't possible.

New team members and instrument

Excitingly, at the very end of 2020 we received our latest instrument: the Zeiss Elyra 7 Lattice Structured Illumination Microscope (SIM)! Building on technology recognised by the 2014 Nobel Prize in Chemistry, this will complement our existing setups brilliantly by allowing rapid gentle SIM imaging at 60- or 120-nanometre resolution and also single-molecule localisation microscopy down to 10s of nanometres. This will allow researchers at the Institute to pinpoint important cancer mechanisms in living cells and tissues down to a sub-organelle level. Nikki Paul joined the BAIR team at the beginning of 2020, coming to us with a wealth of experience in imaging cancer cell biology. She has rapidly got to grips with developing and transferring advanced imaging methodology in the Institute. Towards the end of 2020, we were also excited to welcome Peter Thomason, another expert imaging cancer cell biologist to join our team part time and he will join full time in 2021.



A cancerous duct in a mouse model of pancreatic ductal adenocarcioma surrounded by cancer associated fibroblasts. *Image by Dale Watt.*

BIOINFORMATICS AND COMPUTATIONAL BIOLOGY

Head
Crispin Miller

Scientific Officers
Ann Hedley
Matthew Neilson
Robin Shaw

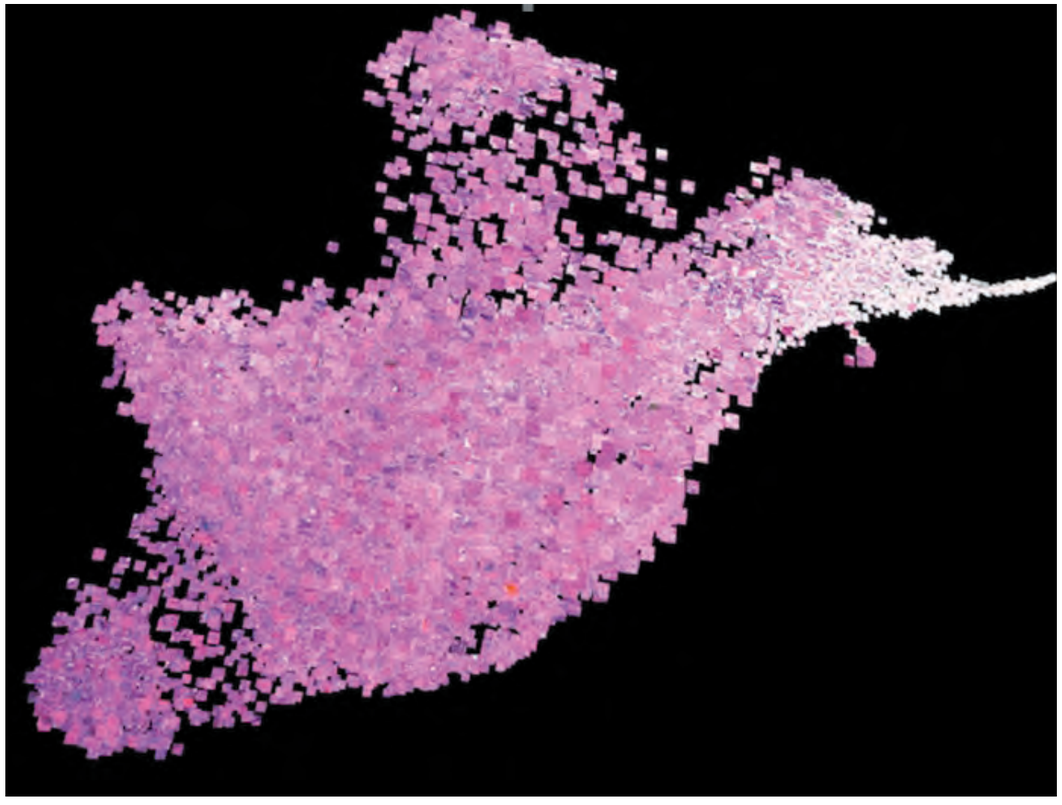
The Bioinformatics and Computational Biology unit provides numerical expertise across the Institute. A major aspect of our work continues to centre on the analysis of high-throughput 'omics data, including a wide range of next-generation sequencing, proteomics and metabolomics datasets. Our remit also 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 datasets 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.



This image shows colon tumours (each little square is a cancer image) being classified by self-supervised learning. *Image by Rob Insall*

METABOLOMICS

Head
David Sumpton

Graduate Student
Rachel Harris

Metabolism is a cornerstone 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 to the Institute's researchers, 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 Exactive 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 Quadrupole instrument that broadens the sensitivity and specificity of the detection for specific metabolites of interest. In addition, an Agilent GC-MS Triple Quadrupole instrument provides complementary coverage to our LC-MS systems.

This has proven to be a challenging year with the requirement to operate differently and safely under the necessary COVID restrictions. It has also been a year of change with Gillian Mackay stepping down as head of the facility. At the beginning of February, David Sumpton assumed leadership of the facility. David has worked with LC-MS at the institute since 2007 and within the metabolomics facility for the past five years. 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 and custom analysis when needed. We offer expertise and assistance in data analysis, data interpretation and experimental design. We also offer training in the data analysis of targeted metabolomics experiments. Increasingly, we are making use of a more complex untargeted analysis to glean more from our LC-MS analysis. We are continuously striving to further develop these methods.

Our ongoing PhD project in collaboration with Saverio Tardito's group provides one means to improve upon our methodologies. The project's aim is to study the metabolism of BRAF mutant melanoma for therapeutic gain. Mutations in BRAF are the most frequent found in melanoma, and BRAF inhibitors provide an excellent first line of therapy; 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 if 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 apply targeted and untargeted metabolomics to melanoma cells grown in culture and to tumours *in vivo*.

These methodologies are being put to further use by Saverio's team. In particular, our untargeted approach has aided their investigation into the role of glutamine synthetase in β-catenin-driven hepatocellular carcinoma.

We have continued to support Alexei Varquez's research into the role of formate metabolism in cancer. A highlight this year was helping to dissect the mechanism by which formate induces pyrimidine synthesis via mTORC1-dependent phosphorylation of carbamoyl phosphate synthetase (CAD).

Recent work led by Owen Sansom's group has highlighted the importance of metabolite uptake and availability. One project showed that targeting cystine import with an xCT transporter–lowering MEK inhibitor, in combination with statins, can cause acute tumour cell death. Another project making use of our targeted metabolomics in *APC* and *KRAS* mutant genetically engineered mouse models (GEMMs) of colorectal cancer, describes how the mutations drive essential amino acid import via specific upregulation of amino acid transporters to fuel the proliferative demands of the cancer cells. Notably, this work was also carried out in collaboration with Cancer Research UK's Grand Challenge Rosetta project that aims to better understand the spatial distribution of metabolites in tumour tissue. The facility is continuing to provide support to the Grand Challenge team by providing complementary LC-MS analysis and helping to validate the data produced from the novel mass spectrometric imaging techniques.

We also worked with Stephen Tait's group to investigate an off-target effect of Venetoclax, a potent BH3-mimetic. We found Venetoclax induced metabolic reprogramming independent of its inhibition of BCL-2. Investigating this further, the group uncovered that the Venetoclax-induced metabolic rewiring was dependent upon the integrated stress response and ATF4 transcription factor.

Hing Leung's group employed both our metabolomics and our updated lipidomics platforms in their study of castration-resistant prostate cancer. Using a proteomic screening, the mitochondrial 2,4-dienoyl-CoA reductase (DECR1), was identified as an important enzyme associated with the development of therapy resistance. We showed that DECR1 deletion impaired lipid metabolism, which induced ER stress and ultimately reduced tumour growth.

Publications listed on page 106

PROTEOMICS

Head
Sara Zanivan

Scientific Officers
Maja Bailey[1]
Kelly Hodge
Grigorios Koulouras
Sergio Lilla

[1]CRUK Early Detection and Diagnosis Primer Award

Proteins constitute half of the cell (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 proteomes analyses. We have strong expertise in quantitative analysis of secretomes (extracellular matrix, extracellular vesicles and conditioned media) and posttranslational modifications, including cysteine oxidation. For the latter, we have developed SICyLIA, a method that enables the quantification of cysteine oxidation levels at global scale with no enrichment steps required (van der Reest, Lilla et al. Nat Commun 2018) and that has been fundamental to answer different biological questions (Port *et al.* Cancer Discov 2018, Hernandez-Fernaud, Ruengeler *et al.* Nat Commun 2017, Cao X *et al.* J Cell Sci 2020).

This year, we have worked to expand our portfolio, including developing the SICyLIA technology for multiple samples comparison, and we have made good progress to establish an innovative platform for plasma redox proteomics for early detection of cancer.

During 2020, 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 to enrich the quality of the data that the facility can provide.

www.beatson.gla.ac.uk/transgenic_models

TRANSGENIC MODELS OF CANCER

Head
Karen Blyth

Associate Scientist
Kirsteen Campbell

Scientific Officers
Jayanthi Anand
Dimitris Athineos
Laura Galbraith

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

Modelling cancer *in vivo*

The Beatson Institute is renowned for its application of *in vivo* modelling to address key cancer questions. At the core of this is the Transgenic Models Lab, which facilitates collaborative science with many of our colleagues at the Beatson Institute and the University of Glasgow, as well as external research groups. 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. So interrogating aspects of this multifaceted behaviour in a plastic dish has obvious limitations. It is important therefore to use physiologically relevant 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. Our lab utilises genetically engineered mouse models sympathetic to the same genetic alterations in human cancers such as breast, colorectal, pancreatic and prostate cancer. We also have expertise in orthotopic xenograft models, and in syngeneic allograft models permitting interrogation of immune interactions with primary and metastatic tumour cells.

Research Collaborations

As for so many, this year has been challenging and unique, forcing us to adapt to working at home and putting some of our research on hold. Yet it is with immense pride that we can say this has not deterred stimulating Zoom conversations around ongoing and future research collaborations, nor our dedicated team in managing to conduct essential research when access to the lab was permitted. It has been gratifying to collaborate with David Bryant and his group on a variety of projects. For example, using orthotopic models of prostate cancer, we have been interrogating the role of the ARF GTPase Exchange Factor, IQSEC1 in cancer cell invasion and its ability to drive metastasis *in vivo*. We have also been trying to refine these models, and by combining bioluminescence and ultrasound imaging we can derive more subtle information about the metastatic growth of these prostate tumours that would ordinarily be missed (Figure 1).

Targeting cancer cell metabolism presents an important opportunity for novel therapeutic means, and to this end we have continued our long-standing collaboration with Oliver Maddocks at the University of Glasgow. In particular we have been exploring amino acid vulnerabilities such as the polyamine pathway and associated cysteine dependencies in tumour cells (Zhang *et al.*, *Nature Metabolism*, 2020), as well as the role of tryptophan metabolism in providing nutrient sources for pancreatic cancers. It is critical that we can translate the findings in the laboratory to the whole animal and confirm that tumours *in situ* adapt to the same pressures of metabolic rewiring. Using metabolic tracing *in vivo* and dietary intervention, we can probe these adaptations, as well as how we might be able to circumvent them. Increased formate is one such way that cancer cells can adjust to the increased energy demands, as we showed with Alexei Vazquez (Meiser *et al.*, *Nat Commun* 2018; Oizel *et al.*, *Cell Death Dis* 2020).

In other studies, we demonstrated with Michael Olson the relevance of pliable cancer cells towards increased tumourigencitiy (Rudzka *et al.*, *Small GTPases*, 2020); how the MSP-RON axis stimulates cancer cell growth in breast cancer models with Seth Coffelt (Millar *et al.*, *Mol Oncol.*, 2020); and we continue to collaborate with Gareth Inman's group studying the potential utility of targeting TGFβ-signalling in metastatic cutaneous squamous cell carcinoma (Dayal *et al.*, *Br J Dermatol*, 2020). Projects are also ongoing with Sara Zanivan and Seth Coffelt on models of breast and ovarian cancer interrogating the role of the tumour microenvironment, and in particular the interactions with the immune system and cancer-associated fibroblasts. This has involved applying advanced imaging techniques in exciting collaborations with Leo Carlin's group to study the dynamics of metastatic seeding of breast cancer cells and the interactions with the tumour microenvironment.

Resources and Innovations

In addition to our exciting collaborative projects briefly discussed above, our lab trains and supervises researchers at the Institute in the many complex cancer models (e.g. breast cancer, pancreatic cancer, lung cancer, 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 group, 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 renal capsule delivery, orthotopic prostate delivery, mammary intraductal delivery and primary tumour removal. In all our approaches we continually promote the 3Rs, refining our models and exploring replacement models such as mammary organoids.

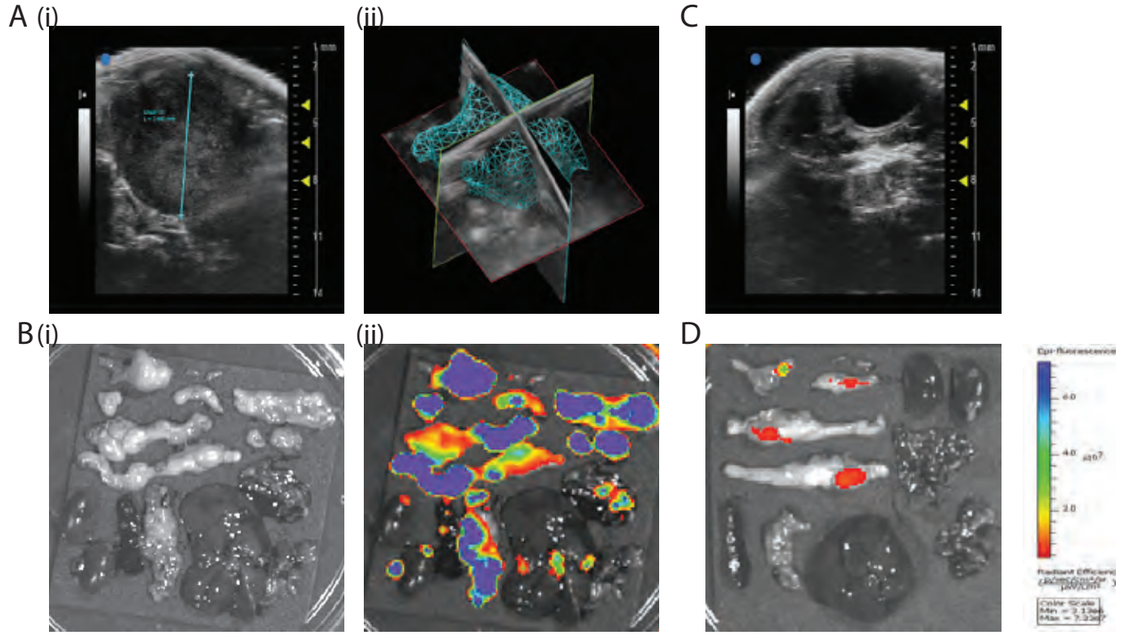


Figure 1
Multimodality imaging in a model of metastatic prostate cancer
(A)(i) An ultrasound image of a male mouse 8 weeks post intra-prostatic injection with human prostate cancer cells (PC3). The blue line denotes the diameter of the prostate tumour while (ii) shows the same tumour, this time in a 3D view re-constructed from multiple 2D cross-sections. This allows for the rendering, using Vevo-lab software, of a 3D volume as evidenced from the blue mesh shown in the image. **(B)(i)** A greyscale image of organs harvested from the same mouse depicted in **(A)**. **(ii)** The PC3 cells were tagged with fluorescent markers and using the IVIS Spectrum it is possible to detect fluorescent signal from tumour cells and identify the primary tumour in the prostate (top left), and also track and identify sites of metastasis as evidenced by fluorescent signal present. **(C)&(D)** Ultrasound and IVIS images (respectively) for a second male mouse 8 weeks post intra-prostatic injection with PC3 cells. No prostate tumour was detectable by ultrasound imaging **(C)**, however IVIS imaging **(D)** showed a weak signal in a small area of the prostate (top left). There is also a signal detected in some of the proximal organs (epididymal fat & lumbar lymph nodes) suggesting sites of metastasis. The difference is striking between **(B)&(D)** highlighting the sensitivity of the IVIS. The scale to the right of **(D)** is for radiant efficiency and applies to IVIS images presented.

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 methods to investigate the consequences of genetic alterations in contributing to the onset and progression of cancer. We can introduce precise genetic changes into specialised stem cells using techniques such as gene targeting or genome editing. These methods allow us to build accurate models of human cancers by the introducing into stem cells identical genetic alterations to those discovered in cancers. Refinements in the technology allows us to introduce multiple genetic changes into cells at the same time. This allows us to analyse how combinations of mutations can interact to enable the development of cancer.

Improving our models of human cancer

We use embryonic stem cells to enable us to decipher the role that different mutations can play in causing growth of cancers. The cells have high rates of homologous recombination, which allow us to introduce specific genetic alterations into these cells with relatively high efficiency. This allows us to effectively copy the exact genetic changes uncovered at the root of human cancers and incorporate these into our models. This can give us an understanding of how these genetic changes can change the function of proteins in intact cells and tissues. Stem cells can also be differentiated into a wide variety of different specialised cells found in different tissues. This allows us to establish how these genetic changes affect the behaviour of cells from the particular tissue in which the mutation was originally uncovered. For example, we can analyse how mutations uncovered in liver cancers affect the biology of liver cells.

A TAZ KO mouse model of Barth syndrome

One such genetic alteration, which specifically affects heart cells, is a truncating mutation in the gene Tafazzin (*Taz*). This mutation also causes an inherited mitochondrial disorder called Barth Syndrome (BTHS). BTHS is a rare mitochondrial disease resulting in a range of variable clinical features including cardiomyopathy, neutropenia, muscle myopathy and metabolic defects. Barth syndrome is caused by mutations in the *Taz* gene. Here at the Beatson Institute, we generated the first conditional model of this gene in order to allow its further analysis and study. The *Taz* KO cells exhibit abnormal mitochondrial function as a result of a defect in the mitochondrial membrane. As a result of these defects, *Taz* KO heart cells are not able to work as effectively as wild-type (WT) cells, identical to the clinical consequences of BTHS seen in affected individuals. As well as this, animals carrying a *Taz* KO mutation show a reduction in growth and an inability to gain weight, even when being fed a high-fat diet.

Mitochondria in Cancer

Mitochondria are responsible for regulating the production of cellular energy, providing amino acid building blocks for new cells as well as controlling redox homeostasis and apoptosis. Functioning mitochondria are therefore essential for tumourigenesis, and the depletion of mitochondria from tumour cells has been shown to compromise this process. For this reason, we were interested in what effect the *Taz* KO allele would have on tumour progression.

Taz KO and Hepatocellular Carcinoma (HCC)

HCC is the most common form of liver cancer, most often found in people with long-term liver diseases and common in people who drink large amounts of alcohol or who have an accumulation of fat in the liver. As animals carrying a *Taz* KO mutation are shown to be resistant to the effects of fatty diet, we believed this was a good cancer model to use for our investigation. We collaborated with another group at the Institute in order to breed the *Taz* KO allele onto the inducible Myc/BCat model of HCC. In this study the proto-oncogene MYC is overexpressed and B-Catenin is mutated directly in hepatocytes of these animals using a liver-specific adenovirus. Over the course of five months and following sampling, it was established that animals carrying the *Taz* KO allele had a significantly lower tumour burden within the liver relative to mice wild-type at the *Taz* locus (Figure 1). The tumours within the Taz KO mice were found to be similar immunohistologically to those found within the WT mice but were smaller, suggesting either these were appearing later or growing slower. Ongoing analysis is underway to investigate this further.

Additionally, this year we have continued to collaborate with a number of other groups at the Institute to generate a wide variety of different new models. These include point mutations, conditional knockouts and protein tags. In addition, we have established new methods that allow the replacement of entire endogenous stem cell genes with their human equivalent. This ensures that the genetic alterations that we make in stem cells are carried out in the precise genomic context, and as a consequence, these changes more accurately model the outcome of the mutations associated with human disease.

Publications listed on page 111

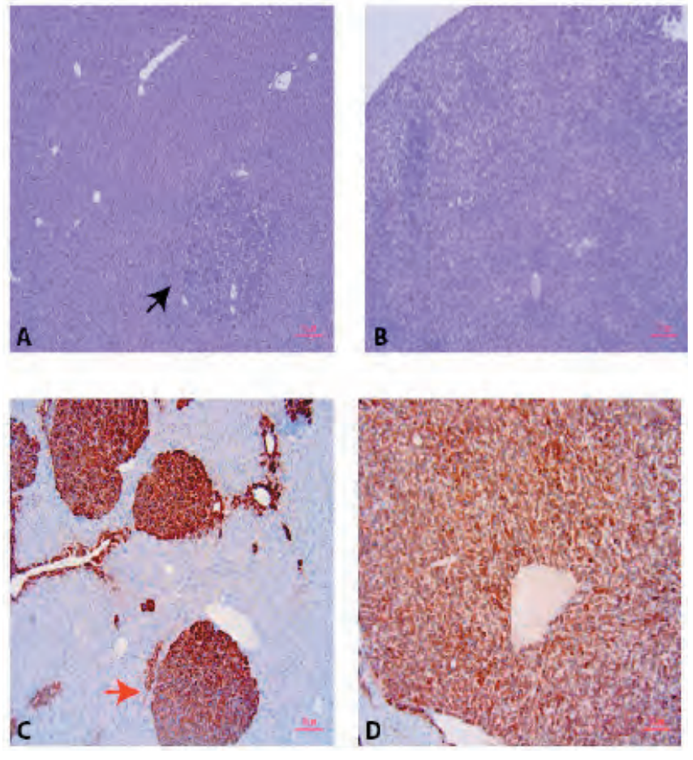


Figure 1
Liver tissue histology
H&E staining of liver tissue taken from induced mice either *Taz* KO (A) or *Taz* WT (B). WT tissue shown to be almost entirely tumour tissue compared with KO where smaller tumours are present (black arrow). Glutamine Synthase (GS) staining of liver tissue taken from either *Taz* KO (C) or *Taz* WT (D) showing that both *Taz* KO and *Taz* WT tumours exhibit β-catenin activation; however, much smaller growths of tumour tissue are present within the *Taz* KO sample (red arrow).

TRANSLATIONAL MOLECULAR IMAGING

Head
David Lewis

Staff Scientist
Gaurav Malviya

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

Medical Physicist
Caroline Findlay[1]

Scientific Officers
Emma Johnson
Agata Mrowinska

[1]Beatson Cancer Charity

Translational Molecular Imaging (TMI) advances novel imaging technologies and serves 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 Centre, 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 and clinical imaging, which are further supported by a wide network of expert collaborators. The TMI drives collaborative imaging research across this network with the focus on developing and applying innovative imaging technologies such as new PET radiotracers and MRI methodology to aid the visualisation and understanding of 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, 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 clinical imaging biomarkers for new applications such as tumour classification and personalised cancer therapy.

PET radiochemistry

In 2020, we finished installation of the [^{11}C]HCN production on our bespoke SYNTHRA Radiopharmaceutical Synthesizer and expanded tracer availability by developing improved radiolabelling of [^{18}F]-labelled FSPG, a tracer for imaging the cystine/glutamate antiporter xCT (SLC7A11).

The R&D radiochemistry platform is now fully equipped for development of novel carbon-11 and fluorine-18 labelled PET probes starting from a range of radiolabelled precursors. This platform allows us to embark on a programme developing a range of carbon-11 labelled amino acids for *in vivo* metabolic studies, including the first amino acid under development, [^{11}C]leucine.

Despite the challenging conditions in 2020, we have continued to support the extensive imaging programmes in the TMI. The list of available radiotracers includes: [^{11}C]acetate, [^{18}F] fluoro-ethyl-tyrosine (FET), [^{11}C]methionine, [^{18}F] fluoro-thymidine (FLT), [^{18}F]terafluoroborate (TFB), [^{18}F] fluorodeoxyglucose (FDG) and (4S)-4-(3-[^{18}F]fluoropropyl)-L-glutamate (FSPG).

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 – the first clinical non-FDG tracer in Glasgow. We have continued to support PhD students from the Department of Chemistry at the University of Glasgow in their radiopharmaceutical development. In collaboration with Prof Andrew Sutherland's laboratory, we have facilitated experiments developing a novel fluorine 18 labelled PARP inhibitor.

Preclinical and translational imaging

In 2020, we published a collaborative study with Prof Hing Leung's laboratory showing that persistent uptake of an [^{18}F]-labelled analogue of the amino acid L-leucine, ^{18}F Fluciclovine, signifies tumour resistance to androgen deprivation therapy (ADT), an effect, at least in part, mediated by upregulated tumour expression of the L-type amino acid transporter LAT1 following chronic ADT (Figure 1). Early detection of castration-resistant prostate cancer using ^{18}F Fluciclovine PET imaging would facilitate patient stratification onto more personalised treatment. The PET/MRI figure published in this manuscript received the Image of the Month Award from the Scottish Imaging Network: A Platform for Scientific Excellence (SINAPSE).

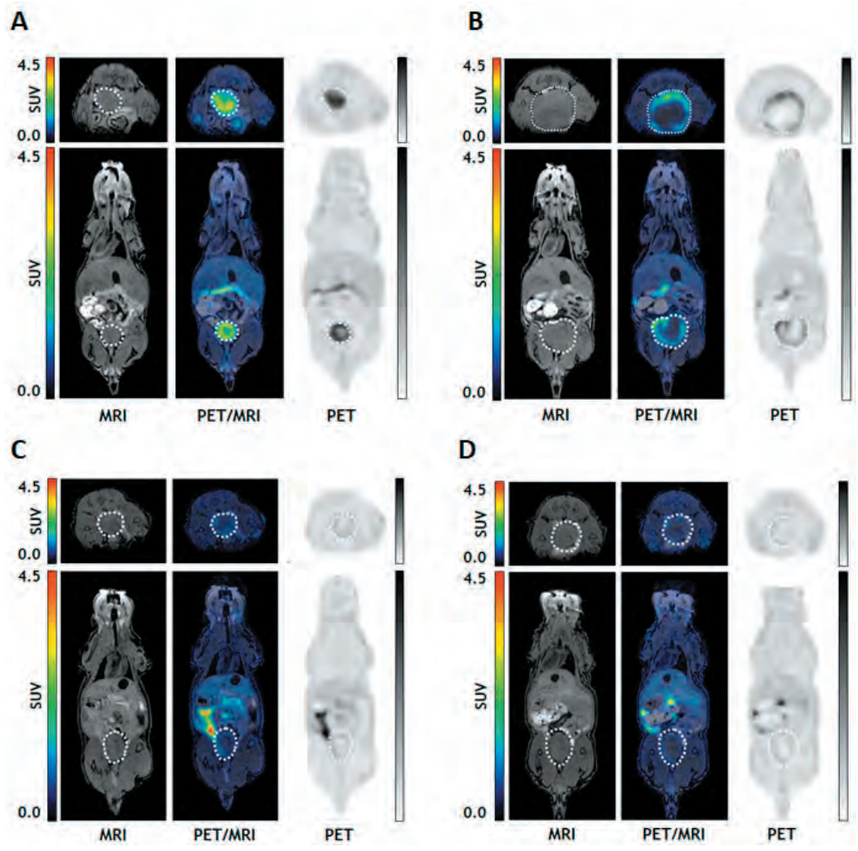


Figure 1
^{18}F-Fluciclovine PET metabolic imaging reveals prostate cancer tumour heterogeneity
Repeated ^{18}F-Fluciclovine PET/MRI imaging on mice bearing CWR22Res (androgen-responsive) orthograft (A) Scan 1 (before ADT) and (B) Scan 2 (after ADT) or 22Rv1 (castration-resistant) orthograft (C) Scan 1 and (D) Scan 2 (both following ADT). Images presented in axial and sagittal imaged field of view (upper panel and lower panel, respectively). (Malviya G et al. EJNMMI Res 2020; 10(1):143)

In collaboration with Dr Douglas Strathdee's laboratory, Transgenic Technology, we investigated *in vivo* metabolic changes in a mouse model of Barth syndrome (Taffazin knockout) using PET/MRI. Using dynamic [^{11}C]acetate and [^{18}F]FDG PET imaging, we identified an oxidative deficiency in the hearts of Taffazin knockout mice and a corresponding switch to higher myocardial glucose consumption. These biomarkers are potentially valuable in identifying metabolic deficits in Barth syndrome patients and for subsequent monitoring of novel therapies.

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 with Prof Owen Sansom's laboratory, we are exploring the role of PET/MRI for phenotyping subtypes of colon cancer. Using the state-of the-art colon cancer models at the Beatson Institute, we are developing spatial and temporal imaging biomarkers for stratifying colon cancer. Ongoing multiplexed PET imaging probing glucose, nucleotide, amino acid and fatty acid metabolism has shown subtype specific differences in imaging phenotype. We aim to further validate this work and identify subtype heterogeneity and plasticity using non-invasive imaging.

Publications listed on page 112

HISTOLOGY

Colin Nixon

Barbara Cadden
Denise McPhee
Gemma Thomson
Mark Hughes
Saira Ghafoor
Shauna Currie Kerr
Tess Aktinson
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.

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, immunohistochemical/ immunofluorescence staining methods or *in situ* hybridisation techniques.

A comprehensive immunohistochemistry service is offered. The immunohistochemistry service offers a large repertoire of previously validated antibodies that can be stained using any of our five autostainers in order to provide 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 method 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 probes 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.

A recent advancement with the *in situ* hybridisation technique means that when a probe is available or one can be specifically designed to 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 fully automated large-capacity Leica Aperio AT2 slide scanner which is capable of capturing 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 the 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. These slides can be appropriately stained, allowing cellular material to be identified and separated and permitting 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

LABORATORY OPERATIONS

Head
Scott Kelso

Laboratory operations cover a number of different functions who 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 been a challenging year for everyone in the Institute due to the COVID pandemic; however, the whole Laboratory Operations team have responded superbly, showing flexibility and resilience. There have been significant changes to the building to implement COVID restrictions, but the team have continued to deliver a high-quality service to our researchers throughout the period.

Going forward, we aim to deliver a strategy of continuous improvement, ensuring best value for all the money spent, whilst retaining a high-quality service for the research groups.

Building Facilities

Alistair Wilson, Andrew Hosie, Mark Deegan

Building Facilities manage the outsourced services provision for catering, cleaning and janitorial services. We provide 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. Use of the online helpdesk facility continues to be an effective means of logging reactive calls for maintenance and repair.

This year we have been heavily involved in preparing the building for COVID-19, implementing one-way routes and distancing measures whilst also completing a project to upgrade the electrical distribution boards in the main research building.

We have also carried out a number of functional moves to create space for a new research group whilst continuing to support the needs of our research staff.

Laboratory Management

Laura Bence, Richard Selkirk, Karen Thomas, James Dyball

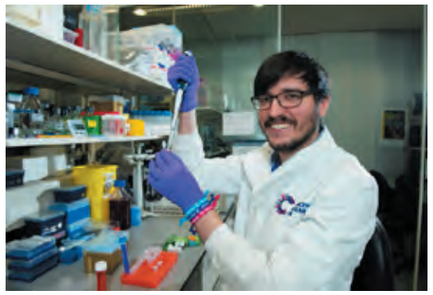
The Laboratory Management team is responsible for providing a number of vital support roles to the Institute. One of the primary roles for the team is the provision of advice, training and information to all staff on health and safety issues, especially with regard to risk assessments and appropriate control measures necessary for laboratory work involving biological, chemical, radiation and genetic modification processes. Safety in regard to fire risk is also managed. As safety plays an important part of everyday life in the laboratory, and in running building services, it is essential that health and safety processes are reviewed and monitored regularly, that any training needs are met and deficiencies rectified and that adequate provision is made to fulfil the Institute's legal obligations to staff. All staff and students are required to have a safety update once a year and new starts attend a series of safety inductions. In addition, a number of training sessions are arranged for new PhD students. During the COVID pandemic a number of online/remote training sessions have been developed instead of face-to-face training.

Another major role of Laboratory Management is to oversee the replacement and purchase of equipment to facilitate the needs of researchers. This year, we arranged a number of demos of new equipment, some showing new technology, to enable researchers to see first-hand the latest state-of-the art technology, which resulted in the purchase of several new pieces of equipment. A number of online training seminars and webinars were also organised to enable researchers to gain knowledge of new and improved techniques using the equipment and technologies already present within the Institute.

Laboratory Management also coordinate the service and maintenance of core equipment and of any systems that these require. The provision of gas supplies, such as carbon dioxide or nitrogen gas, to equipment is carefully managed and coordinated to ensure gas safety is maintained and that systems meet with regulatory compliance. The Lab Management team are proactive in ensuring that equipment breakdowns are kept to a minimum and are being dealt with as efficiently and effectively as possible. Service contracts for core equipment are reviewed annually and procured centrally to ensure costs are kept as low as reasonably practical. We have effective procurement processes and liaise with Cancer Research UK Purchasing to take advantage of any centralised agreements. We also have a good relationship with suppliers to ensure we achieve best prices and discounts for goods. This year we have also actively engaged with a number of our key suppliers to achieve further discounts from them. A further essential role is the monitoring of all outgoing orders to ensure compliance with Institute safety procedures, particularly those relating to COSHH. In addition, assistance is given to users 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. We are also currently progressing a 'Green' policy for recycling and reducing energy costs.

Lab Management work closely with the Stores facility to ensure that, by maintaining a good relationship with suppliers, preferential pricing for laboratory consumables is obtained. As a result of these negotiations, a better turnaround time is received from suppliers with considerable cost savings for the Institute. Lab Management also liaise with Stores to acquire free samples of new products to ensure the best and most appropriate products are used by the researchers.

Laboratory Operations management team: Laura Bence, Richard Selkirk, Scott Kelso, Angela Miller, Alistair Wilson, Billy Clark.



Researcher George Skalka launched Cancer Research UK's campaign for World Cancer Day in Scotland

Laboratory Support Services & Stores

Laboratory Support Services

Angela Miller, **Tracy Shields**, Elizabeth Cheetham, Dilhani Kahawela, Kirstie McPherson, Jonny Sawers, Linda Scott, Conor Gilbey

Laboratory Services provides a vital service, supporting the research undertaken in the Institute. The team work 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, tissue culture solutions and *Drosophila* fly food 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.

Stores

Angela Miller, Michael McTaggart, George Monteith

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 on a self-service basis with automatic cost centre allocation. A porter service is run to deliver external orders to the researchers, while outgoing samples or materials are processed by Stores for courier collection. 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 free 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 and Reagent Services

Billy 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.

Reagent Services provide a diverse range of support to the research groups. Servicing of biological safety cabinets is coordinated every six months. 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. We coordinate batch testing of serum. The facility provides a range of commonly used buffers, for example 10X TBST and bacterial growth reagents. Each product is tested for suitability of use and sterility where possible before being released for general stock. The preparation of antibiotic bacterial culture plates has been automated using a MEDIACLAVE (INTEGRA Biosciences AG) to sterilise and dispense into the plates.

PUBLICATIONS

Imran Ahmad (page 12)
Models of Advanced Prostate Cancer

Primary Research Papers

Dinneen, E. et al.
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Loveridge CJ, Slater S, Campbell KJ, Nam NA, Knight J, Ahmad I, Hedley A, Lilla S, Repiscak P, Patel R, Salji M, Fleming J, Mitchell L, Nixon C, Strathdee D, Neilson M, Ntala C, Bryson S, Zanivan S, Edwards J, Robson CN, Goodyear CS, Blyth K, Leung HY.
BRF1 accelerates prostate tumourigenesis and perturbs immune infiltration. ***Oncogene***. 2020; 39: 1797-1806.

Patel R, Brzezinska EA, Repiscak P, Ahmad I, Mui E, Gao M, Blomme A, Harle V, Tan EH, Malviya G, Mrowinska A, Loveridge CJ, Rushworth LK, Edwards J, Ntala C, Nixon C, Hedley A, Mackay G, Tardito S, Sansom OJ, Leung HY.
Activation of beta-catenin cooperates with loss of Pten to drive AR-independent castration-resistant prostate cancer. ***Cancer Research***. 2020; 80: 576-590.

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Enhanced Quality and Effectiveness of Transurethral Resection of Bladder Tumour in Non-muscle-invasive Bladder Cancer: A Multicentre Real-world Experience from Scotland's Quality Performance Indicators Programme. ***European Urology***. 2020

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RUNX1 marks a luminal castration-resistant lineage established at the onset of prostate development. ***eLife***. 2020; 9: e60225.

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Is MRI Enough or IMRIE study: a multicentre evaluation of pre-biopsy multi-parametric MRI compared to biopsy. ***European Urology Focus***. 2020

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Hartley A, Leung HY, Ahmad I.
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Tom Bird (page 14)
Liver Disease and Regeneration

Primary Research Papers

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Vizioli MG, Liu T, Miller KN, Robertson NA, Gilroy K, Lagnado AB, Perez-Garcia A, Kiourtis C, Dasgupta N, Lei X, Kruger PJ, Nixon C, Clark W, Jurk D, Bird TG, Passos JF, Berger SL, Dou Z, Adams PD.
Mitochondria-to-nucleus retrograde signaling drives formation of cytoplasmic chromatin and inflammation in senescence. ***Genes & Development***. 2020; 34:428-445.

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The landscape of gene mutations in cirrhosis and hepatocellular carcinoma. ***Journal of Hepatology***. 2020; 72: 990-1002

Nehme J, Borghesan M, Mackedenski S, Bird TG, Demaria M.
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Karen Blyth (page 16)
In Vivo Cancer Biology

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Dynamic ROS control by TIGAR regulates the initiation and progression of pancreatic cancer. ***Cancer Cell***. 2020; 37: 168-182 e164.

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Dhayade S, Pietzke M, Wiesheu R, Tait-Mulder J, Athineos D, Sumpton D, Coffelt S, Blyth K, Vazquez A.
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Mevel R, Steiner I, Mason S, Galbraith LCA, Patel R, Fadlullah MZH, Ahmad I, Leung HY, Oliveira P, Blyth K, Baena E, Lacaud G.
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Millar R, Kilbey A, Remak SJ, Severson TM, Dhayade S, Sandilands E, Foster K, Bryant DM, Blyth K, Coffelt SB.
The MSP-RON axis stimulates cancer cell growth in models of triple negative breast cancer. ***Molecular oncology***. 2020; 14: 1868-1880

Oizel K, Tait-Mulder J, Fernandez-de-Cossio-Diaz J, Pietzke M, Brunton H, Lilla S, Dhayade S, Athineos D, Blanco GR, Sumpton D, Mackay GM, Blyth K, Zanivan SR, Meiser J, Vazquez A.
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Zhang T, Bauer C, Newman AC, Uribe AH, Athineos D, Blyth K, Maddocks ODK. Polyamine pathway activity promotes cysteine essentiality in cancer cells. ***Nature Metabolism***. 2020; 2: 1062-1076.

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Crispin Miller (page 44 & 76)

Bioinformatics and Computational Biology

Primary Research Papers

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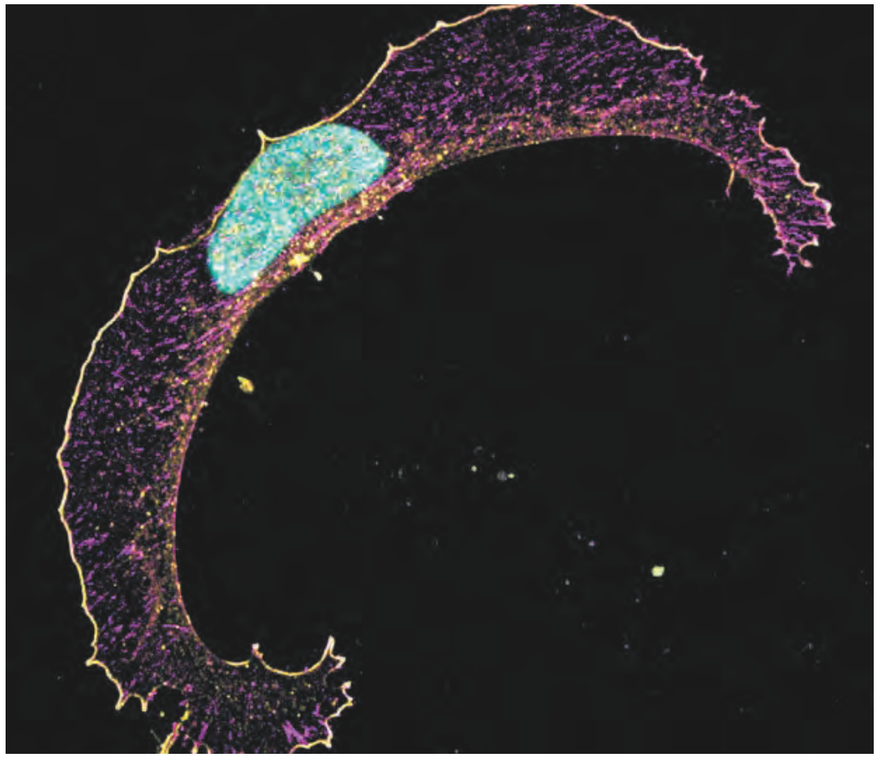

Image by Anh Le

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***. 2020.

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Rooney N, Mason SM, McDonald L, Dabritz JHM, Campbell KJ, Hedley A, Howard S, Athineos D, Nixon C, Clark W, Leach JDG, Sansom OJ, Edwards J, Cameron ER, Blyth K. RUNX1 is a driver of renal cell Carcinoma correlating with clinical outcome. ***Cancer Research***. 2020; 80: 2325-2339.

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***. 2020: 1-17.

David Bryant (page 18)

Molecular Control of Epithelial Polarity

Primary Research Papers

Millar R, Kilbey A, Remak SJ, Severson TM, Dhayade S, Sandilands E, Foster K, Bryant DM, Blyth K, Coffelt SB. The MSP-RON axis stimulates cancer cell growth in models of triple negative breast cancer. ***Molecular oncology***. 2020; 14: 1868-1880

Martin Bushell (page 20)

RNA and Translational Control in Cancer

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Leo Carlin (page 22)

Leukocyte Dynamics

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Seth Coffelt (page 26)

Immune Cells and Metastasis

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Payam Gammage (page 28)
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Danny Huang (page 30)
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Gareth Inman (page 32)
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Robert Insall (page 34)
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Hing Leung (page 38)
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David Lewis (page 40)

Molecular Imaging

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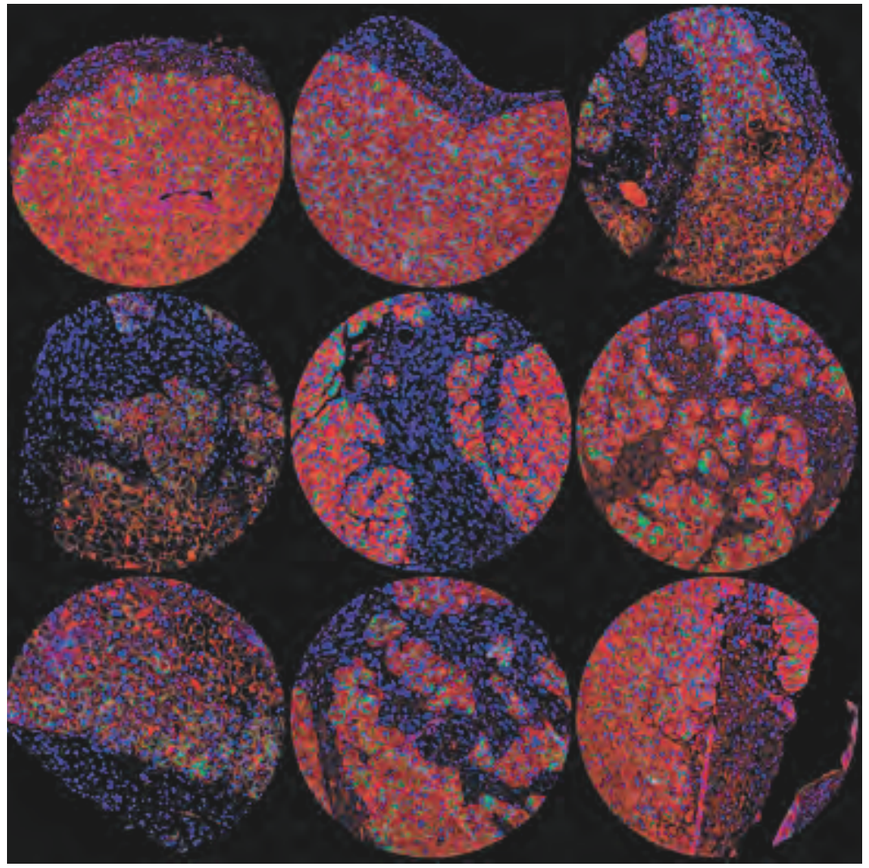
Image by Amelie Juin



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Laura Machesky (page 42)

Migration, Invasion and Metastasis

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Metabolomics (page 78)

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Precision-Panc Preclinical Lab

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Ed Roberts (page 52)
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Owen Sansom (page 56)
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Stephen Tait (page 60)

Mitochondria and Cancer Cell Death

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David Lewis (page 86)

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Tumour Microenvironment and Proteomics

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John Paul Career Award

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

The winner of this year's award was **Christos Kiourtis** from Tom Bird's group. He has been investigating the role of senescence in acute liver injury.

Theses

Barthet, Valentin Jérôme Antoine (2020)
Investigating the tumour suppressive role of autophagy-related genes in liver cancer formation. PhD thesis, University of Glasgow, Beatson Institute.

Le, Hoang Anh (2020)
CYRI-A is recruited to macropinocytic cups and mediates integrin uptake, limiting invasive migration. PhD thesis, University of Glasgow, Beatson Institute.

Leach, Joshua (2020)
Investigating right-sided colon cancer and the involvement of inflammation, the microbiome, and foetal stem cell-like plasticity. PhD thesis, University of Glasgow, Beatson Institute.

Laing, Sarah (2020)
The role of MYC and KRAS in lung cancer. PhD thesis, University of Glasgow, Beatson Institute.

Magnussen, Helge Magnus (2020)
Structural characterisation of MDM2 RING domain: E2-ubiquitin binding and activation by phosphorylation. PhD thesis, University of Glasgow, Beatson Institute.

Naylor, Gregory (2020)
The role of apoptotic cell morphology in anti-tumour immunity. PhD thesis, University of Glasgow, Beatson Institute.

Novo, David (2020)
An investigation into the role of mutant p53-dependent extracellular vesicles and their impact on the extracellular matrix. PhD thesis, University of Glasgow, Beatson Institute.

Ntala, Chara (2020)
Immune profiling of the tumour microenvironment in prostate cancer. PhD thesis, University of Glasgow, Beatson Institute.

Phinichkusolchit, Narisa (2021)
ROCK1-mediated apoptotic blebbing in genetic models of tissue homeostasis and tumourigenesis. PhD thesis, University of Glasgow, Beatson Institute.

Roca-Portoles, Alba (2020)
Investigation of non-canonical effects of BH3-mimetic compounds. PhD thesis, University of Glasgow, Beatson Institute.

Sánchez Martínez, Rafael (2020)
Characterisation and Role of SLFN5 in Castration Resistant Prostate Cancer. PhD thesis, University of Glasgow, Beatson Institute.

CONFERENCES AND WORKSHOPS

Cancer Research UK Beatson International Cancer Conference

Due to the pandemic, we took the difficult decision to postpone this year's conference. However, we are delighted to be able to welcome delegates to the conference in 2021, which will take place virtually.

Cartography of Cancer: Mapping Tumours in 3D 2020

12–13 July 2021
Much of our understanding about the molecular mechanisms of cancer initiation, progression and spread stems from methods that involve the dissociation of tissue and, unfortunately, the loss of spatial information either within the tumour, or in relation to the tumour microenvironment. Recent technological advances are now making it possible to preserve spatial coordinates and still extract functional molecular information. We can now interrogate cancer genetics, metabolism, microenvironment and immunology in space and time with unprecedented breadth and depth. This is underpinned by emerging technologies to address fundamental questions about 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 perform 'the cartography of cancer'.

For the 2021 Virtual Beatson International Cancer Conference, we have developed a strong programme themed around these five key research areas and are encouraging early-career researchers to submit their abstracts for consideration as soon as possible. We are looking forward to world-leading speakers with a keynote lecture by Josephine Bunch.

From left to right: Laura Torrens, Tom Bird and Miryam Müller

Accelerator Award Liver Workshop

27–28 February 2020
The HUNTER preclinical models workshop was a focused practical workshop aiming to foster discussion, optimisation and training in state of the art animal preclinical models systems. There were formal talks, discussion and poster sessions and a series of practical demonstrations including HCC GEMMs, intravital imaging, orthotopic transplantation, organoids culture and liver slice culture over a two day programme combined with a lively social event and dinner.

Scottish Cancer Models Network

17 September 2020
The Scottish Cancer Models Network is a new forum to foster collaboration and innovation in cancer research among academic, clinical and industry partners in Scotland, with a vision to improve cancer care, treatments and outcomes for patients. This first meeting featured scientific talks from Glasgow and Edinburgh researchers, and provided a forum for career mentoring for trainees.

SEMINARS

The following seminars were held virtually at the Cancer Research UK Beatson Institute throughout 2020. Due to the pandemic, many of our seminars from external speakers unfortunately had to be cancelled. However, we had a fantastic series of virtual seminars given by group leaders within the Institute.

January

Jennifer Lippincott-Schwartz, Howard Hughes Medical Institute, Ashburn, VA, USA

Alexis Gautreau, CNRS, Ecole Polytechnique, Paris, France

Kay Macleod, University of Chicago, USA
Jumpei F Yamagishi, University of Tokyo, Japan

February

Tim Witney, King's College London and University College London, UK

Tariq Enver, UCL Cancer Institute, University College London, UK

Ed Tate, Imperial College London / Francis Crick Institute, London, UK

Luke Boulter, University of Edinburgh, UK

August

Shukry Habib, King's College London, UK

September

Martin Hemberg, Wellcome Sanger Institute, Hinxton, UK

December

Neta Erez, Department of Pathology, Aviv University, Tel Aviv, Israel

STUDENTSHIPS AND POSTDOCTORAL FELLOWSHIPS

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.

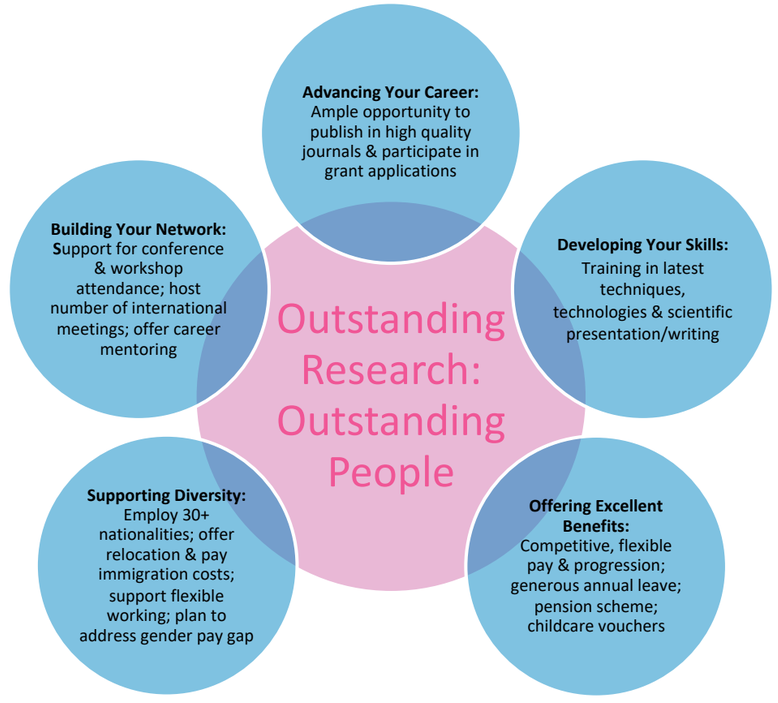
Our students are fully integrated with University of Glasgow graduate school (www.gla.ac.uk/colleges/mvls/graduateschool) and are allocated primary and secondary supervisors who are jointly responsible for supporting and monitoring their progress. The primary supervisor is responsible for developing 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 Laura Machesky), 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.

POSTDOC OPPORTUNITIES AT THE BEATSON



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.

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.

Building on the successful implementation in 2015 of the new finance system, the team has continued to develop and improve the range of management information available, including the introduction of monthly reforecasting and the continued development of the five-year forecasting and scenario-planning model.

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

Our mission is to enable cancer discovery for patient benefit by providing a professional human resources service to our stakeholders to allow them to efficiently and effectively manage the Institute's resources. 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 2020 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 through the introduction of our first Employee Assistance Programme. In addition to this, HR also worked with Finance colleagues to implement a new pension plan for new starts as the costs of remaining in the University pension scheme became prohibitive in the longer term.

There was no pay rise for staff in 2020, but through careful planning, the introduction of shift working and the good will and motivation of our staff the Institute has had a productive year and we look forward to working with staff to realise our Equality, Diversity and Inclusion ambitions in 2021 and to getting our staff development efforts back on track as well.

Administration

Sheila McNeill (Administration & Internal Communications Manager), Rebecca Gebbie, Barbara Laing, Sarah Price, Catriona Entwistle, Shona McCall

The Administration team, headed by the Admin & Internal Communications Manager, 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 running of the main 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 2020, the team took a leading role in organising a joint advanced technologies retreat with the other CRUK Institutes prior to lockdown and a number of scientific reviews, most of which needed to be moved online. In March, we moved to working from home but continued to support our researchers in a variety of ways: Catherine Winchester was particularly busy helping those preparing grants and manuscripts for submission. She also promoted the Institute's approach to research integrity to a number of different external groups; Angela Kelsey returned from maternity leave to her role supporting PhD students, including the 13 new ones that started in October; Kate started a new joint role with us and the Drug Discovery Unit with a particular focus on boosting the Institute's social media profile and supporting interactions with pharma; Fiona continued to support the ACRC Accelerator as well as the Glasgow Colorectal Cancer Initiative.

Cancer Research UK, Commercial Partnerships Translation Executive

Natasha Tian PhD

Cancer Research UK manages intellectual property and commercialisation through its Commercial Partnerships Team, formerly known as Cancer Research Technology, to develop promising CRUK-funded research into new cancer therapeutics, diagnostics and enabling technologies. The Commercial Partnerships Team's strong network across academia, and particularly industry, enables the translation of promising research into commercial propositions, mainly through industry collaborations, licensing intellectual property or through the creation of spin-out companies, to deliver patient benefit and commercial value that will support further cancer research.

CRUK's subsidiary Cancer Research Technology Ltd, an oncology-focused technology transfer and development company, has exclusive rights to more than £450m of world-class cancer research annually and leveraged £20 million in industry funding to progress promising cancer research last year. This includes the recent major 3-year collaboration between the Institute and Novartis, supported by CRUK and the CRT Pioneer Fund, to develop novel therapeutics targeting RAS. Since a substantial amount of the funding for the Institute comes from CRUK, a dedicated CRUK Commercial Partnerships Translation Executive manages all translational research and intellectual property–related matters on behalf of the Institute.

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.

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

To determine the gender pay gap, the Government requires companies to measure the average earnings of all male and female employees, regardless of role and working hours, and show the percentage difference between the two. Compared to 2019, the mean hourly pay gap between females and males increased by 3.3% points and the median hourly pay gap increased by 4.0% points in 2020, disappointingly reversing the progress made in 2019.

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

Hourly pay gap	2018	2019	2020
Mean	11.50%	9.9%	13.2%
Median	8.01%	7.1%	11.10%

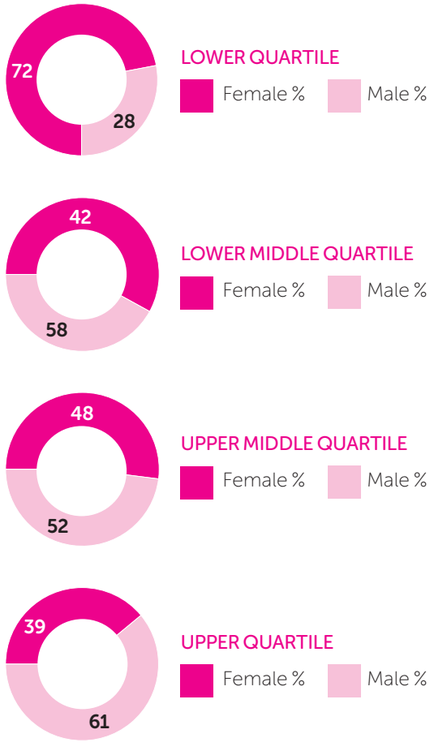
Gender pay gap vs equal pay

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?

Our workforce is 45% male and 55% female. When we rank the pay of our staff into four quartiles we can see that there is a majority of females in the lower and lower middle quartiles. The number of females in the lower middle quartile and upper middle quartile rose by 9% points and 6% points respectively in 2019/20, mainly due to the number of females we recruited to these quartiles in this period – 65% female compared to 35% male. The good news is that several new female appointees are early-career management positions with development potential. This in part explains why our pay gender mean and median differential increased in 2020. In addition, the percentage of women in the upper quartile reduced slightly by 3% points. This is because we had several senior female staff either leave or transfer (as a result of promotion) to another employer on a hybrid contract* (and therefore not feature in our pay gender figures).

*Hybrid contracts are a symbiotic arrangement with the University of Glasgow, whereby well cited senior scientific researchers are employed by the University but under the Terms and Conditions of the CRUK Beatson Institute. This allows the University to benefit from their scientific papers and publications for REF purposes and allows senior scientific researchers access to other sources of external grants and funding.



Comparison of quartiles over past 3 years – 2018 to 2020:

	LOWER QUARTILE	LOWER MIDDLE QUARTILE	UPPER MIDDLE QUARTILE	UPPER QUARTILE
F-2018	71%	50%	44%	41%
F-2019	71%	49%	46%	42%
F-2020	72%	58%	52%	39%
M-2018	29%	50%	56%	59%
M-2019	29%	51%	54%	58%
M-2020	28%	42%	48%	61%

What are we doing to close our gender pay gap?

The CRUK Beatson Institute is committed to reducing its gender pay gap through actions identified in our 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% 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. One of the main reasons for this is, we believe, the need for more certainty and support when starting a family. In recent years, a small 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 been well received; however, there is more work to be done to encourage more flexible working for Postdoctoral Researchers. There seems to be a reluctance to requesting more flexible or part-time working patterns amongst such staff and we believe that being able to achieve a better work-life balance might encourage female Postdoctoral Scientists to remain in science and as a consequence achieve their potential and more independent research positions.

Taking Action

This is the fourth year that the Institute has reported its gender diversity and we can see that in 2020 pay gender differentials have increased despite our best efforts over the past three years to reduce them. Our ability to address these issues in 2020/21 has been limited, due to the COVID-19 pandemic and a cut in our budgets. We did not offer any pay awards in 2020 and we will continue to be limited financially in 2021. In 2020, we did increase our balance of female to male Postdoctoral Researchers, with two thirds of newly appointed Postdocs being female.

Areas for improvement

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

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

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

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.

In Summary

Increasing diversity 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, Royal College of Surgeons Edinburgh, Tenovus, University of Glasgow Paterson Endowment Fund, Wellcome Trust

Karen Blyth
Breast Cancer Now

Martin Bushell
AstraZeneca, BBSRC, Celgene, Medical Research Council

Leo Carlin
Breast Cancer Now

Drug Discovery Unit
Celgene

Danny Huang
AstraZeneca, Biotechnology and Biological Sciences Research Council, European Research Council

Gareth Inman
BSF, DEBRA, SANOFI

Robert Insall
EPSRC Physics of Life

Hing Leung
European Community, GU GL, Prostate Cancer Foundation, Prostate Cancer UK

Laura Machesky
ESPRC Physics of Life, Medical Research Council, Saudi Government

Jennifer Morton
Elstar, Medical Research Council, Pancreatic Cancer UK, RedX, UCB

Jim Norman
West of Scotland Women's Bowling Association

Kevin Ryan
The Kay Kendall Leukaemia Fund

Owen Sansom
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Alexei Vazquez
European Community

Sara Zanivan
Breast Cancer Now

Institute of Cancer Sciences, University of Glasgow

David Bryant
EssenBio, GU GL, Royal Society

Seth Coffelt
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David Lewis
Beatson Cancer Charity, GU GL

Daniel Murphy
British Lung Foundation, Chief Scientist Office, Medical Research Council, GU GL, Mick Knighton Mesothelioma Research Fund, Pancreatic Cancer UK, Puma Biotechnologies

Stephen Tait
GU GL , Prostate Cancer UK

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.

Accord Hospice / Mrs N Paternostro
Mr Iain Anderson - In memory of his wife
Mrs J Arthur - In memory of Mrs C Anderson
Biotek Ltd
Mrs J Brock
Mrs Margaret G Brown
Mrs Gladys Buick
Charities Aid Foundation
Charities Trust
Clyde Travel
Mrs Isabella Coutts - Bequest of her aunt, Mrs Mary Forbes
Mrs S Dempster
Angela Dunn
Eastwood Probus Club
Easy Fundraising Ltd
The Edrington Group
ELECTA CHAPTER No.27 O.E.S
Equiniti Ltd
Enterprise RAC UK Ltd
Erskine Golf Club Ltd
Mr & Mrs Fraser - In memory of Mrs C Anderson
Fergusons Chartered Accountants re James Inglis Trust
Greenock District Ladies Bowling Association
Avril Haddow
Hillpark Ladies Bowling Club
Teresa Hull
J & D Lawson Ltd - In memory of Mrs C Anderson
Linda Jaffrey
Miss Charlotte Johnston
Legacy of the Late Annie McNab
Legacy of the Estate of Lady Irene Wallace of Campsie
Legacy of the Late Isabella Maclean English
Legacy of Mrs June McFarlane
Legacy of the Estate of Jessie Davis Robertson
Legacy of Mr Steven Alexander McLean
Legacy of the Late Mr Henry Wilson
C N Leoid - In memory of Mrs C Anderson
Janet Lyke
Mrs Pauline Martin
Mr & Mrs McEwan
Mr & Mrs G McIntyre - In memory of their son
Mrs Fiona McNeill & Family
Mrs Margaret Meikle
Mosshead Primary School
M Muir
G & L O'Neill
Mrs Sarah Percy
Port Ban Holiday Park Ltd
The Robertson Trust
Share Gift
Mrs Marjorie Sellar
Mrs M Slimming & Dr T Slimming MBE
Mrs Ann Soutar - From the Walter Soutar Fund
St Leonard's Parish Church, Ayr
St Modan's High School
Mrs Joan Taylor
Mr John Teevan (Darrows)
Jacqueline Thomson - In memory of her Father
Thornhill Gardening Society
Mr & Mrs Turner - In memory of Mrs C Anderson
Mrs J Whiteford & Miss A R Paterson
West of Scotland Women's Bowling Association

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Patrons

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

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

Cancer Research UK
Cancer Research UK is a registered charity in England and Wales (1089464), Scotland (SC041666) and the Isle of Man (1103).
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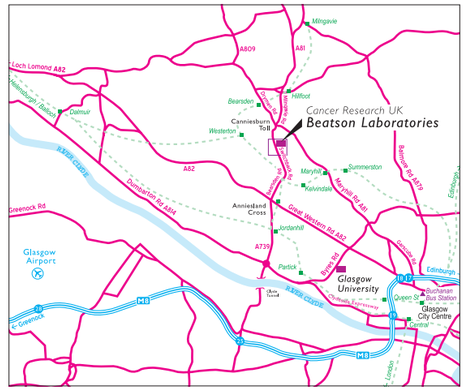

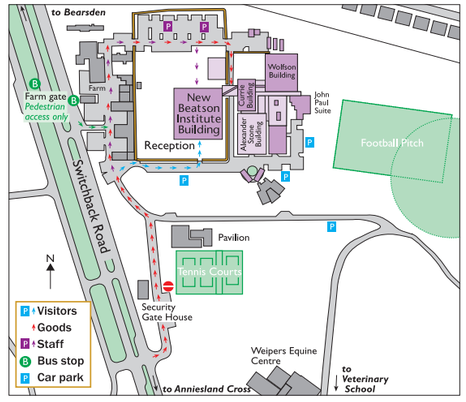

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