SCIENTIFIC REPORT 2019

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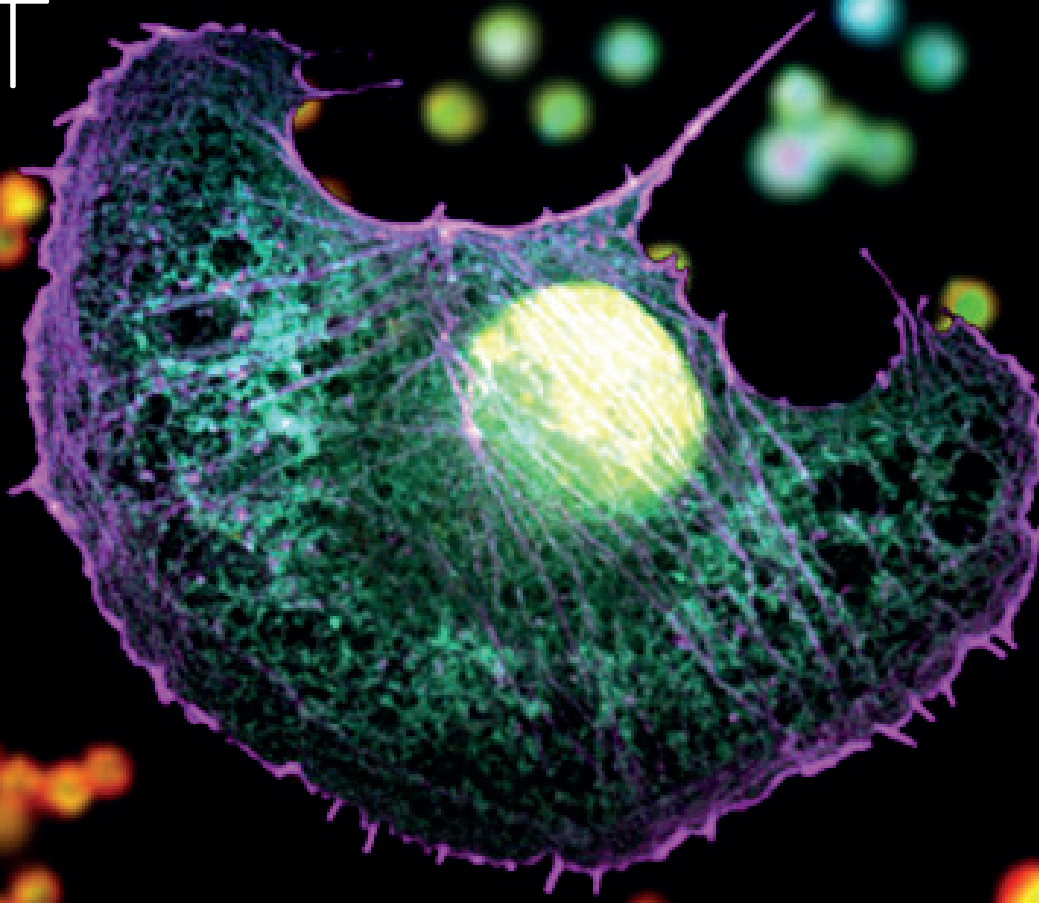

CANCER RESEARCH UK | BEATSON INSTITUTE

COVER IMAGE

B16-F1 melanoma cell labelled with GFP, phalloidin (actin) and DAPI for the nucleus. Phagocytic beads used and labelled. Image taken with Zeiss 880 LSM with Airyscan.

Image by Savvas Nikolaou

SCIENTIFIC REPORT 2019

BEATSON INSTITUTE

University of Glasgow

CANCER RESEARCH UK | BEATSON INSTITUTE

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

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DIRECTOR'S INTRODUCTION

Director of the Cancer Research UK Beatson Institute

Professor Owen Sansom FRSE, FMedSci

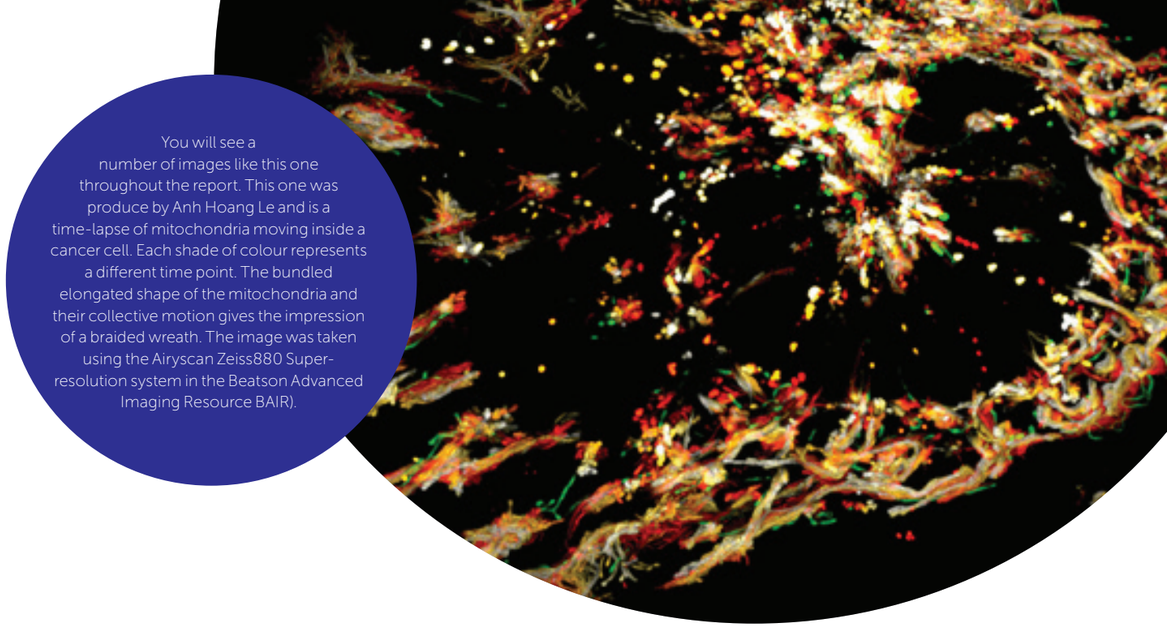
2019 was another highly successful year for the Institute as a whole as well as for many of our individual researchers. We continued to do high risk-high gain discovery research and to apply it, wherever possible, to relevant pre-clinical and clinical studies.

Our vision is to be a centre of excellence for discovery cancer research and to maximise the translation of our findings and expertise for the benefit of cancer patients. Driven by CRUK's principal strategic aim of increasing our understanding of cancer, we focus on two main areas of cancer research: **energetic stress and cancer metabolism** and **microenvironment and metastasis**. We are building a third area aimed at understanding the **biology of early disease** in order to develop a precision prevention approach. This year, we have made excellent progress in the latter, developing models of hepatocellular cancer and mesothelioma to underpin two funded CRUK Accelerator Awards: PREDICT-Meso and HUNTER. The CRUK Accelerator Award ACRCelerate also continued to make good progress in coordinating a pre-clinical network of colorectal cancer models to reinvigorate stratified medicine trials in this devastating disease.

On an individual level, I would also like to congratulate Karen Blyth, Jen Morton and Sara Zanivan who were all appointed to University of Glasgow Professorships this year. In addition, Jen was promoted to Senior Staff Scientist in June and deservedly so. Jen is doing internationally leading work in both modelling and understanding the biology of pancreatic cancer. She is thus a great asset to the Institute and the wider cancer research community. Karen and Sara also had very successful Senior Staff Scientist reviews in October, underlining the excellence of their own research programmes as well as their invaluable contribution to the collaborative science that is such an important feature of the Institute. This led the panel to note how well the Senior Staff Scientist model is working for us.

The same panel also reviewed our Advanced Technologies teams and was hugely impressed by all of them, including the staff running them and the volume of activity. I am very pleased with the outcomes of these important reviews and extremely grateful to the panel members for all their efforts and input, including the chairs Dave Adams (Cambridge) and Margaret Frame (Edinburgh).

Members of our Advanced Technologies teams, Stephen Bell, Margaret O'Prey, Brenda McGuire and Sheila Bryson receiving their long service awards from the Director, Owen Sansom in March.



You will see a number of images like this one throughout the report. This one was produce by Anh Hoang Le and is a time-lapse of mitochondria moving inside a cancer cell. Each shade of colour represents a different time point. The bundled elongated shape of the mitochondria and their collective motion gives the impression of a braided wreath. The image was taken using the Airyscan Zeiss880 Super-resolution system in the Beatson Advanced Imaging Resource BAIR).

We also held a Scientific Advisory Group meeting for our Drug Discovery Unit in May, chaired by Peter Parker from the Crick. This was an excellent opportunity for us to discuss our portfolio of targets and strategy for applying our discovery science to the clinic. Again, we are very grateful to the panel members for the amount of time and excellent advice they were able to offer. Progress within the DDU was underlined towards the end of the year when we were able announce that it had entered into an important collaboration with Novartis to progress its ground breaking work on the development of KRAS inhibitors.

I would also like to congratulate two of our Senior Group Leaders, Danny Huang and Jim Norman who both had very successful outcomes to their Quinquennial Reviews at Cancer Research UK this year, with their future research programmes scoring very highly. In addition, one of our intermediate clinician scientists, Tom Bird was awarded the British Society of Gastroenterology's Sir Francis Avery Jones Award 2019.

This year, we welcomed new Junior Group Leaders, Ed Roberts (from UCSF in the US) and Payam Gammage (from the MRC Cancer Unit, Cambridge, UK) to the Institute. Ed is studying migratory dendritic cells and the role they play in the body's immune response to cancer, while Payam Payam is developing novel approaches to manipulate the mitochondrial genome and using these to address the relationship between perturbed mitochondrial function and cancer progression.

Within the University of Glasgow, Chiara Braconi (from ICR, London, UK) was appointed to a Readership in Hepatobiliary Cancer and a Lord Kelvin/Adam Smith (LKAS) Fellowship, adding to our growing strength in the biology of early disease and liver cancer.

Scott Kelso also joined us as our new Head of Laboratory Operations, overseeing buildings, health and safety, lab support services and lab management. As well as managing these multidisciplinary teams, he will be reviewing space usage within our building and scoping out potential solutions to our future space needs.

There were many and varied opportunities for scientific discourse at the Institute this year, including a mouse models symposium with plenary speaker Karin de Visser; the Beatson International Cancer Conference on protein dynamics in cancer; our first ACRCelerate workshop; joint workshops with our Edinburgh colleagues on imaging and artificial intelligence; on brain cancer; and for our early-career researchers. The Institute's approach to research integrity training was also presented at the World Conference on Research Integrity in Hong Kong.

Finally, we hosted very successful visits by CRUK Chief Executive Officer, Michelle Mitchell and CRUK Trustees, which included important discussions of our research strategy. We were also active in organising a joint CRUK Institutes retreat that has started some useful discussions of how the Institutes might work together, especially in promoting themselves as a 'brand'.

RESEARCH HIGHLIGHTS

We made excellent progress in all of our research areas in 2019. This is highlighted by numerous high quality publications in each of our themes that drive our understanding of cancer forward and identify new therapeutic nodes.

Energetic Stress and 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 identified key differences in tissue culture media that alter tumour cell dependencies allowing them to develop physiological media (now sold by Ximbio, Vande Voorde *et al.* Science Advances 2019).

They also described novel combinations that might target growth of our two main tumour types; pancreatic and colorectal cancer. In pancreas, Allen-Petersen *et al.* (Cancer Research, 2019) provided evidence for combined targeting of PP2A and mTOR, Halbrook *et al.* (Cell Metabolism, 2019) demonstrated targeting macrophages enhances gemcitabine treatment and McGregor *et al.* (Cancer Research, 2019) showed how statin-induced ROS production could be exploited. In colon, Kaymak *et al.* (Cancer Research, 2019) described how mevalonate pathway inhibitors could be combined with treatments that induce metabolic stress.

Furthermore, our scientists made key mechanistic insights that may allow us to target translation; mTORC1 activation requires DRAM-1 (Beaumatin *et al.* Molecular Cell 2019), eIF4A2's mechanism of action (Wilczynska *et al.* Genome Biology 2019) and vulnerabilities caused by increased protein synthesis (Schmidt *et al.* Nature Cell Biology 2019).

Microenvironment and Metastasis

For us to be able to stop recurrence and target metastasis, we need excellent predictive models that recapitulate human cancer to allow us to be able to work out the significance of targeting different cells in the tumour microenvironment. This year, the Sansom group developed novel models of colorectal cancer (CRC) that recapitulate the worst prognostic subtypes of the disease (Jackstadt *et al.* Cancer Cell 2019). This was an unmet need in the CRC field. Importantly, they were able to show that neutrophils (one of our focus immune cell types at the Beatson) were required for metastasis via the TGFβ pathway. Work is now underway to translate this clinically within the ACRCelerator platform for both colon and rectal cancer. This year, ACRCelerate, led by Owen Sansom, continued to consolidate model systems across the UK, Spain and Italy, in order to drive a new era of stratified medicine trials in CRC.

We have also shown clear efficacy of CXCR2 inhibition (which targets neutrophils) and checkpoint inhibition in hepatocellular carcinoma (HCC) models. This pre-clinical data supported a successful CRUK application for an HCC trial, CUBIC led by Jeff Evans. Pre-clinically, the Carlin group identified key determinants for neutrophil activation in the lung (one of the key metastatic sites, collaborating on McCormick *et al.* Journal of Immunology 2019, Patel *et al.* Science Immunology 2019) and these could provide further targets.

Other work by Beatson groups this year has demonstrated key roles for fibroblasts (in pancreas, Pereira *et al.* Trends in Cancer 2019, Kugeratski et al. Science Signalling 2019), LPA (Juin *et al.* Developmental Cell 2019, Auciello *et al.* Cancer Discovery 2019), RAC (Amato *et al.* Current Biology 2019) and WNT signalling in invasion and/or metastasis (Wellenstein *et al.* Nature 2019).

Biology of Early Disease

We continue to use our cutting-edge model systems to understand the early events of carcinogenesis. In colorectal cancer, Lgr5+ stem cells are thought to be a key cell of origin and APC loss is the most frequent initiating event. We have identified key pathways that are required for Lgr5 cells to be transformed; BCL9/9l (Gay *et al.* Nature Communications 2019) and RAL GTPases (Johansson *et al.* Cell Stem Cell 2019).

Within hepatocellular carcinoma, we have identified key determinants for senescence, which we think is an important tumour suppressive pathway in this tissue during transformation (Teo *et al.* Cell Reports 2019).

We have also been successful in obtaining grant funding using our early models of disease to see if they will be informative for early detection (Kevin Ryan and Sara Zanivan) or for identifying those patients with early disease that are more likely to progress (Daniel Murphy/Kevin Blyth: PREDICT-Meso).

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

www.beatson.gla.ac.uk/imran_ahmad

MODELS OF ADVANCED PROSTATE CANCER

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Consultant Urological Surgeon
(NHS Greater Glasgow & Clyde)

Research Scientist
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[1] CRUK Glasgow Centre

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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 Pparγ activation in metastatic prostate cancer

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

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

Our data suggests that prostate cancer patients could be stratified in terms of PPARγ/FASN and PTEN levels to identify patients with aggressive prostate cancer who may respond favourably to PPARγ/FASN inhibition (low PTEN/high pAKT expression), a finding that has potential to guide the design of future clinical trials. Ongoing research by our group has demonstrated 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.

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

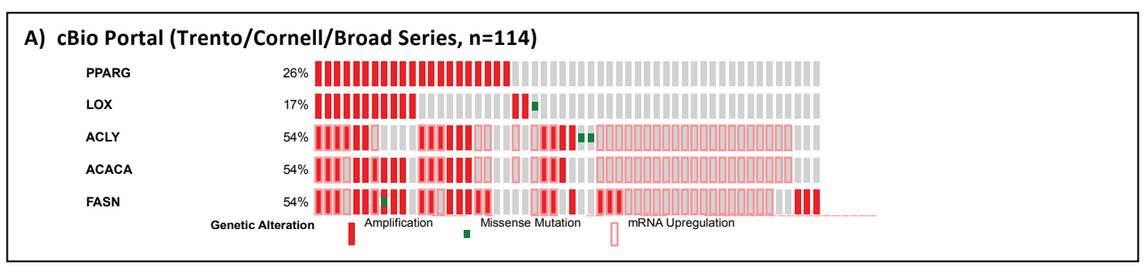
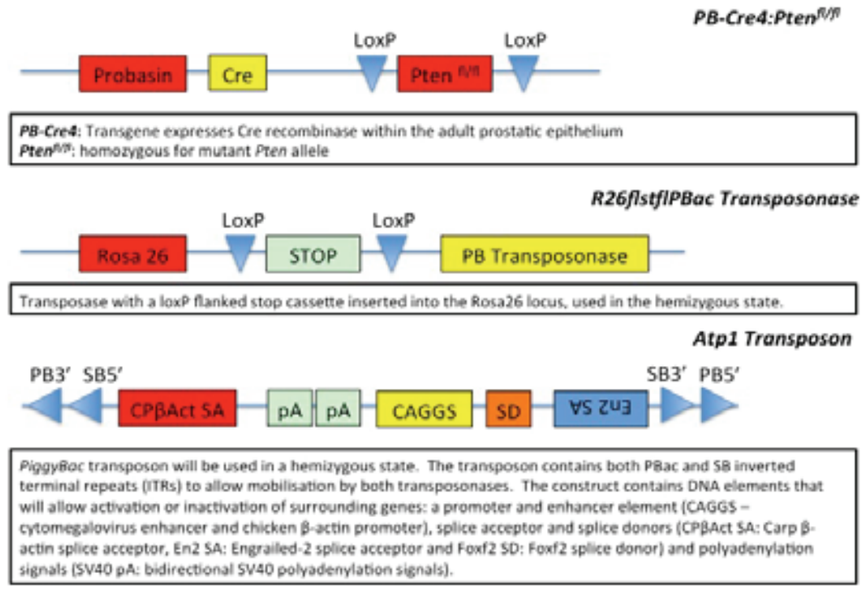


Figure 2
Genetic modifications of the PiggyBac mice.



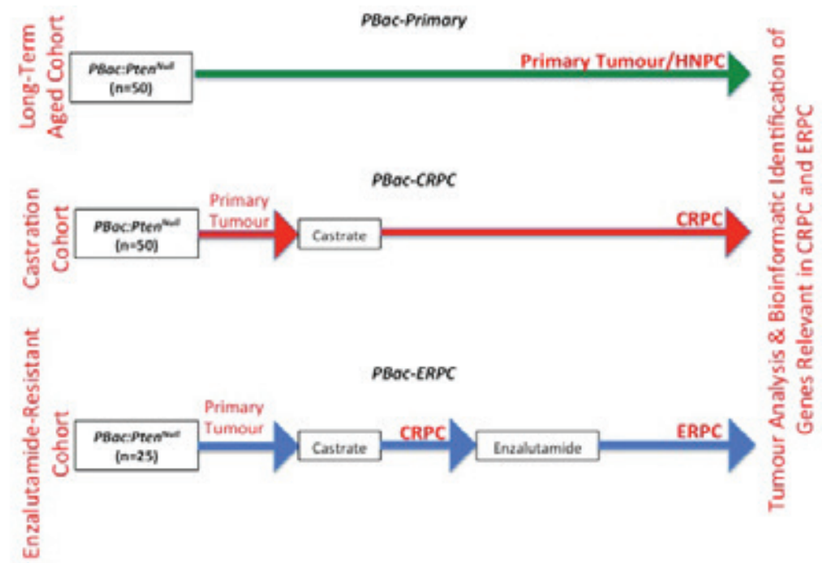
Identification and validation of new therapeutic targets in castrate-resistant prostate cancer

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.

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



www.beatson.gla.ac.uk/tom_bird

LIVER DISEASE AND REGENERATION

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[1]Wellcome Trust
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Paterson Endowment Fund
[3]CRUK HUNTER ACCELERATOR

Liver cancer is the third most common cause of cancer-related death worldwide and is increasing at alarming rates in many areas of the globe. Working at the interface between clinical care in the NHS and the development of preclinical models to study liver biology, the focus of our group is to understand dysregulated liver regeneration during cancer development. Our aim is 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 ageing, 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 specific sub-types of HCC.

Current pharmacological therapy for HCC is only minimally effective, and no current treatment is directed to specific molecular forms of the disease. We have developed, and continue to expand, a suite of models of HCC to study how hepatocytes escape the normal controls governing regulated regeneration, aiming to identify novel therapeutic targets. This aims to guide human clinical trials to target specific therapies to specific subtypes of liver cancer.

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 zones of the liver where we believe that the regenerative cells reside. Activation of the Wnt pathway is sufficient to cause hepatocytes to divide and the liver to grow. Nonetheless this activation is insufficient to effectively lead to tumour formation alone. 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.

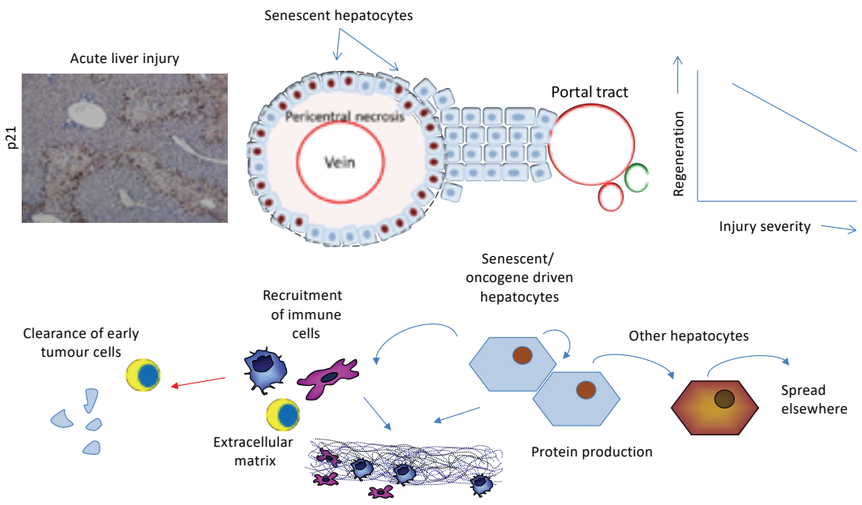

Figure 1
Damage-induced senescence in the liver
Following acute insult (e.g. paracetamol toxicity) senescence develops in hepatocytes, normally the regenerative cell of the liver. Instead of local regeneration, hepatocellular proliferation is activated elsewhere. However, when injury becomes increasingly severe liver regeneration reduces progressively. The senescent cells are able to affect their environment, 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 anticancer therapies.

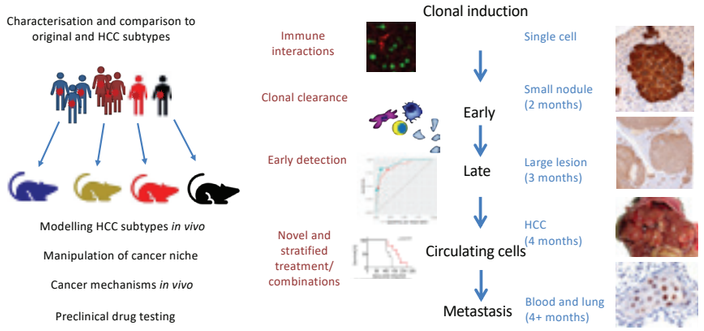

Figure 2
Human HCCs can be grouped into different functional and genetic subclasses
We are mimicking the genetic alterations in human HCC subclasses using *in vivo* models in the mouse. Our strategy is to induce clonal hepatocytes with these targeted genetic alterations and then follow the clones as 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.

We are investigating the nature of β-catenin and other 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. This state leaves many of the functional abilities of the hepatocyte preserved but, as we have shown, renders them incapable of participating in regeneration. In severe liver injury, we have reported that senescence may occur in response to injury and activation of immune cells (Figure 1). Ongoing work suggests senescent hepatocytes are dramatically altered in their mechanisms of protein production and their interaction with their environment, including immune activation, matrix deposition and the induction of senescence in other cells. Moving forward, we are now studying ways to interfere with such spreading senescence as a mean to develop treatments for human diseases including fulminant liver failure.

Transformation of regenerative hepatocytes into malignancy

β-catenin mutants are just one example of a cancer driver in HCC. We have developed a number of models of HCC utilising combinations of targeted cancer drivers in a clonal population of hepatocytes. These genetic targets have been chosen to mimic the genetic changes most frequently occurring in a variety of human HCC subtypes creating models for each subtype of human cancer (Figure 2). We are then able to 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 to other organs and responses to therapies. Using the advanced technologies facilities within the CRUK Beatson Institute, we are able to track and characterise tumours as they develop using a combination of preclinical imaging and analysis of mRNA transcription, protein expression and secretion. 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. The 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.

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. We 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. Integrating these with newer novel biomarkers, including those being studied in our collaboration with Sara Zanivan, is an additional future goal to aid accurate and early detection of HCC.

Publications listed on page 92

IN VIVO CANCER BIOLOGY

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[1]Funded by Breast Cancer Now

Our lab uses *in vivo* models to recapitulate human cancer and interrogate aspects of the disease within a biological context. Validating *in vitro* discoveries in physiologically relevant models in this way will expedite novel therapeutic approaches for patient benefit. The group has expertise in modelling different cancer types but has a specific interest in breast cancer, and how metabolic pathways and certain signalling nodes such as the RUNX/CBFβ transcriptional complex and pro-survival factor MCL-1, contribute to tumour progression and metastasis.

The RUNX/CBFβ transcriptional complex in breast cancer.

The Blyth lab has had a long-standing interest in the role of the *RUNX* genes (specifically *RUNX1* and *RUNX2*) in epithelial cancers. Whilst *RUNX1* and its obligate DNA binding partner CBFβ are widely associated with haematopoietic malignancies, our work and large-scale genomic studies have revealed that loss and gain of RUNX function segregates with different forms of breast cancer and correlates with clinical outcome (Rooney *et al*, 2017). RUNX1 is particularly intriguing in this regard with a proposed suppressor role in ER-positive breast cancer cells yet is a pro-oncogenic effector in ER-negative cells. Using models of breast cancer, we have been investigating the importance of the *Runx* genes *in vivo*. 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. 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, we have found that RUNX1 is critical for regulating mammary stemness whereby loss of RUNX1 potentiates mammosphere capability, while overexpressed RUNX1 has the reverse effect. Current work in the lab is investigating the transcriptional signatures of *RUNX*-deleted mammary tumours as well as the function of CBFβin mammary tumourigenesis.

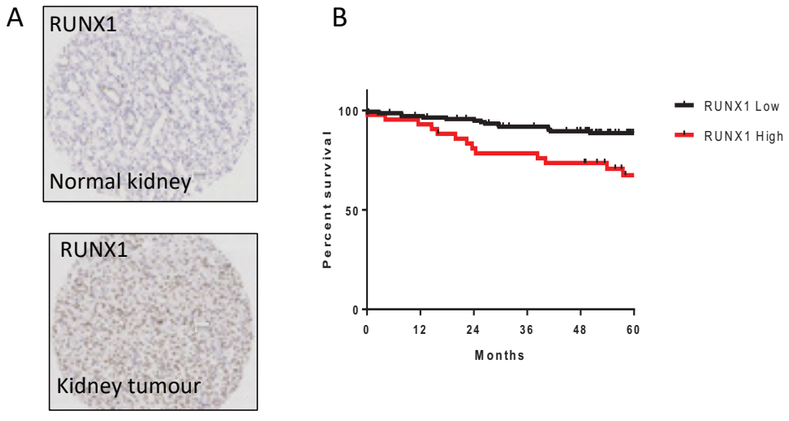

Figure 1
Renal cell carcinoma tissue was stained for RUNX1 (A). Strong expression was observed in cancer tissue compared to normal kidney. Patients whose tumours stained high for RUNX1 had reduced disease free survival as shown in (B) with 68% of RUNX1-high patients alive at 5 years compared to 88% of RUNX1-low patients.

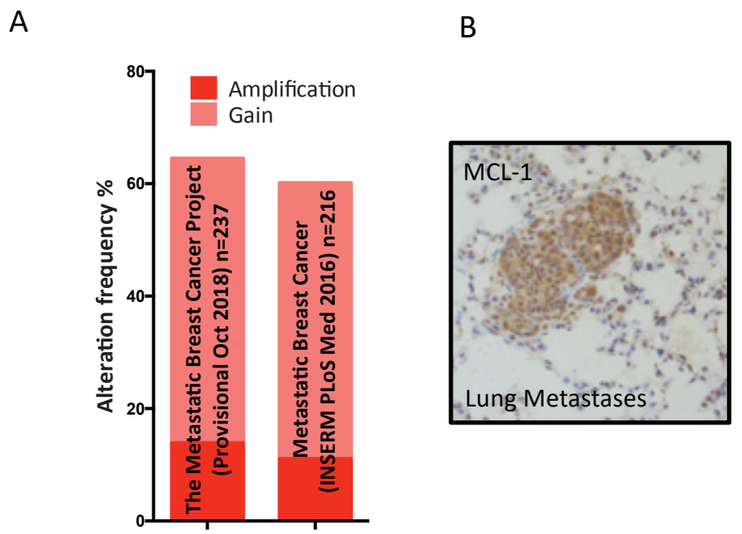

Figure 2
High MCL-1 in metastatic breast cancer. **A)** Analysis of The Cancer Genome Atlas data reveals that increased *MCL1* occurs in over 60% of metastatic breast cancers. **B)** MCL-1 staining reveals high expression in lung metastases in mouse models of breast cancer.

RUNX1 is a novel driver of renal cell carcinoma relating to poor clinical outcome

We were fascinated by the observation in The Cancer Genome Atlas dataset that *RUNX* genes are altered in a significant percentage of renal cell carcinoma (RCC). Interestingly, unlike breast cancer, alterations almost exclusively result in *RUNX1* overexpression. This has led us to unequivocally show that high RUNX1 or high RUNX2 expression in a cohort of RCC patients correlates with lower patient survival (Figure 1). By deleting RUNX1 in RCC cells we used RNAseq analysis to find a RUNX1 regulated gene signature with predominant changes in cell adhesion. Consistent with these findings, RUNX1 was important for driving cell migration, cell cycle progression and tumourigenesis in an orthotopic xenograft model. We also found that RUNX1 was overexpressed in a genetic *in vivo* model of kidney cancer where deletion of RUNX1 was enough to decrease tumour cell proliferation and improve tumour-free survival, identifying for the first time a novel role for the *RUNX* genes in kidney cancer.

MCL-1 as a prognostic indicator and drug target in breast cancer

Whilst earlier diagnosis and new treatments have resulted in significant improvement in breast cancer outcome over recent decades, it remains the second biggest cause of cancer death in women. In particular, alternative treatments are required for patients that fail to respond to existing therapies or have advanced disease. MCL-1 is a pro-survival protein best known for its role in cancers of the blood, but we have shown that high levels of MCL-1 at diagnosis are associated with poor outcome in breast cancer (Campbell et al, 2018). Through a combination of *in vitro* and *in vivo* approaches we have found that genetic or pharmaceutical targeting of MCL-1 can inhibit primary breast cancer growth and that MCL-1 is important for breast cancer stem cells. High levels of MCL-1 are prevalent in both metastatic and treatment resistant breast cancer (Figure 2) and we are developing *in vivo* models to determine whether targeting MCL-1 could improve outcome in advanced disease.

Publications listed on page 92

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MOLECULAR CONTROL OF EPITHELIAL POLARITY

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Mohammed Mansour[2]

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Emma Sandilands[1]

Graduate Students
Erin Cumming[3]
Eva Freckmann[4]
Konstantina Nikolatou[5]

[1]University of Glasgow
[2]Left during 2019
[3]University of Glasgow MVLS Doctoral Training Program
[4]University of Glasgow Industrial PhD Partnership
[5]CRUK Glasgow Centre

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

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

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

Traditionally, how cells move 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' (spheroids) 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 a newly formed 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.

In collaboration with the HICAR facility, our group is developing an integrated platform for high-throughput, high-content, live imaging–based analysis of spheroid invasion. We have developed lentiviral shRNA arrays to manipulate gene expression in spheroids in massive parallel.

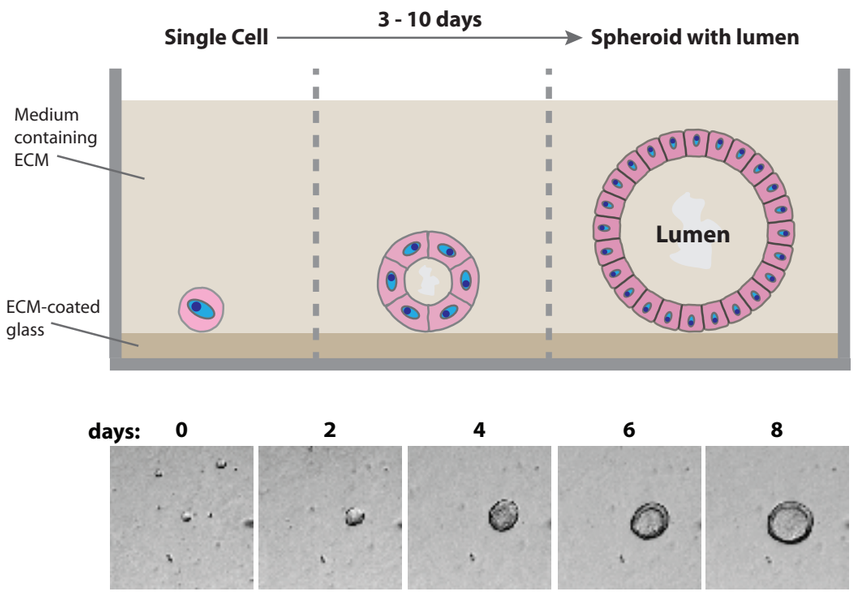

Figure 1
By culturing cells on glass-bottomed chambers coated with extracellular matrix (ECM), we direct the self-assembly of single cells into multicellular spheroid structures with a single, central lumen. This process occurs over 10 days, allowing us to study the dynamics of tissue formation.

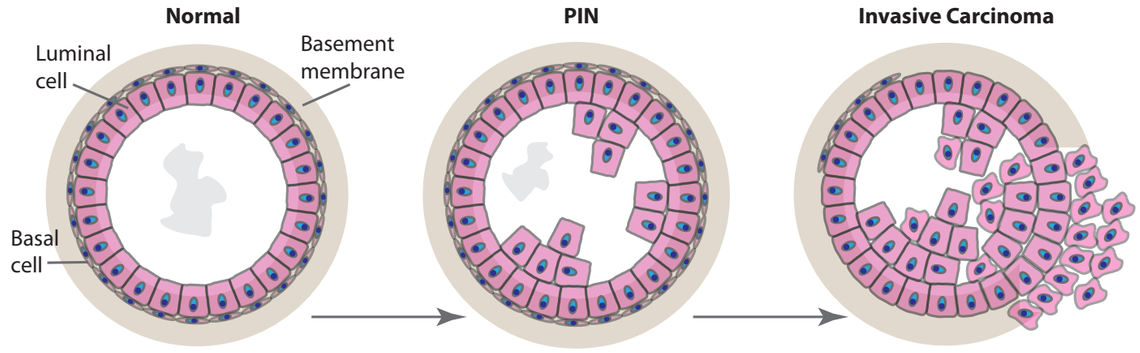

Figure 2
3D cultures of cells to form cysts (also called spheroids or organoids) also allows us to model the loss of normal tissue architecture that occurs in cancer. For example, the progressive disrupted organisation of Normal, to Prostatic Intraepithelial Neoplasia (PIN), to Invasive Carcinoma typifies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

We have coupled this to semi-automated multi-day imaging (both live and fixed) of spheroid invasion. Our analyses have been aided by the introduction of machine learning algorithms to classify different invasion phenotypes in a robust, quantitative method. This presents an exciting new possibility to examine, in a rapid fashion, the factors that regulate tissue formation and its disruption in cancer. We have used this to investigate mapping of signalling networks, and gene expression clusters stratifying particular cancer patient subgroups.

ARF GTPase circuits controlling cell invasion

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

In collaboration with the Leung group, we are interrogating their function in prostate cancer cell invasion from spheroids. We find that many ARFome family members assumed as redundant have highly divergent and sometime opposing roles in invasion, and show that there may indeed be specificity of signalling between family members. In addition, we are focusing on how alternate splicing of ARFome proteins can contribute to divergent functions of such alternately spliced isoforms. This is an important preamble to identify which, and how selectively, ARF GTPases may be targets for future therapeutic inhibition studies.

Podocalyxin function in collective cancer cell invasion

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

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

Understanding the effect of common genomic alterations on epithelial–stromal crosstalk in ovarian and endometrial cancer

In collaboration with the Zanivan group, we are developing a novel 3D co-culture of fibroblasts and epithelial cells to understand the role of epithelial–stromal crosstalk in ovarian cancer. Our approach combines quantitative mass spectrometry and high-throughput image analysis to understand how common genomic alterations in ovarian cancer affect both fibroblast and epithelial organisation. Our aim is to understand whether there are specific, druggable signalling events between fibroblasts and ovarian cancer cells that control cancer progression.

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RNA AND TRANSLATIONAL CONTROL IN CANCER

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[7]MRC Toxicology Unit

The dysregulation of protein synthesis in the tumour clone and the supporting stroma is essential for induction of oncogenic gene programmes which are required for the malignant phenotype. The tumour clone requires efficient production of proteins that drive cell 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 clone. 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 the mRNAs encoding key proteins required for these processes are first selected for decoding by ribosomes and then how the decoding process is achieved. This research has uncovered that the gene expression programmes operating within these different cellular states are fundamentally distinct, imposed via different recruitment mechanisms, and require a profound shift in the decoding mechanisms.

RNA helicases and mRNA selection

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, from ribosome recruitment to the mRNA, through translation of the mRNA into protein, to mRNA decay. One such critical RNA helicase is eIF4A1 which, as part of the eIF4F complex, is responsible for the rate-limiting initial steps leading to the decoding of mRNAs. eIF4A1 is specifically required for selection of mRNAs encoding oncogenic proteins within the highly proliferating tumour cell clone due to the high degree of secondary structure within their 5′ untranslated region. Our recent work has uncovered unforeseen mechanistic insights on how this helicase functions to promote oncogenic gene expression. We find that eIF4A1 exists in a monomeric as well as multimeric form *in vivo* and *in vitro* (Fig 1A). Moreover, it is only the multimeric state that unwinds RNA efficiently (Figure 1B). Our ongoing work supports the idea that different eIF4A1 subunits perform distinct enzymatic activities within this complex (Fig 1C). Our most recent work has shown how inhibition of eIF4A1 causes specific changes in secondary structure within the 5′ untranslated region of a subset of mRNAs (Figure 1D). These results challenge the current view of how eIF4A1 carries out its oncogenic function in controlling gene expression. Ongoing activities within the lab aim at defining how the changes in mRNA structure map to eIF4A1 binding sites within mRNA. This will provide us with valuable information as to the functional capacity the factor to control oncogenic gene expression.

eIF4A1 has a closely related paralogue, eIF4A2, which was believed to function interchangeably with eIF4A1 within the eIF4F complex. However, our studies have highlighted that eIF4A2 is primarily expressed in the supporting stromal compartment of the tumour, unlike eIF4A1 which is predominately expressed in the tumour clone. While we are currently investigating the role of eIF4A2 within the stromal compartment, two publications in the last year from the laboratory have shed new light on the importance of eIF4A2 in a very different aspect of gene expression control. We have shown that eIF4A2 does not interact with the eIF4F complex, but rather with an RNA binding complex central to mRNA decay, the CCR4-NOT complex (Wilczynska *et al.*, Genome Biology 2019; Meijer *et al.*, Science 2013; 340: 82–5, Meijer *et al*, NAR 2019). eIF4A2 behaves like inhibited eIF4A1 in terms of its RNA-binding specificity (Figure 2A). This results in

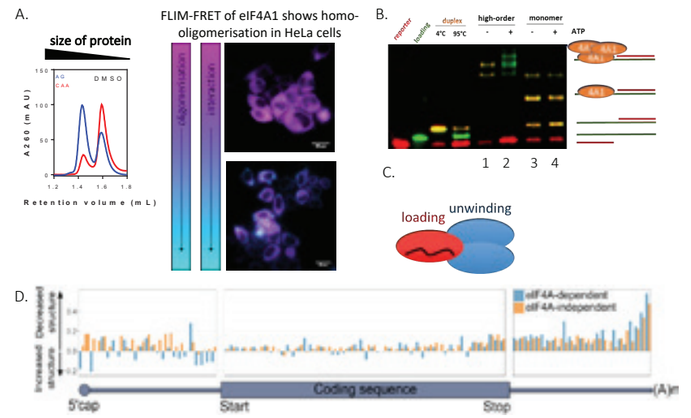

Figure 1
eIF4A1 forms higher-order structures.
A) Gel filtration reveals that eIF4A1 oligomers form differentially for different RNA oligos. This is confirmed by FLIM-FRET in HeLa cells. **B)** EMSA shows that only high-order eIF4A1 complexes separate an RNA duplex, while monomeric eIF4A1 is completely inactive. **C)** We propose that the subunits of the eIF4A1 oligomer perform different functions in the complex. **D)** Inhibition of eIF4A1 leads to local rearrangement in the structure of 5′UTRs of dependent mRNAs.

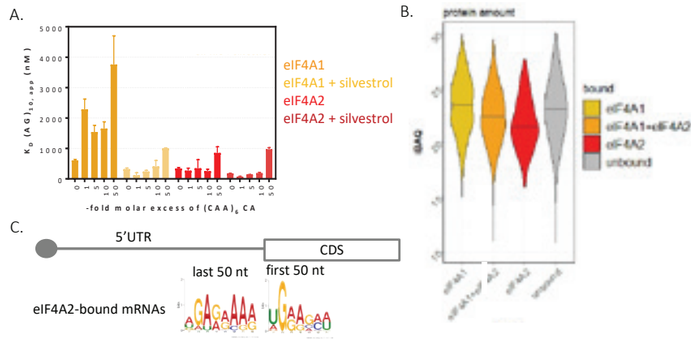

Figure 2
eIF4A2 behaves like a translation repressor on specific mRNAs.
A) eIF4A2 has the RNA specificity of inhibited eIF4A1. **B)** eIF4A2-bound mRNAs are expressed at lower levels than those bound by eIF4A1. **C)** eIF4A2-bound mRNAs are enriched for purine motifs around the start codon of the coding region.

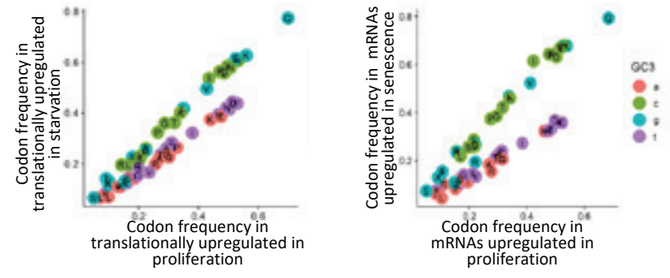

Figure 3
Synonymous codon patterns are conserved between similar cellular states.
mRNAs regulated on the translation level show distinct patterns of synonymous codon usage in starvation and proliferation (left panel). A similar pattern is observed when comparing mRNAs differentially expressed in an oncogene-induced senescence model.

the mRNAs that eIF4A2 binds being translationally repressed (Figure 2B). These mRNAs have a specific enrichment of high-affinity sequences around the start codon.

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

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:

1. Recent studies have highlighted the impact of distinct synonymous codons on the expression of proteins with the same amino acid sequence and the half-lives of mRNAs that encode them.

2. The expression of synonymous tRNAs is profoundly different in tumours compared to normal tissues. Moreover, the anticodon signature of tRNAs in cancer cells specifically matches the codon composition of mRNAs required for cell proliferation (Gingold *et al.*, Cell 2014; 158: 1281–92). 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 matching synonymous tRNAs. In fact, this may represent a feedforward loop preserving the neoplastic phenotype.
We have demonstrated that the synonymous codon usage diverges in mRNAs that are differentially translated in serum starvation conditions compared to proliferative conditions (Figure 3). Extending our observations into cancer models that are the focus of multiple groups at the Institute, we have documented a similar signature.

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 determine the role of these helicases in this process.

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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 recurrence, 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 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. 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 (which also produces and modulates matrikines – chemoactive products of matrix degradation). 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 contribute to both tumour antagonism and tumour-promoting inflammation, 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.

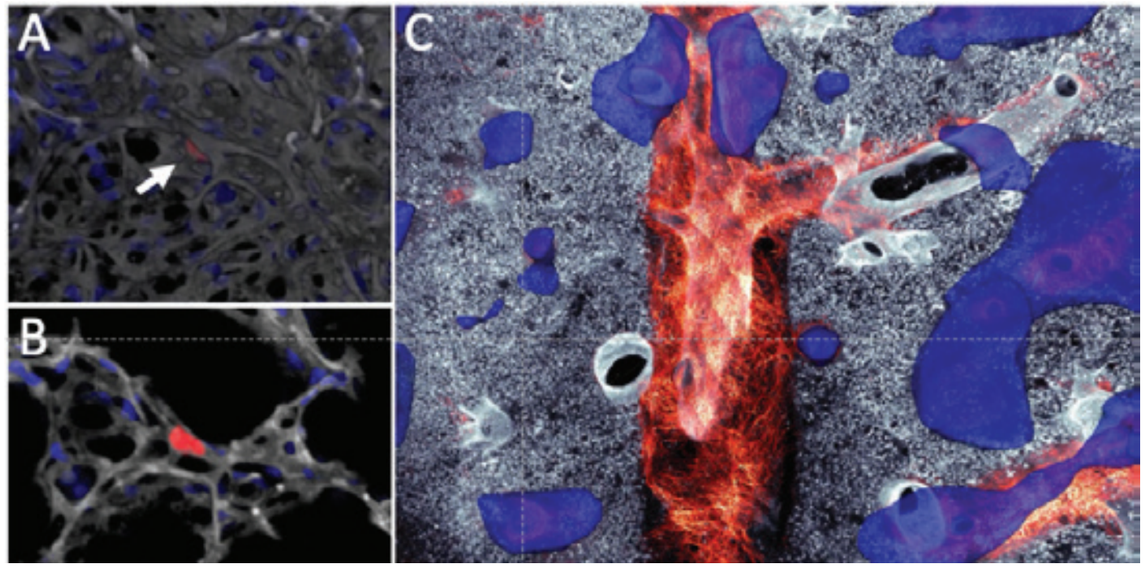

Figure 1
Investigating early and late breast cancer metastasis using advanced light microscopy.
A) 3D reconstruction of single metastatic cancer cell in a large area of lung capillary bed (arrowhead points to location).
B) Single optical section from A clearly localising tumour cell in the capillary.
C) Localisation of large areas of experimental metastasis (outlined blue) in lung (tissue autofluorescence; white) also showing fibrillar collagen (Second Harmonic Generation; 'Glow' orange).

By looking across multiple, relevant, cancer models, we aim to do two 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 chemo-therapeutic 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 models. For example, in collaboration with the Coffelt, Blyth and Roberts labs, we have developed advanced optical clearing and multiplexed imaging approaches to monitor models of breast cancer metastasis to the lung (Figure 1).

This year we celebrated another PhD graduation. Well done to Dr Judith Secklehner who came with us from London and passed her viva in December. Judith was one of the first members of our group and in addition to her own project discovering fundamental mechanisms that regulate neutrophil behaviour in the lung, she laid the groundwork for many of the innovative techniques we now use routinely in the lab.

Publications listed on page 93

IMMUNE CELLS AND METASTASIS

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[4]Marie Curie Fellowship
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[8]Pancreatic Cancer UK Future Leader Academy
[9]William Forrest Charitable Trust PhD Research Fund
[10]University of Glasgow

Our lab focuses on immune evasion mechanisms in cancer progression and metastasis, using genetically engineered mouse models of cancer. Currently, we are focused on breast, colon and pancreatic cancers. In each of these cancer types, we have uncovered unique contributions of γδ T cells in promoting cancer progression and metastasis that are discussed below and summarised in Figure 1A. We also initiated projects in ovarian cancer to determine the immune response to anti-cancer therapy.

Over the past year, we welcomed two new members to the lab, Wilma and Josip. We were fortunate to secure two new grants from Breast Cancer Now written together with Leo Carlin, Ed Roberts and Karen Blyth. Other accolades for the lab include:

- Best poster prize at the British Society for Immunology West of Scotland Immunology Group Showcase, Glasgow, awarded to Sarah Edwards
- Selected talk for the British Society for Immunology annual congress in Liverpool awarded to Sarah Edwards
- Selected talk for the International PhD Student Cancer Conference at Netherlands Cancer Institute, Amsterdam, awarded to Mark Lawrence

Breast cancer

Our work aimed at understanding the regulation and function of γδ T cells in mammary tumour metastasis centred on two key projects. First, we continued to analyse and validate the single cell RNA sequencing dataset we generated in 2018. With the help of Kristina Kirschner (University of Glasgow), Ann Hedley and Crispin Miller, we have asked questions about γδ T cell diversity in the lungs of wild-type and mammary tumour-bearing mice. This analysis has yielded important information on γδ T cell subsets and how tumours influence these subsets. We have found that mammary tumours increase γδ T cell diversity in the lung (Figure 1B). Interestingly, anti-tumour γδ T cells were suppressed in tumour-bearing mice. We have isolated these cells from mice, expanded them *ex vivo,* characterised their surface marker expression and tested their killing ability (Figure 1C). Second, we have continued our efforts to delineate the function of NKG2D – a cancer cell recognition molecule, normally expressed on NK cells – on γδ T cells. We have found that stimulation of NKG2D signalling fails to modulate expression of IL-17, but increased survival of γδ T cells. Future work will determine whether these unique functions are important in breast cancer metastasis.

This year we also completed a project on targeting Recepteur d'origine nantais (RON), a receptor tyrosine kinase encoded by the *Mst1r* gene that shares high homology with the cMET oncogene. We found that RON and its ligand, Macrophage-stimulating protein (MSP), were upregulated in triple-negative breast cancer models and inhibiting this signalling axis can delay cancer progression.

Colorectal cancer

In collaboration with Owen Sansom's lab and Adrian Hayday (Francis Crick Institute), we were investigating the role of γδ T cells in various mouse models that recapitulate distinct molecular subtypes of colon cancer. To understand the role of gut-resident γδ T cells in cancer progression, we crossed *Btnl1*$^{-/-}$ mice, which lack intra-epithelial γδ T cells, with *Villin-Cre;Apc*$^{F/+}$;*Kras*^{G12D} mice. Overall survival of these mice, tumour burden and tumour size was the same as controls, suggesting that gut-resident γδ T cells failed to influence tumour formation and progression in this model. However, other experiments have uncovered a compensatory mechanism between CD8 T cells and γδ T cells. In the metastatic Villin-Cre;KrasG12D;Trp53F/F;Nicd1F/+ model and, in a similar fashion to our observations in metastatic mammary tumour models, we found that the increase in IL-17-producing γδ T cells occurs in visceral organs in addition to the primary tumour. Transplantation of tumour-derived organoids into the colon of γδ T cell-deficient mice has shown that γδ T cells were required for cancer progression in this model. Over the next year, we aim to determine the mechanisms on γδ T cell function in these various models of colorectal cancer.

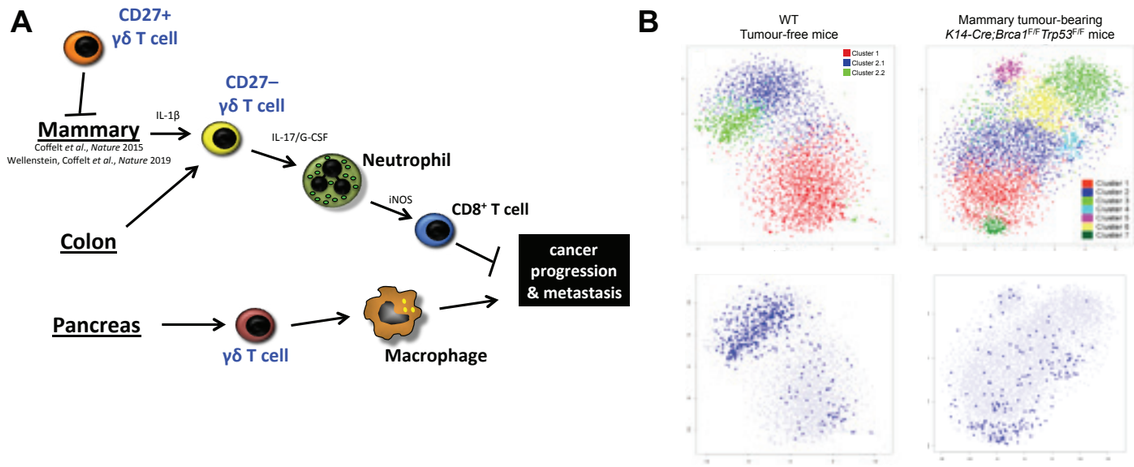

Figure 1
γδ T cells in mammary, pancreatic and colon cancers
A) Summary of γδ T cell function in various tumour types. **B)** CD3$^+$γδTCR$^+$ cells were sorted from murine lungs and loaded onto the Chrominum 10X platform. Upper row: tSNE analysis of lung γδ T cells revealing 3 clusters of cells in WT mice and 7 clusters in mammary tumour-bearing mice. Lower row: Feature plots of *Cd27* mRNA expression from scRNAseq data shown in B (where blue = expression, grey = no expression). These data show enrichment of *Cd27* in Clusters 2.1 and 2.2 from WT mice, and Cluster 7 of mammary tumour-bearing mice. (C) Proportion of dead *K14-Cre;Trp53*$^{F/F}$ (KP) or *K14-Cre;Brca1*$^{F/F}$;*Trp53*$^{F/F}$ (KB1P) cancer cells *in vitro* (assessed by DAPI$^+$ cells using flow cytometry) after treatment with Cisplatin or co-culture with splenic CD27$^+$ γδ T cells for 24 hours (n=3).

Pancreatic cancer

We have generated more data on γδ T cell function in the *Kras*^{G12D};*Trp53*^{R172H};*Pdx1-Cre* (KPC) model over the past year. We showed that γδ T cells regulate cancer progression and liver metastasis, as tumour-bearing mice lacking γδ T cells exhibited a delay in the transition to carcinoma and fewer secondary tumours in the liver. Interestingly, pancreatic tumours in γδ T cell-deficient mice contained less macrophages than control tumours. We have found that this reduction in macrophages was not due to the ability to recruit monocytes, and our new hypothesis is that γδ T cells regulate monocyte to macrophage differentiation.

Ovarian cancer

We initiated new projects in ovarian cancer this year by asking questions about immune response to PARP inhibitors. We are currently investigating how myeloid cells and lymphocytes participate in the anti-cancer efficacy of PARP inhibitors using the ID8 model of high-grade serous ovarian cancer.

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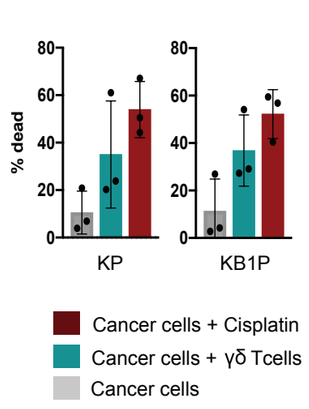

www.beatson.gla.ac.uk/payam_gammage

MITOCHONDRIAL ONCOGENETICS

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Research Scientist
Andrew Shaw

Graduate Student
Mahnoor Mahmood

Mutation of oncogenes and tumour suppressors in nuclear DNA are well-described routes to cancer initiation and progression. However, the role of commonly detected mutations and copy number variations of mitochondrial DNA (mtDNA) in cancer remains broadly undefined. Mitochondria perform numerous metabolic functions, relying on faithful expression and maintenance of mtDNA, a small, separate genome from the nuclear DNA that is contained exclusively within mitochondria. Mutations of mtDNA can lead to profound metabolic dysfunction - one of the earliest identified hallmarks of cancer - and are present in >50% of tumours. In order to understand the possible links between mtDNA mutations and metabolic dysfunction in cancer, our lab studies the impact of mtDNA mutations across 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.

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.

Determining the role 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.

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, in mitochondria 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. By developing our understanding of mtDNA copy number regulation, identifying the molecular mechanisms underlying this process, we hope to design future therapeutic strategies underpinned by manipulation of mtDNA copy number.

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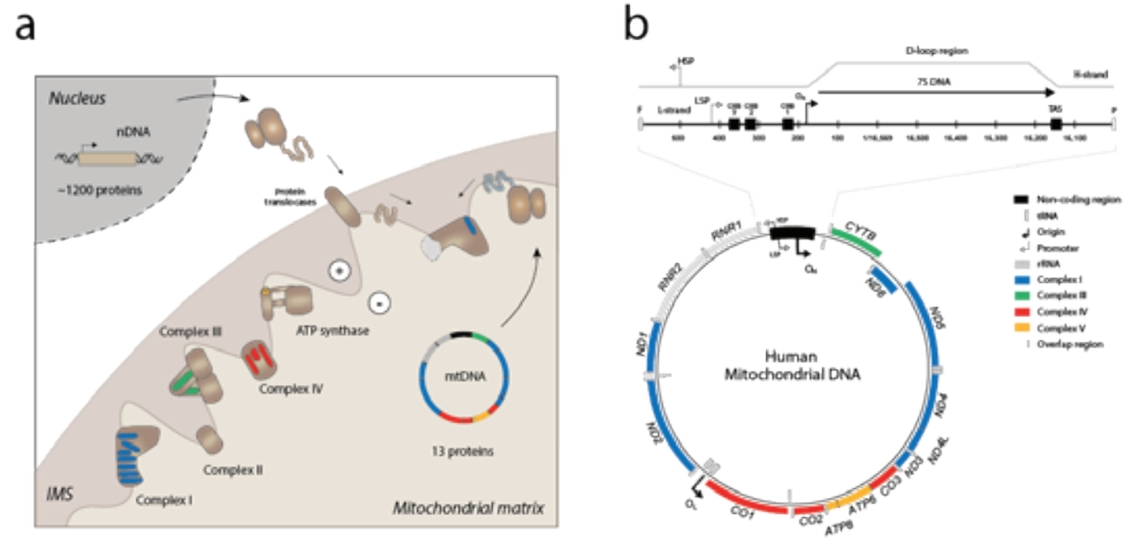

Figure 1
Genetic composition of human mitochondria.
A) Dual-genome origins of the mitochondrial electron transport chain (ETC). The ETC comprises ~ 90individual protein subunits, encoded by both nuclear (nDNA) and mitochondrial genomes (mtDNA). Assembly of a functional ETC requires co-ordinated regulation and expression of these components by the two separate genomes. Beyond the 13 ETC proteins encoded in mtDNA, the remainder of the human mitochondrial proteome is encoded in and expressed from the nuclear genome. Import of nuclear-encoded proteins through membrane-embedded protein translocases into the mitochondrial matrix requires a membrane potential between the intermembrane space (IMS) and the matrix (white circles). Nuclear encoded components coloured brown, mitochondria-encoded components in blue, red, green, and yellow by complex. Complex III is shown as a dimer. **B)** Annotated genetic features of human mtDNA. Eleven mRNAs (two overlapping) encode 13 polypeptides forming essential components of the ETC. These are expressed using an altered genetic code, enabled by a full complement of 22 mitochondria-specific tRNAs also encoded in mtDNA. Resulting proteins are co-translationally inserted into the inner mitochondrial membrane (IMM) by mitochondrial ribosomes, which contain structural RNA components of exclusive mitochondrial origin (12SrRNA, 16S rRNA, and mt-tRNA^{Val}). An expanded view of the displacement loop (D-loop) and major non-coding region (NCR), incorporating 7SDNA, with indication of key loci for mtDNA transcription (heavy strand promoter, HSP; light strand promoter, LSP), replication (origin of heavy strand, O_H) and other prominent elements relevant to these functions (conserved sequence block 1–3, CSB 1–3; termination-associated sequence, TAS).

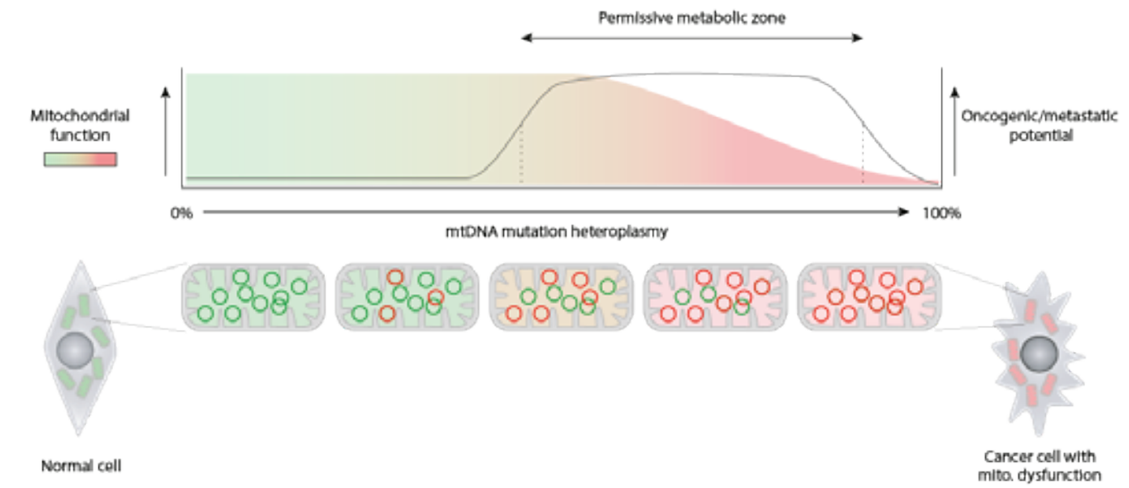

Figure 2
A model for 'oncogenic/metastatic licensing' through mtDNA mutation-derived mitochondrial dysfunction.
Although mitochondrial dysfunction can be advantageous to cancer cells, and possibly oncogenic to normal cells, total ablation of mitochondrial function is likely detrimental to both. The genetic and metabolic plasticity afforded to cells bearing heteroplasmic mutations permits greater oncogenic/metastatic potential once a threshold for heteroplasmy-induced mitochondrial dysfunction is reached. A 'permissive metabolic zone' of heteroplasmy-induced mitochondrial dysfunction is proposed. Green circles, wild-type mtDNA; red circles, mutant mtDNA.

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 X-ray crystallography 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 understanding their regulation and function, with a particular focus on RING E3s that have been linked to cancer, such as CBL and MDM2.

CBL proteins (CBLs) are RING E3s that negatively regulate RTKs, tyrosine kinases and a host of other proteins by promoting their ubiquitination and subsequent degradation by the proteasome or via endocytosis. Mutations in c-CBL have been observed in human patients with myeloproliferative diseases (MPD) and conditional knock-in mice expressing these c-CBL mutants developed leukaemia. More recently, the inhibition of CBL-B in immune cells has shown to activate their anti-metastatic activity. Over the past year, we have investigated the mechanism by which CBL mutants exert oncogenesis and have developed tools for targeting CBLs.

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. Studies have shown that the inhibition of MDM2-p53 interaction could stabilise p53, resulting in elevated p53 activity that promotes cell cycle arrest and apoptosis in cancer cells. This has led to the development of small-molecule inhibitors targeting MDM2's N-terminal p53-binding domain. However, these compounds exhibit high toxicity due to high levels of p53 activity, and therefore we explored whether targeting the RING domain is a suitable strategy to target MDM2-p53 axis.

Targeting RING E3s with ubiquitin variants

RING E3s generally contain a catalytic domain and a substrate-binding module that are important for mediating substrate ubiquitination. The catalytic domain of RING E3s, commonly known as the RING domain, promotes Ub transfer by binding and stabilising the E2~Ub conjugate in a closed conformation to facilitate catalysis ((Figure 2a). Due to the small surface area, targeting the RING domain remains a major challenge. The substrate-binding domain varies between different RING E3s. In contrast to the RING domain, the substrate-binding domain often contain pocket or groove to recognise certain amino acid motif or post-translational modification on the substrate, and therefore can be targeted by small molecules or peptides.

Figure 1

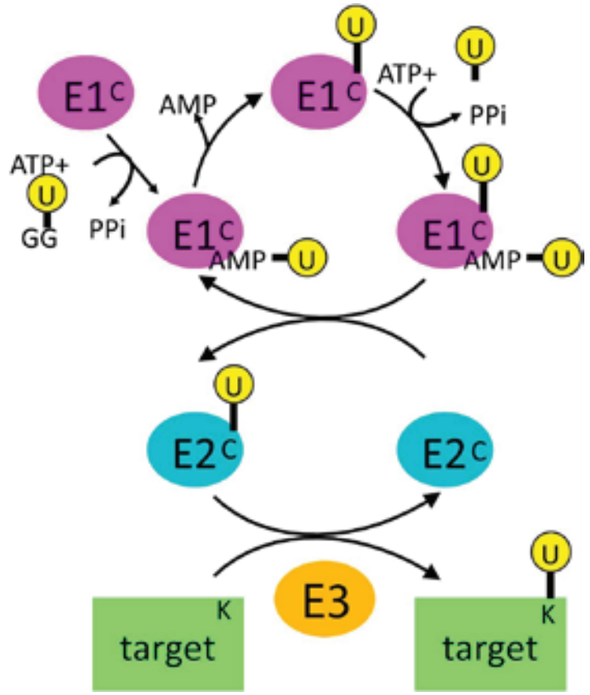

Figure 1
Enzymatic cascade for Ub modifications

Figure 2

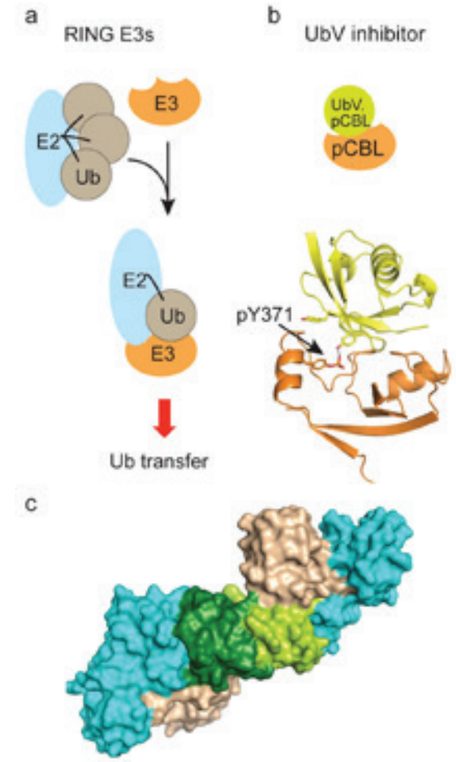

Figure 2
Mechanism and targeting of RING E3s
A) Activation of the E2~Ub complex by the RING domain. The RING domain binds the E2~Ub complex and promotes the formation of the closed E2~Ub complex to facilitate Ub transfer.
B) UbV.pCBL functions as an inhibitor. The crystal structure of the pCBL RING domain bound to UbV.pCBL reveals that phosphoTyr371 interacts with UbV.pCBL.
C) Surface representation of MDM2 homodimer (light and dark green) bound to UbcH5B (cyan) covalently linked to Ub (wheat).

To develop tools for targeting RING E3s, we collaborated with Professor Sachev Sidhu's lab at the University of Toronto, where they have developed phage-displayed ubiquitin variant (UbV) and peptide libraries. The UbV library contains native Ub sequence that was randomised to generate billions of ubiquitin variant sequences. We have applied phage-displayed UbV approach to identify UbVs that bind selectively to the catalytic RING domains of several RING E3s (Gabrielsen *et al.* 2017 Molecular Cell, 19, 456-70). For example, we identified UbV.pCBL, which acts as an inhibitor by binding selectively to the Tyr371-phosphorylated CBL RING domain to compete against E2~Ub binding (Figure 2b). Our work showed that some UbVs have acquired the ability to form non-covalent dimers. Through mutagenesis studies, we found that a single amino acid substitution of Ub's Gly10 to either Ala or Val is sufficient to convert Ub from a monomer to a dimer. These results can be used to develop next-generation phage-display libraries of UbVs to engineer new interfaces for protein recognition (Gabrielsen *et al.* 2019 Structure, 27, 1452-59).

Targeting MDM2 RING domain

We have determined the crystal structure of MDM2-MDMX RING dimer bound to an E2 UbcH5B covalently linked to Ub (Nomura *et al.* 2017 Nature Structural and Molecular Biology, 24, 578-587). The structure reveals the mechanism of E2~Ub activation by the MDM2-MDMX heterodimer. Guided by the crystal structure, we designed MDM2 mutants that prevent E2~Ub binding, without altering the RING domain structure. These mutants lost MDM2's E3 activity and were unable to ubiquitinate and degrade p53. However, they retained the ability to bind p53, thereby limiting p53's transcription activity. Cells expressing these mutants retained basal p53 levels and therefore responded more quickly to cellular stress than cells expressing wild-type MDM2. This work reveals a ligase-independent role of MDM2 in regulating p53 and suggests that inhibition of the MDM2 E3 ligase activity may be a gentler approach to activate p53.

Recently, we have identified UbVs that target the MDM2 RING domain and are exploring whether they are suitable for targeting the MDM2-p53 axis. Furthermore, we investigated the role of DNA-damage induced phospho-regulation of MDM2 and determined the crystal structure of the MDM2 RING homodimer bound to UbcH5B covalently linked to Ub (Figure 2c). Structural and biochemical studies revealed how phosphorylation serves to stabilise the activated E2~Ub complex enhancing MDM2 E3 activity and promoting its ubiquitination and degradation. This work suggests alternate strategies to modulate MDM2 E3 activity.

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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). As well as having vital normal physiological functions during development and in adult life, these factors play pivotal roles in cancer biology, acting as potent tumour suppressors or tumour promoters in a context-dependent manner. Work in our laboratory seeks to understand how, when and where TGFβ superfamily signalling regulates tumour progression. Dysregulation of TGFβ signalling is particularly prevalent in squamous cell cancers, and this has led us to begin to decipher the molecular landscape of cutaneous squamous cell carcinoma progression and its similarities with other squamous tumour subtypes.

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). Consistent with these findings immunohistochemical analysis of PO_4–SMAD2 and PO4–SMAD3 levels in a human tissue microarray revealed reduced levels in larger and invasive tumours indicating that impaired canonical TGFβ signalling activity may represent a common feature of primary invasive human cSCC and suggests that the loss of TGFβ-mediated tumour suppression provides a significant growth advantage for cSCC tumour cells (Rose *et al.*, Oncotarget. 2018 Feb 22;9(18):14552-14566).

The genetic 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 1). 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, Claude Chelala (Barts Cancer Institute), Charlotte Proby (University of Dundee), Mike Stratton and Ludmil Alexandrov (Sanger Institute), 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 (Figure 1). 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. We identified 22 significantly mutated genes which were called by at least two of three algorithms. Clonality analysis indicated that tumours can be entirely clonal or contain up to 11 subclones, further illustrating the complexity of cSCC. Mutational signature analysis revealed a novel mutational signature (Signature 32). This correlated with exposure to azathioprine, which was used as an immunosuppressant drug in the organ transplant patients from which many of the tumours we sequenced were isolated (Inman *et al.*, Nat Commun. 2018 Sep 10;9(1):3667).

We have developed and characterised at the molecular and biological level a panel of patient derived human cSCC cell lines (PDCLs) derived from non-metastatic and metastatic primary tumours and from metastases. (Hassan *et al.*, Int J Mol Sci. 2019 Jul 12;20(14)). We have established a suite of biological assays to investigate the proliferative, migration and invasion properties of these cells *in vitro* (Figure 2) and *in vivo* in sub-cutaneous xenografts. We are using these cell lines to investigate the function of potential driver genes, pathways and processes identified in our molecular profiling studies 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 (Greater Glasgow and Clyde NHS) 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.

Targeting tumour promoting TGFβ signalling

As well as having 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 itself is maintained in ~70% of tumours, and we are investigating if this may drive tumour progression and represent a potential therapeutic target. In collaboration with AstraZeneca, we have characterised two novel small molecule kinase inhibitors which act to inhibit the type 1 receptors of the TGFβ superfamily (Spender *et al.*, Mol Pharmacol. 2019 Feb;95(2):222-234) and found that they may inhibit proliferation of some PDCLs.

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. 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 Andrew South (Thomas Jefferson University, Philadelphia, Pennsylvania) and have found that this may act to promote proliferation and invasion of cSCC cells in some patients.

Our efforts are now focused 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 cSCC.

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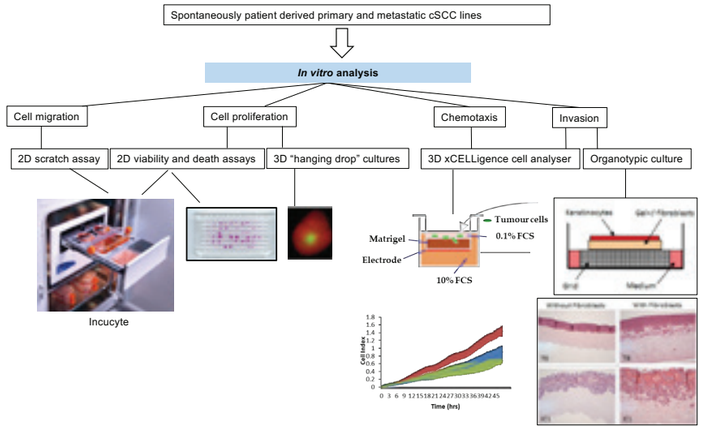

Figure 2
In vitro preclinical models of cSCC.
We have a panel of patient derived cSCC cell lines derived from non-metastatic and metastatic primary tumours and from metastases. We utilise a suite of *in vitro* assays to measure cell proliferation in monolayer cultures using the Incucyte live cell analyser, with proliferation additionally measured in 3D anchorage–independent cultures in soft agar, methylcellulose and as hanging drop spheroids. The xCELLigence real-time cell analyser is used for 3D measurement of chemotaxis and invasion. Collagen:matrigel and fibrin gel organotypic cultures are a well-established method of measuring invasion in our laboratory and have clearly shown the key role of normal human fibroblasts in promoting SCC invasion.

Figure 1
Disease progression of human cSCC.
Images illustrating disease progression (Courtesy of Charlotte Proby, University of Dundee). We are performing molecular profiling of human disease progression using NGS approaches, Immunohistochemistry and spatial transcriptomics.

CELL MIGRATION AND CHEMOTAXIS

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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 use every possible tool, in particular mathematical analysis and computational techniques such as modelling and machine learning, based around data we obtain from microscopy and biochemistry.

We ask questions of two distinct types. The first 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 particularly subtle type of chemotaxis, in which cells steer themselves, by creating and manipulating gradients of signals in their environments. The second 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. The lab contains mathematicians, computer scientists, biochemists, microscopists and geneticists. We see one of our chief jobs as spreading true multidisciplinarity – mathematicians do cell biology experiments, and biochemists use mathematical models and computational tools. However, our strategy is always based around cell migration – what drives it and most importantly how it is steered.

Mechanisms underlying chemotaxis: Pseudopods and self-generated gradients

Chemotaxis is emerging as 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, donated neutrophils and Dictyostelium.

We are collaborating 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.

Our experiments are currently focused on identifying the activators and other proteins that regulate each component of the complex. We are using the Institute's expertise in mass spectrometry to identify proteins that crosslink to SCAR in living cells at different migration rates. We have set up a system that allows us to purify biochemically useful amounts of SCAR complex from living cells. This is a unique facility. We have now found that SCAR is phosphorylated at multiple sites when it is activated; this is an exciting result, because it has never been possible before to identify the active molecule. We are now seeking to understand what regulates this phosphorylation, and how it connects to upstream signalling molecules such as receptors and G-proteins. The behaviour of SCAR's relative WASP is slightly anomalous – there is a high degree of consensus among cell biologists about how it is controlled, but the standard view does a poor job of explaining the observed behaviour. We are therefore mutating and dissecting WASP to see how it works.

Publications listed on page 96

SPATIAL SEGREGATION OF SIGNALLING

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At equilibrium, entropy (disorder) reaches maximum, since disorder is more probable than order. Life exists away from equilibrium and our cells have developed mechanisms to counteract disorder. Spatial and temporal segregation/organisation of proteins of opposite functions is crucial for controlling cell signalling output, e.g. separating phosphatases and kinases or the small GTPases regulators, guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). Our group is interested in studying self-organised cellular compartments that participate in cell signalling without being separated from the rest of the cell by membranes, such as the primary cilium and the immunological synapse. We combine structural biology, biochemistry and cell biology to investigate the atomic basis of cellular mechanisms that maintain and regulate the distinct composition of the cilia and immunological synapses in space and time. We take advantage of the similarities between cilia and immunological synapses and cross-test information that we generate from both cellular structures. We aim to develop cancer therapeutics by manipulating the spatial organisation of these protein networks and hence the output of their signalling compartments.

Mutations in the small GTPase ARL3 cause Joubert syndrome

The cilium is a hair-like protrusion on almost every cell in our body and functions as a signalling antenna. Concentrating signalling proteins and receptors inside the cilium is key to its function, and dysfunctional cilia result in many developmental diseases, collectively called ciliopathies. Previously, together with other groups, we have managed to identify and characterise the machinery that transports and concentrates those signalling proteins in the cilium (Ismail *et al.*, Nat Chem Biol. 2011; 7: 942, Ismail *et al.*, EMBO J. 2012; 31: 4085, Watzlich *et al.*, EMBO Rep. 2013; 14: 465, Fansa *et al.*, Nat Commun. 2016; 7: 11360).

In collaboration with John Sayer (Newcastle University), we have reported *ARL3* missense variants as likely cause of Joubert syndrome (JBTS), making *ARL3* a novel JBTS gene. We showed *in vitro* and in cells from patients that missense variants of *ARL3* arginine at position 149 disrupt the known interaction between ARL3 and ARL13B. This prevents the activation of ARL3 and in turn the correct release of intra-ciliary cargoes. We propose ARL3 as a hub within the network of ciliopathy-associated genes, whereby perturbation of ARL3 results in the mislocalisation of multiple ciliary proteins, including INPP5E and NPHP3.

T cells repurpose ciliary machinery to traffic and concentrate LCK at the immunological synapse

Upon the engagement of a T cell receptor (TCR) with an antigen-presenting cell, LCK phosphorylates the immunoreceptor tyrosine-based activation motifs (ITAMs), initiating TCR signalling. Autophosphorylation of LCK tyrosine 394 (Y394) is critical for its kinase activity in cells. However, *in vitro*, phosphorylated Y394 results in only a two-fold increase of its catalytic activity, suggesting an additional layer of regulation. In this project, we showed that phosphorylation of LCK Y394 does not only alter the catalytic rate but also regulates its interaction with the ciliary UNC119A and thus LCK trafficking. UNC119A interacts with the unique domain of LCK with a high affinity, compared to other SRC family kinases. We showed that the release at the target membrane is under the control of the ciliary ARL3/ARL13B. The UNC119A N terminus acts as a 'regulatory arm' by binding the LCK kinase domain, an interaction inhibited by LCK Y394 phosphorylation, thus together with the ARL3/ARL13B machinery ensuring immune synapse focusing of active LCK. We finally proposed in this study that the ciliary machinery has been repurposed by T cells to generate and maintain polarised segregation of signals at the immune synapse.

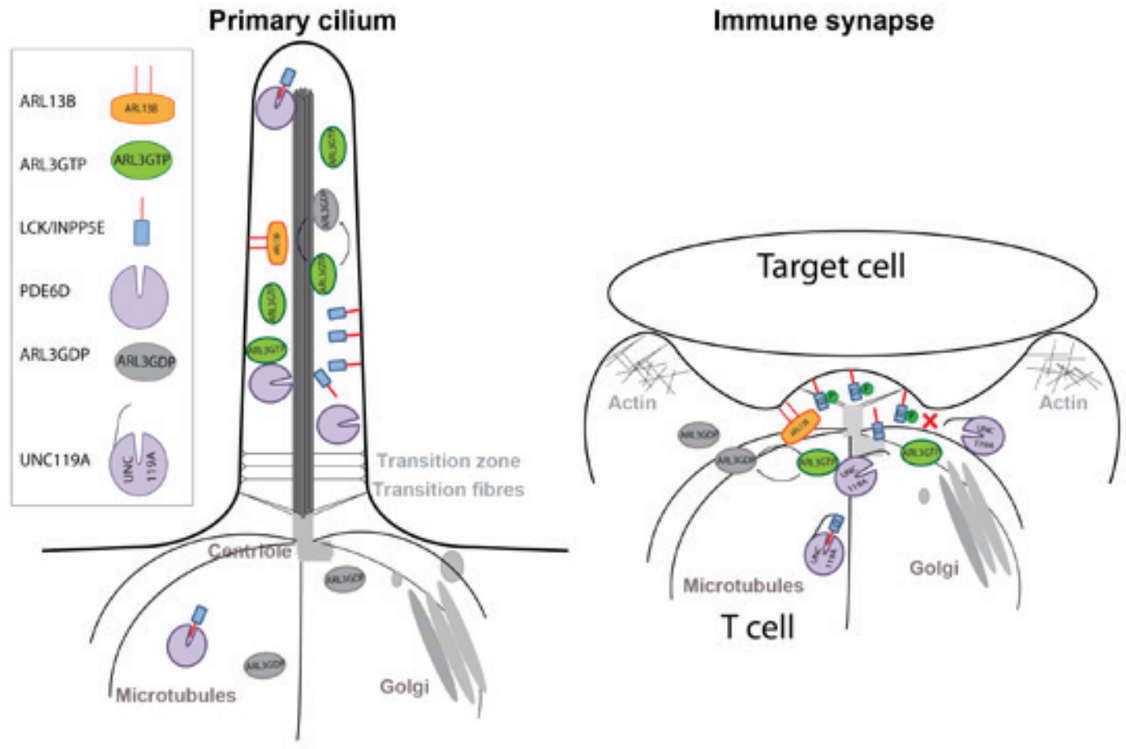

Figure 1
The primary cilium and the immunological synapse share the machinery for shuttling lipid-modified cargo
Lipid modified proteins are solubilised by GDI-like solubilising factors (GSFs). Solubilised cargoes are released by ARL3GT. Localisation of guanine exchange factors (GEFs) such as ARL13B at the immunological synapse or primary cilium drives activation of ARL3.

Pioneer Award

Farnesylation plays an important role in proteins of the Ras superfamily. This family includes well known oncoproteins such as N- and KRas, which regulate critical cellular processes. Farnesylation targets these proteins to the cell membrane as well as internal membranes. Only at these membranes are Ras family proteins active and perform their biological functions. The lab was awarded a Pioneer Award from CRUK to develop small molecules to selectively stop different Ras proteins from being targeted to membranes and hence inhibit their oncogenic activities.

Publications listed on page 96

PROSTATE CANCER BIOLOGY

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[5]Horizon 2020 ITN, in conjunction with GenomeScan, the Netherlands
[6]in conjunction with Nuclear Medicine, NHS Greater Glasgow and Clyde

Prostate cancer affects one in eight men in the developed world, and now accounts for more cancer related deaths in men than females dying from breast cancer. Despite improvement in patient survival with novel androgen receptor inhibitors and taxane chemotherapy, a significant proportion of patients with advanced disease still die within five years of diagnosis. In this report, we highlight recent publications arising from our research. Collectively, our findings open up new therapeutic opportunities for future development. In addition, our ongoing research also exploits adaptive cancer metabolism to tackle treatment resistant prostate cancer.

Developing novel combination approaches to tackle treatment resistance in prostate cancer

Taxane chemotherapy remains a key treatment option for advanced prostate cancer. We carried out two innovative screens to identify new therapies to enhance docetaxel treatment. Both screens have revealed novel approaches to treat chemo-resistance in prostate cancer.

In the first instance, we performed a drug repurposing screen using our treatment resistant prostate cancer cell models. Just under 900 drugs were screened, and cellular viability was assayed by nuclear counting using High Content Imaging Analysis. Mebendazole, an anthelmintic drug, was identified as a clinical candidate to enhance the efficacy of docetaxel in advanced prostate cancer.

Combined docetaxel and mebendazole treatment dramatically reduced cell cycle progression with increased G2/M mitotic block and enhanced cell death. Strikingly, following combined treatment, no prostate cancer cells were observed to divide correctly, forming multipolar spindles that resulted in aneuploid daughter cells or arrest in prometaphase. In our publication (British Journal of Cancer, https://doi.org/10.1038/s41416-019-0681-5), we confirmed enhanced tumour response to combined docetaxel and mebendazole treatment in both *in vitro* and *in vivo* models.The concept of combined mebendazole and docetaxel treatment therefore warrants formal clinical evaluation. We are actively planning the relevant design of a clinical efficacy study.

Applying a novel preclinical prostate orthograft model developed in our laboratory, we performed an *in vivo* genome wide CRISPR screen. We employed a murine prostate cancer cell model derived from a genetically engineered mouse model of invasive prostate cancer, and orthotopically implanted the cancer cells to result in tumour formation. We further applied docetaxel treatment to tumour bearing mice in order to probe/'screen' for genes that may influence tumour response to chemotherapy (Figure 1).

TCEAL1 (Transcription Elongation Factor A-like 1) was the top negatively selected gene from this screen, suggesting that it might be an essential gene for prostate cancer cells to survive docetaxel treatment. Little is currently known about the function of TCEAL1. TCEAL1 is considered to be a phosphoprotein similar to transcription factor SII, promoting or repressing target promoter activities in a context-dependent manner. Upon suppression of TCEAL1 expression, we were able to confirm enhanced response to docetaxel in multiple human prostate cancer cell lines, including PC3M, LNCaP, CWR22 and DU145. We are now investigating the underlying molecular mechanism, through which we hope to nominate new strategies to enhance tumour response to docetaxel.

Improved understanding of the molecular basis driving aggressive prostate cancer will provide new therapeutic opportunities. We have previously shown that activation of the WNT pathway is implicated in resistance to hormone treatment (or androgen deprivation therapy, ADT) in prostate cancer (European Urology,

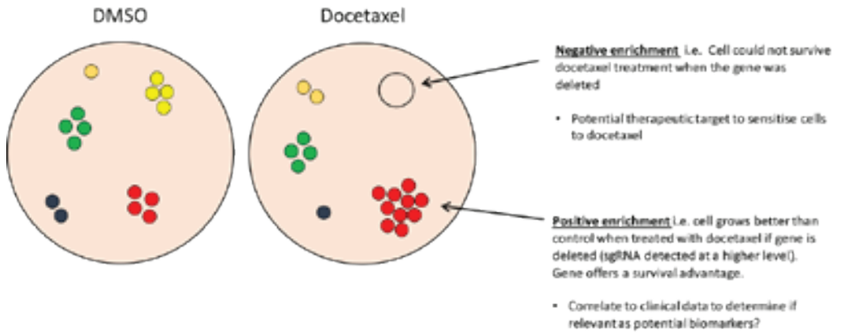

Figure 1
Cartoon illustration of positive or negative enriched genes following docetaxel treatment in an *in vivo* CRISPR screen.

https://doi.org/10.1016/j.eururo.2013.08.011). We therefore applied clinically relevant murine prostate cancer models to investigate the significance of β-catenin activation in prostate cancer progression and treatment resistance (Cancer Research, https://doi.org/10.1158/0008-5472.CAN-19-1684).

β-catenin activation (in both prostatic luminal and basal subpopulations) co-operated with Pten loss to drive androgen receptor (AR) independent castration-resistant prostate cancer. Prostate tumours with β-catenin activation relied on the non-canonical WNT ligand WNT5a for sustained growth. Overall, WNT/β-catenin and AR signalling are reciprocally inhibited (Figure 2). We showed that WNT5a mediated WNT signalling repressed AR expression and maintained the expression of c-Myc, an oncogenic effector of β-catenin activation. In a proof of concept efficacy *in vivo* experiment, we applied combined suppression of the WNT and AR pathways. We showed that inhibition of the WNT/β-catenin signalling by LGK974 (a potent inhibitor of porcupine which is required for WNT ligand palmitoylation during the secretory process) functions in concert with AR blockade, producing enhanced anti-tumoral effects.

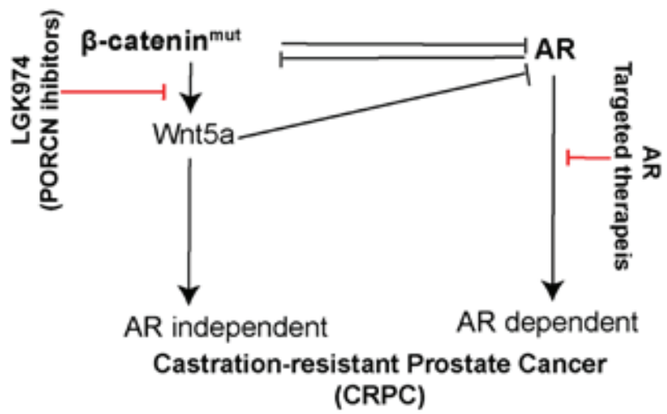

Figure 2
Illustration of the reciprocal interactions between the WNT and AR signalling pathways, with emphasis on the impact of combined suppression of both pathways. (Cancer Research, https://doi.org/10.1158/0008-5472.CAN-19-1684)

AR-mediated rewiring of cancer lipid metabolism to support resistance to AR inhibitors

Despite the clinical success of AR-targeted therapies, reactivation of AR signalling remains the main driver of castration-resistant prostate cancer (CRPC) progression. The human AR-expressing and hormone-dependent LNCaP prostate cancer cells were chronically exposed to multiple AR inhibitors (ARI) to generate CRPC cell models. Combined proteomics and metabolomics analyses revealed shared adoptive metabolic phenotype among the ARI-resistant cells with perturbed glucose and fatty acid metabolism. This metabolic rewiring occurs in an AR-dependent manner and ultimately leads to a profound reorganisation of the cellular lipidome of the resistant cells; as evidenced by a strong accumulation of multiple classes of sphingolipids and polyunsaturated triglycerides.

To exploit this phenotype, we delineated a subset of proteins consistently associated with ARI resistance 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 resistance to novel inhibitors of the AR pathway.

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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.

Visualising metabolic heterogeneity and plasticity in lung cancer

We know that tumours are not single entities but are rather complex ecosystems with regional genetic, microenvironmental and metabolic landscapes. This tumour heterogeneity contributes to treatment failure due to the pre-existence of drug resistant clones and tumour niches. Understanding and countering this complexity is an increasingly important task if we want to develop effective treatment strategies. To address this, we have developed several multiplexed technologies such as dual-isotope positron emission tomography, dual-isotope autoradiography and dual-isotope tracking (DIOPTRA) to visualise and evaluate heterogeneity, in particular regional tumour metabolism.

Applying these technologies to unravel heterogeneity in genetically engineered mouse models of lung adenocarcinoma, we have non-invasively identified two spatially heterogeneous metabolic subtypes (Figure 1). One subtype is characterised by high uptake of the radiolabelled tracer [^{18}F]FDG, a surrogate of high glucose uptake in tumour cells. The other subtype is characterised by high [^{11}C]acetate uptake, representing elevated *de novo* fatty acid synthesis. Molecular profiling of these two imaging phenotypes showed that they have distinct transcriptional, proteomic and metabolic signatures. Regions with higher glucose consumption are more proliferative with activation of cell cycle genes, Myc targets and the unfolded protein response. While regions of high acetate uptake have signatures for fatty acid metabolism, reactive oxygen species, tricarboxylic acid (TCA) cycle and oxidative phosphorylation.

We traced [U-^{13}C]glucose and [U-^{13}C] isotopologues to establish metabolic pathway activity in each subtype. FDG-avid tumours utilise glucose for synthesis of serine and glycine, while using acetate to replenish the TCA cycle intermediates. In contrast, acetate-avid tumours use glucose for TCA anaplerosis and glutamine biosynthesis while using acetate for synthesis of palmitate. Each subtype, therefore, uses distinct metabolic pathways suggesting different vulnerabilities.

Notably, these tumour subtypes differentially express the ubiquitin proteasome system and the downstream regulator of the unfolded protein response, XBP1. We speculated that each tumour subtype would respond differently to the proteasome inhibitor Bortezomib. We monitored the metabolic phenotype of each lung tumour using longitudinal [^{18}F]FDG and [1-^{11}C]acetate PET imaging during a three-week course of treatment. In the tumours of mice administered vehicle control the uptake of both tracers increased over time as did metabolic heterogeneity, that is the extent of differential tracer uptake. In Bortezomib-treated mice, tumour [1-^{11}C]acetate uptake was static over three weeks while the uptake of [^{18}F]FDG increased; heterogeneity of [1-^{11}C]acetate and [^{18}F]FDG uptake was reduced compared to control mice. These results suggested that Bortezomib restricted the tumour capacity for acetate utilisation, compensated by increased glucose use, possibly indicating an emergent therapeutic susceptibility.

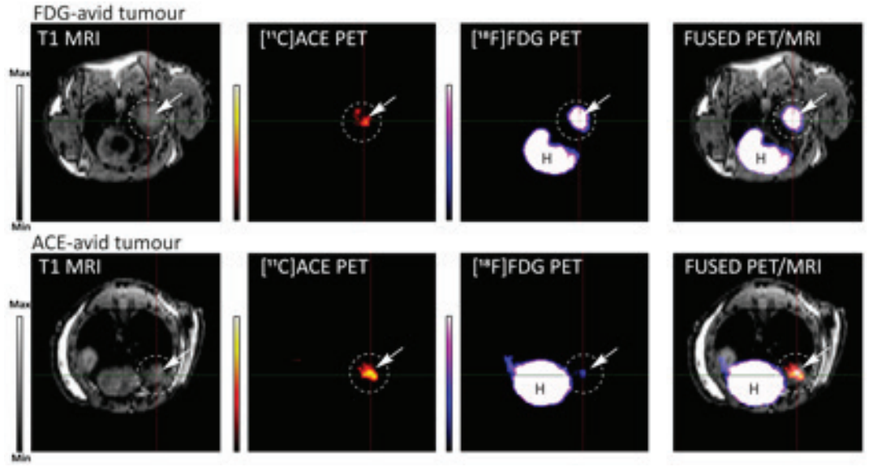

Figure 1
Imaging metabolic heterogeneity in a mouse model of lung cancer
Dual [^{18}F]FDG and [1-^{11}C]acetate PET/MRI imaging of the Kras$^{G12D/+}$ *p53*$^{-/-}$ mouse model of lung adenocarcinoma. Tumours with distinct metabolic features, high glucose uptake (FDG-avid tumour, *top panel*) and fatty acid synthesis (ACE-avid tumour, *bottom panel*) respectively, are evident within an individual mouse.

These results show that not only can metabolic imaging identify distinct metabolic phenotypes with different underlying tumour biology, but also imaging can identify early metabolic responses to treatment. Metabolic imaging could guide therapy in lung cancer, where spatial and temporal heterogeneity is likely to play an important role in treatment resistance.

Rapid assessment of tumour metabolic response using sodium iodide symporter (NIS): a radionuclide imaging reporter gene

One of the major challenges in developing cancer treatments is using cancer models that are predictive of drug efficacy in patients. The sensitivity of cancer cells to treatments that target metabolic pathways in particular, is dependent on complex microenvironmental features, which are difficult, if not impossible to accurately recapitulate *in vitro*. *In vivo* cancer models such as genetically engineered mice recapitulate the genetic and metabolic heterogeneity observed in lung cancer but are an expensive and low throughput technology for rapid assessment of drug efficacy.

We have, therefore, explored the use of NIS (sodium iodide symporter protein) as a radionuclide imaging reporter gene to provide a direct and rapid readout of tumour cell response to therapy. Using clonal lines stably expressing NIS, we measured the uptake of radioactive NIS substrates (i.e. [^{18}F]tetrafluoroborate (TFB) and [^{99}mTc]pertechnetate) following treatments targeting glycolysis and oxidative phosphorylation (2-deoxyglucose and oligomycin A, respectively) - both key pathways utilised by tumour cells to regenerate ATP. We were able to demonstrate a rapid (within 30 minutes) decrease in uptake in the treated NIS-expressing cells when compared to controls. This robust effect was measurable in advance of the decrease in cell viability or number. Mechanistically, the above phenomenon is the result of the direct dependence of NIS activity on the sodium gradient across the plasma membrane, generated by Na^+/K^+ ATPase in an ATP-dependent manner.

We further developed NIS in a novel lentiviral vector (LV-PGKCre-EF1StrawNIS) for *in vivo* transduction of somatic cells, allowing imaging of spontaneous lung tumourigenesis. This vector delivered - through intranasal administration - multiple transgenic elements to somatic cells of adult mice with conditional (floxed) oncogenic Kras (LSL-Kras$^{G12D/+}$) and *p53*$^{fl/fl}$ alleles. Mice were longitudinally imaged using ([^{18}F]TFB PET and $^{99m}TcO_4^-$ SPECT). Lesions down to tens of nanolitres could be imaged repeatedly. Imaging following drug therapy identified single initiating lesions with significantly reduced $^{99m}TcO_4^-$ SPECT uptake and high cleaved caspase 3 (CC3) staining, showing that imaging was identifying true responders.

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. This vector comprises a new platform technology and provides a quantitative, 4D readout of cell viability, critical for rapid monitoring of therapeutic efficacy and identifying responders within a heterogeneous tumour.

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MIGRATION, INVASION AND METASTASIS

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Cell migration, mechanosensing and actin cytoskeletal dynamics impact on tumour progression and spread, both in tumour cells and in the surrounding stromal cells. Our group uses molecular cell biology, advanced imaging and mouse models to elucidate how stresses and signals in the tumour microenvironment, such as matrix stiffness, lack of nutrients and gradients of signalling molecules, drive metastatic progression. Using bioengineering, we are also modelling the tumour microenvironment, to recreate the metastatic niche so that its complexity can be better dissected. We currently focus mainly on pancreatic cancer, which is known for its stiff stroma and aggressive metastatic behaviour.

The tumour microenvironment is increasingly understood to be a crucial driver of how cancer evolves and spreads. In general, the cancer microenvironment is a place of stresses and extreme conditions; tumours resemble tissues, but with normal controls deranged, leading to aberrant behaviours. These stresses drive reprogramming of tumour cells at many levels - leading to metastatic dissemination.

A main focus of our group has been to identify drivers of pancreatic cancer dissemination and discover mechanisms by which they promote tumour spread. We focused on the actin nucleating protein N-WASP, a well-known organiser of actin, Arp2/3 complex, Rac1 and Cdc42 in invadopodia. Amelie Juin showed that loss of N-WASP in pancreas opposed metastatic spread in a preclinical model of metastatic pancreatic cancer based on expression of $KRas^{G12D}$ and $p53^{R172H}$ using pancreas-specific Pdx-1::Cre (Juin *et al.*, Dev. Cell, 2019). She went on to discover that the role of N-WASP in pancreatic cancer spread involved a novel role of N-WASP in trafficking the major lysophosphatidic acid receptor LPAR1 back to the plasma membrane after internalisation during chemotactic signalling. Loss of N-WASP in tumour cells greatly reduced their ability to chemotax toward serum and the signalling lipid lysophosphatidic acid (LPA).

The trafficking loop with LPA was further probed using GFP-trap and mass spectrometry analysis, which revealed that the sorting nexin SNX18 was a major binding partner of N-WASP. Further study showed a crucial role of SNX18 in LPAR1 recycling via N-WASP-mediated tubulo-vesicular route through the Rab11 recycling compartment. This trafficking loop is important for chemotactic migration toward LPA and also for seeding of metastatic nodules in the peritoneal cavity. Peritoneal ascites fluid is a rich source of LPA and pancreatic cancer cells showed dependence on LPAR1 for metastatic seeding in an intraperitoneal model. Furthermore, LPA signalling via LPAR1 is linked to activation of RhoA and regulation of contractile function necessary for remodelling of the extracellular matrix during tumour cell invasion. Our study (Juin *et al.*, Dev. Cell, 2019) highlighted the important role of N-WASP in trafficking chemotactic receptors such as LPAR1 as major motivators of invasive migration of cancer cells out of tumours.

Our studies of the CYRI-A/B (Fam49A/B) proteins also continue, with Jamie Whitelaw, Nikolaou and Anh Le. We have explored the role of CYRI-B interaction with Rac1 further, using GFP-trap and mass spectrometry analysis of various Rac1 mutants, including $Rac1^{P29S}$, a common mutation found in melanoma (Whitelaw *et al.*, Comm. Int. Biol., 2019). We are also exploring the role of CYRI-A/B in adhesion and assembly of integrin-based adhesions as well as in pancreatic cancer.

Stresses, such as nutrient depletion and oncogenic mutations, can be major drivers of cancer aggressiveness, so we have begun to explore the crosstalk between metabolic imbalance and cell migration. Vassilis Papalazarou and Nikki Paul have investigated links between cell migration, mechanosensing and energy metabolism. Vassilis discovered that invasive migration can rewire metabolic pathways for ATP generation in cells, including polarisation of mitochondria into invasive pseudopods, production of ATP by oxidative phosphorylation and recycling of ATP by the creatine kinase/creatinine shuttle. This has interesting implications for the potential to intervene in tumour invasion and metastasis by modulation of these metabolic pathways and suggests some new avenues for future exploration. Nikki has performed a screen of metabolic regulators of actin-based motility and is uncovering new connections between key metabolic pathways and invasive cancer cell migration.

In addition to studying stresses that promote tumour cells to escape from the primary tumour, we are interested in what promotes them to settle in new niches and either grow into metastasis or remain dormant. Re-awakening of quiescent or dormant cancer cells is a major problem related to recurrence of disease after primary tumour removal and therapy. In collaboration with Leo Carlin, we have started new projects around mechanosensing and tumour dormancy, with the arrival of James Drew and Sonia Rolo. They will be studying the lung and liver metastatic niches in collaboration with Heather Spence, Savvas Nikolaou and Amelie Juin.

Elaine Ma also joined the group, studying mechanosensing in the metastatic niche and will be designing controllable niche properties for understanding metastasis and tumour dormancy. Hakem Albilasi will study the leukaemia cancer and metastatic niche, with an aim to understand how leukaemia cells change and interact with the bone marrow niche to promote spread and chemoresistance.

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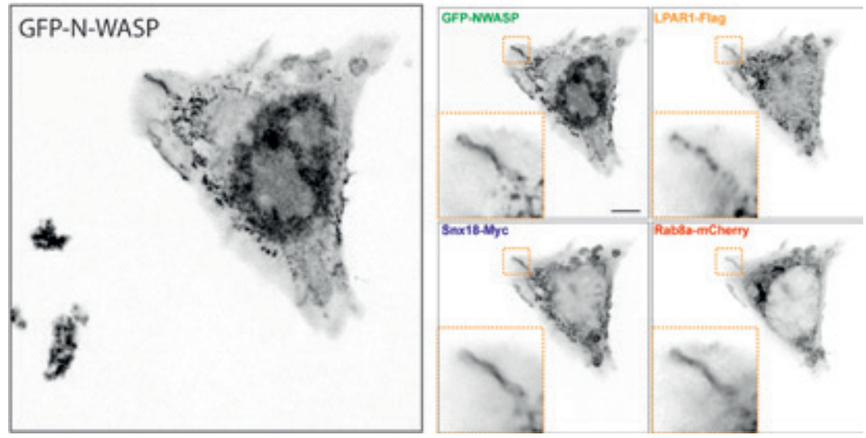

Figure 1
N-WASP localises to tubulovesicular structures in pancreatic cancer cells. The image shows co-localization of GFP-N-WASP, LPAR1, Snx18 and Rab8. LPAR1, the major receptor for LPA in these cells is trafficked through the N-WASP/SNX18 compartment marked also by Rab8 and recycled back to the plasma membrane during signaling.

Photo Credit: Amelie Juin, Dev. Cell 2019 PMID: 31668663.

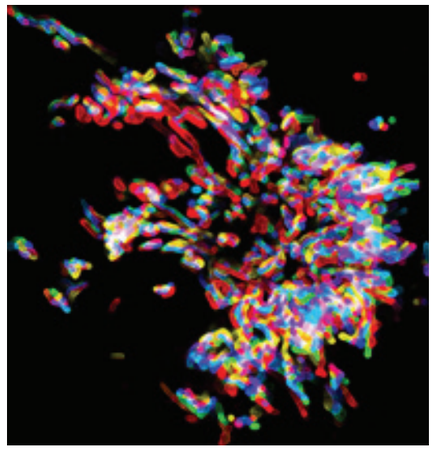

Figure 2

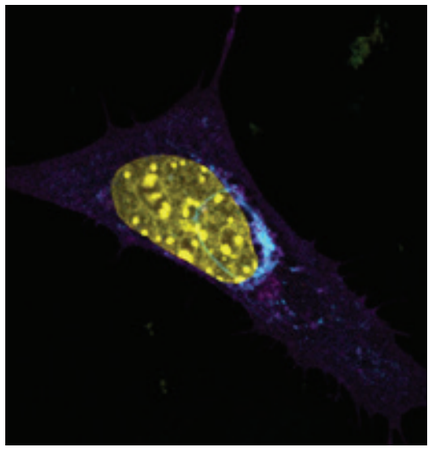

Figure 3

Figure 2
Mitochondria in a pancreatic cancer cell showing false colour to indicate depth of focus. ATP generated from mitochondria powers actin dyanmics and contractile force generation for cancer cell invasion.

Photo Credit: Vassilis Papalazarou and Nikki Paul

Figure 3
Image shows a pancreatic cancer cell expressing SNX18 (magenta), GFP-N-WASP (cyan) And DNA staining with DAPI (yellow). N-WASP co-localises with SNX18 in a tubulovesicular recycling compartment.

Photo Credit: Amelie Juin

COMPUTATIONAL BIOLOGY

Group Leader
Crispin Miller

The Computational Biology Group is focused on how information in the genome encodes the regulatory systems that control protein expression. To do this, we are applying machine learning techniques to tumour sequencing cohorts and asking how these data intersect with multi-omics and single cell sequencing data.

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?

The Computational Biology group was established with my arrival at the Beatson Institute in February 2019. We are in the process of developing our research programme, and student and postdoc recruitment is underway.

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.

A key aim of the group is to use the genomic landscape arising from these *in silico* analyses as a map onto which to project experimental datasets arising from *in vitro* studies. We have already established significant collaborations with the RNA and Translational Control in Cancer Group led by Martin Bushell, and the Advanced Technologies groups that generate many of these experimental datasets.

Rapid advances in technology are also making 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. We are working with Information Services team to establish the next generation of High-Performance Computing infrastructure that will underpin our data science efforts across the Institute.

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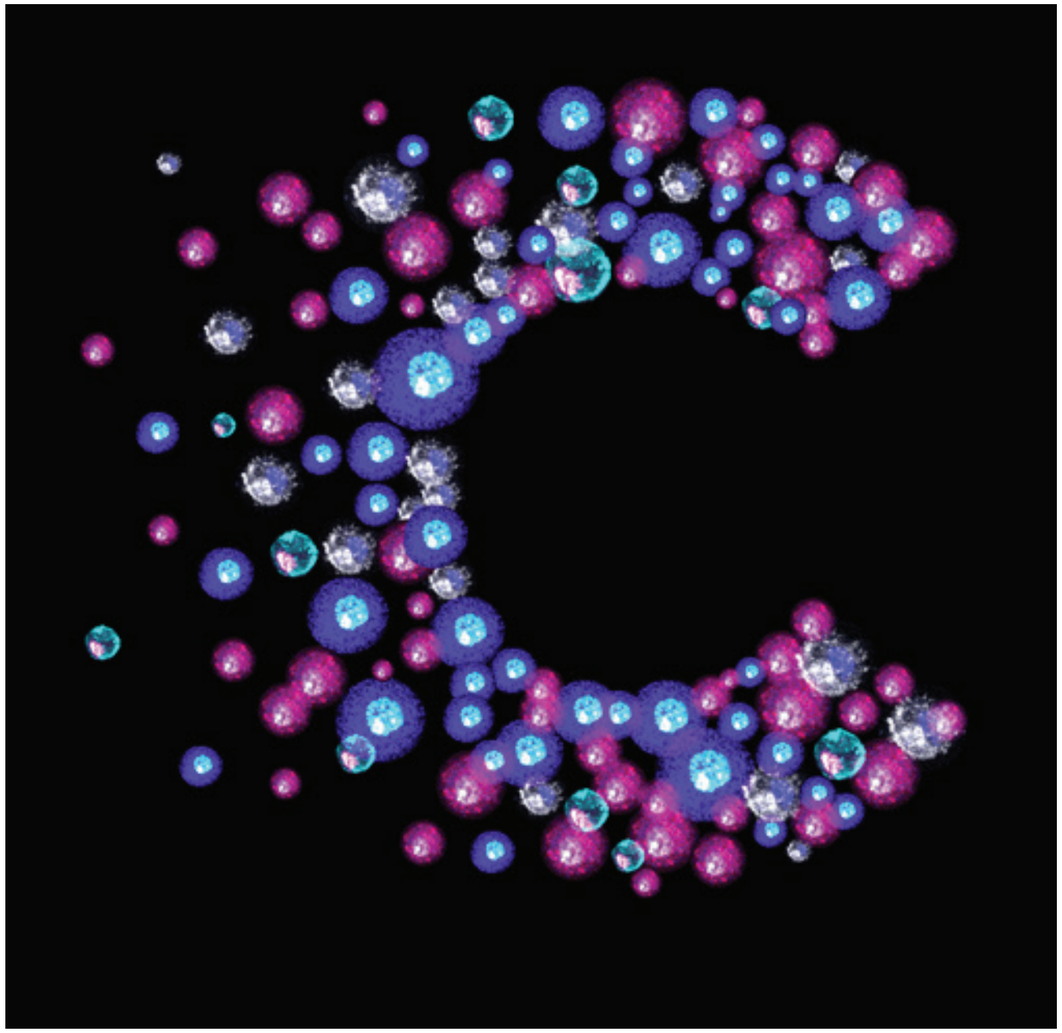

Larval immune cells from the Drosophila melanogaster. Cell nuclei are stained with DAPI and the cell membranes with an anti-NimrodC1 antibody. *Image by Jean-Philippe Parvy*

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PRECISION-PANC PRECLINICAL LAB

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The aim of our lab is to investigate how pancreatic cancer develops and progresses, so that we can identify and test new clinically relevant therapeutic approaches. To do this, we model different genetic subsets of the disease and perform preclinical trials in these models. Importantly, tumours that develop mimic human tumours in terms of genetic and transcriptomic changes, and in terms of the dense desmoplastic and immunosuppressive stroma. The lab is part of CRUK PRECISION-Panc, a multi-disciplinary network aligning pre-clinical discovery and clinical development of 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 next decade. Current therapies are largely ineffective, meaning that only 1% of patients survive 10 years following diagnosis, a situation that has remained virtually unchanged for the past 50 years.

Pancreatic cancer is almost universally driven by activating mutations in *KRAS*. During tumour progression, mutations accumulate in tumour suppressor genes, most commonly *TP53*, *SMAD4* and *CDKN2A*. However, there are other mutations found in subsets of patient tumours that could confer sensitivity to specific targeted therapies if the biological consequences of those mutations were better understood (1). For that reason, part of our work involves modelling mutations in the genes that are mutated in 5-10% of human pancreatic cancer, for example, *KDM6A* and *RNF43*. A further distinguishing feature of pancreatic ductal adenocarcinoma is the dense desmoplastic stromal microenvironment that surrounds and supports the tumour cells and can account for up to 90% of the tumour volume in the human disease. This microenvironment comprises fibroblasts, stellate cells, immune cells, blood vessels, and extracellular matrix proteins. Therefore, it is essential to investigate these aspects of tumour biology *in vivo*, in spontaneous tumours with a physiological microenvironment and immune response.

Targeting signalling downstream of KRAS

By far the most common event driving pancreatic tumourigenesis is *KRAS* mutation, and while inhibiting KRAS itself is challenging, there are druggable downstream signalling pathways. Inhibiting multiple arms of the signalling cascade downstream of RAS is likely required for any therapeutic benefit, so we have focussed on investigating the efficacy of combinatorial approaches in autochthonous models of pancreatic cancer. Because we are able to very accurately monitor tumour growth and response to therapy using high-resolution ultrasound imaging, we can conduct trials in as refined a way as possible (Figure 1, top).

One of the pathways we have focussed on downstream of KRAS is the mTOR pathway. The pathway is negatively regulated by PTEN, and although not typically mutated in pancreatic cancer, ~15–20% of human pancreatic cancers display reduced expression of PTEN, which correlates with reduced survival (2). PTEN loss rapidly accelerates KRAS-driven pancreatic tumourigenesis in mice, and renders tumours sensitive to treatment with mTORC1 inhibition. In contrast, mTORC1 inhibition offers negligible improvement in survival in KPC mice (2, 3). mTORC1 is responsible for the classical functions of mTOR, sensing nutrient stress and regulating transcription and translation, whilst the mTORC2 complex (containing RICTOR) can regulate cell metabolism through activation of Akt and SGK. Interestingly, we previously showed that *Rictor* deletion could significantly extend survival of KPC mice, and when KPC mice were treated with a dual mTORC1/2 inhibitor, survival was significantly prolonged even in mice with late-stage tumours (4). In contrast, we, and others, have observed that inhibiting the MEK/ERK pathway has no therapeutic efficacy in these mice. However, we now find that combined inhibition of MEK and

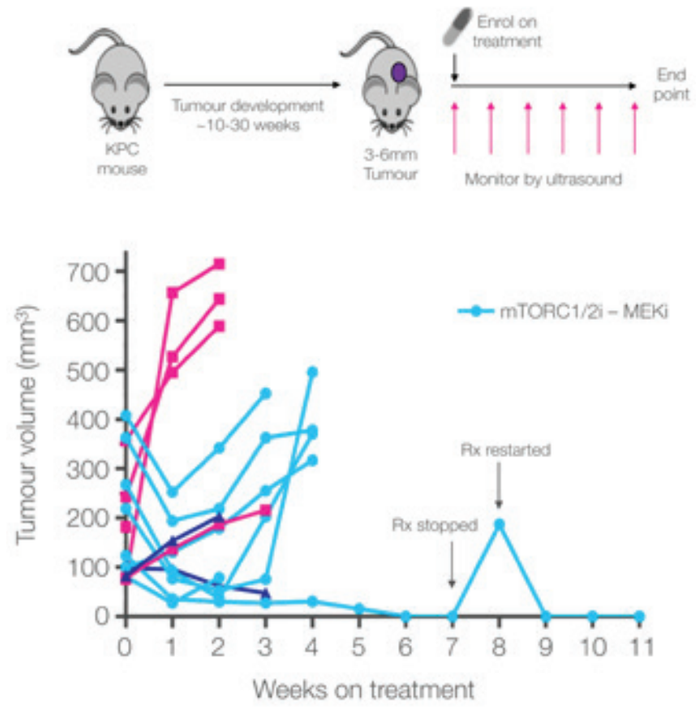

Figure 1
Top panel shows schematic of typical pre-clinical trial design. Bottom panel shows tumour volume, as assessed by high resolution ultrasound imaging, in cohorts of KPC mice treated as indicated.

mTORC1/2 has remarkable effects in pancreatic tumour bearing KPC mice, with durable responses to therapy tumour shrinkage evident in the majority of mice treated (Figure 1). We are currently investigating the resistance mechanisms acquired by these tumours, and exploring further combinations and schedules.

Targeting stromal components

The desmoplastic stroma that is a prominent feature of pancreatic cancer in mice and man is also an area of focus for our lab. Evidence has shown that all components of this stroma play an important role in pancreatic cancer progression, able to influence tumour cell proliferation, survival, metabolism, migration, immune surveillance, and response to chemotherapy (5-9). We are currently using preclinical models to better understand the key signals in the pancreatic cancer microenvironment, how they impinge on other cells in the microenvironment, and how best to modulate them for therapeutic effect. In particular, we are working to understand the role of TGFβ signalling networks in tumour - microenvironment communication, and tumour progression. In order to do this, we are using next-generation models to manipulate genes in different cellular compartments within the tumour, e.g. fibroblasts or different immune cells. This approach allows us to appreciate the complex signalling networks and cellular heterogeneity that is characteristic of this disease (Figure 2).

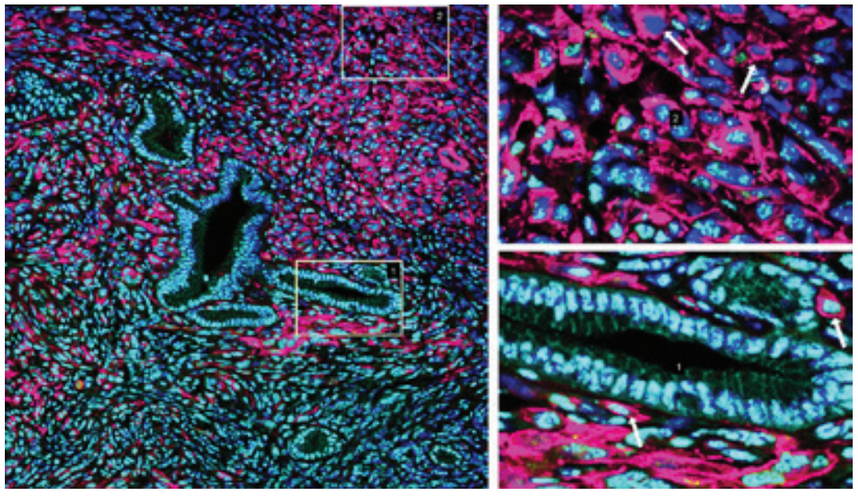

Figure 2
Immunofluorescent staining demonstrating the cellular heterogeneity in pancreatic tumours. Staining shown is for αSMA (magenta), pSmad3 (cyan) and DAPI (blue).

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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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[5]together with Owen Sansom
[6]Scottish Government Chief Scientist Office
[7]Medical Research Council Doctoral Training Programme
[8]Pancreatic Cancer UK Future Leaders Academy

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 screening 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 ccharacterised Rosa26-lsl-MYC^{DM} 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.

Additionally, we have now found that NUAK1 plays a key role in protecting cells from toxic levels of reactive oxygen species (ROS). ROS are naturally produced as by-products of mitochondrial electron transport chain activity, and the elevated metabolic demand of cancer cells can thus increase ROS production. Paradoxically, hypoxia can also elevate ROS production and is moreover a common feature of most cancers. Tumour cells cope with the threat posed by ROS in part by diverting glucose away from the mitochondria but also by increasing pathways that detoxify ROS. We have found that suppression of NUAK1 impairs this latter response, thereby exposing an intrinsic vulnerability in cancer cells. We have determined that acute inhibition of the antioxidant response pathway, via targeted suppression of NUAK1, eradicates MYC-driven adenomas in a genetically engineered mouse model of colorectal cancer. All well as providing strong evidence to support targeting NUAK1 in human colorectal cancer, this observation challenges dietary advice commonly given to patients who already have cancer, in that popular consumption of antioxidant supplements may actually benefit the cancer cells more than the patient.

Paradoxically, canonical activation of NUAK1 requires STK11 (aka LKB1), an erstwhile tumour suppressor associated with Peutz-Jehger's syndrome and a frequently mutated gene in sporadic lung cancer. Curiously, we have found that NUAK1 remains active in LKB1-deficient cells, indicating LKB1-independent mechanisms of NUAK1 regulation. Similar to AMPK, we have found that calcium signalling is required for NUAK1 activity in the absence of LKB1 and have shown that calcium-dependent activation of PKC increases NUAK1 activity. The precise mechanism of activation is as yet unclear and may involve ROS-dependent modification of NUAK1 cysteines. Notably, MYC deregulation sensitises cells to calcium-dependent signalling, in part via transcriptional regulation of multiple proteins involved in the calcium signal transduction pathway. It thus appears that MYC indirectly activates NUAK1 (and potentially other AMPK-related kinases) by enhancing cellular sensitivity to calcium.

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 lab has teamed up with respiratory physician Kevin Blyth to build an international network of clinicians and researchers with the common goal of improving patient outcomes for this dreadful disease. We have developed a new mouse model of mesothelioma that will enable us to investigate the interplay between asbestos-driven chronic inflammation and the major recurring mutations that are commonly found in human mesothelioma. Significantly, intrapleural injection of asbestos dramatically accelerates onset and severity of mesothelioma in our mice, even after homozygous deletion of 3 major tumour suppressor genes, indicating that chronic inflammation continues to contribute to mesothelioma beyond the acquisition of rate-limiting mutations. This startling observation suggests that patients may benefit from interventions that aim to reduce inflammation, in addition to those directly targeting the tumour population.

Major developments in 2019

The lab grew significantly in 2019 resulting from the successful procurement of project grants from the CRUK Early Detection of Cancer committee, the Mick Knighton Mesothelioma Research Fund/ British Lung Foundation and an industrial sponsored research agreement with Puma Biotechnologies (California, USA). We additionally welcomed an independently funded clinical lecturer, Claire Rooney. The lab published one paper in Cancers and collaborated on another in Cell Reports from the lab of Wei-Xing Zong. Katarina Gyuraszova presented our work on development of a new mouse model of mesothelioma at the IASLC World Lung Cancer conference in Barcelona, while I gave invited lectures at Genes & Cancer conference, Cambridge and the METCAN ITN workshop in Leuven, Belgium. The most exciting development of the year was the successful coordination of a new consortium to build resources for mesothelioma research, led by Kevin Blyth and working with Crispin Miller, Sam Janes, Marion MacFarlane, and many others. The consortium has been funded through a CRUK Accelerator Award and will commence work early in 2020.

Outreach activities included invited lectures at the Medical University of Vienna; Technical University of Munich; University of Lyon; Medical Centre Utrecht; and the University of Toronto. I spoke at public events marking Respiratory Health Day (Scottish Government) and Acton Mesothelioma Day (Darlington), and sat on the ICPS advisory panel for UK lung cancer screening. I presented to the CRUK Beatson board of governors and participated in a New England Journal of Medicine forum for product development. Rosa26-lsl-MYC^{DM} mice were deposited with Jaxmice to facilitate distribution to the scientific community.

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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 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.

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

1. To establish how exosomes and metabolites released by tumour cells influence extracellular matrix (ECM) deposition by carcinoma-associated fibroblasts (CAFs) in the primary tumour, and fibroblasts in metastatic target organs (such as the lung and liver) to prime metastatic niches.

2. To investigate how control of ribosome function and the protein synthesis machinery contributes to ECM production to generate oncogenic and metastatic microenvironments

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. Furthermore, a detailed picture of how the ribosome's mRNA translation machinery contributes to the generation of oncogenic and invasive microenvironments will enable us to determine which components of the translation apparatus need to be targeted to treat invasive carcinomas.

How do metabolites and exosomes released by primary tumours influence invasiveness and the priming of metastatic niches?

We have found that several factors - principally metabolites and exosomes - released by tumour cells alter the tumour microenvironment and can profoundly influence ECM deposition in organs that are distant from the primary tumour. Pancreatic adenocarcinoma (PDAC) which express the mutant p53 oncogene release exosomes to promote integrin recycling in fibroblasts which influences ECM deposition by these cells. Thus, by releasing podocalyxin-containing exosomes into the circulation, mutant p53-expressing PDAC influence the deposition of collagen in the lung and liver, and this occurs prior to establishment of metastases in these organs (Figure 1, Novo *et al.* Nat Commun 2018). We are now investigating how these exosome-induced alterations to the lung and liver microenvironments may favour metastatic seeding in these organs. In collaboration with Leo Carlin's group, we are investigating the relationship between the ECM and immune microenvironments in the lungs of PDAC-bearing animals, and how the ECM may influence lung neutrophil populations to promote metastatic seeding (Figure 1).

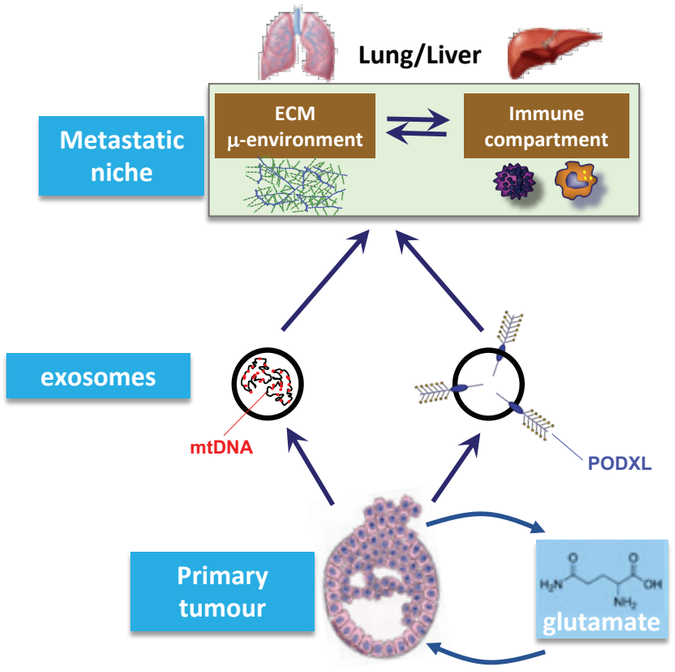

Figure 1
Proposed role of exosomes and metabolites released from primary tumours in priming metastatic niches.
We have shown that exosomes released from primary tumours alter ECM deposition within, and immune cell recruitment to, metastatic target organ (such as the lung and liver) prior to the establishment of metastasis. Exosomes from PDAC display the sialomycin, podocalyxin (PODXL), and that those released by breast cancers contain mitochondrial DNA (mtDNA). We are now investigating mechanistic relationships between these primary tumour-induced alterations to the ECM and immune landscapes in the lung, how this might influence metastatic seeding, and how exosome cargoes (PODXL and mtDNA) mediate these effects.

In breast cancer, we have shown that alterations in glutamine/glutamate metabolism lead to increased invasiveness and the generation of pro-invasive microenvironments (Dornier *et al.* Nat Commun 2017). More recently, we have found that these metabolic alterations promote interaction of mitochondria with endosomes which allows packaging of mitochondrial DNA into exosomes and its release from the cell (Figure 1). These breast tumour-derived, mitochondrial DNA-containing exosomes then influence integrin trafficking and ECM deposition in other cells via a mechanism that involves activation of toll-like receptors. In collaboration with Ed Roberts's group, we are now investigating whether exosomes-containing mitochondrial DNA, which have been released from breast cancer cells with altered metabolism, can influence tumour-antigen presentation and other immune cell functions relevant to metastasis.

How does regulation of the mRNA translation machinery contribute to ECM production to generate oncogenic and pro-invasive microenvironments?

We have previously shown that alterations to the transfer RNA (tRNA) repertoire accompanies tumour-induced fibroblast activation and that this influences ECM deposition by these cells. Indeed, CAFs express increased levels of the initiator methionine tRNA ($tRNA_i^{Met}$) and this leads to increased translation of the mRNAs for certain collagens, thus generating an ECM microenvironment which favours tumour angiogenesis and growth (Clarke *et al.* Curr Biol 2016). In collaboration with Martin Bushell's and Tom Bird's groups, we are now investigating how particular components of the translation initiation machinery influence ECM production and deposition (Figure 2). The eukaryotic initiation factor-4A family consist of 3 closely related paralogues, eIF4A1, eIF4A2 and eIF4A3. We have generated mice in which the gene eIF4A2 is conditionally deleted, and this has enabled us to show that the eIF4A2 paralogue plays an important role in the generation of ECM-rich secretory translatomes. Consistently, in CAFs, it is clear that eIF4A2 supports translation of mRNAs for an ECM-rich secretome which influences the behaviour of cancer cells (Figure 2). We are currently investigating how eIF4A2 controls translation initiation of mRNAs encoding ECM components, and how polysomes translating these mRNAs are recruited to the endoplasmic reticulum. We anticipate that these findings will enable us to develop strategies to oppose the generation of ECM microenvironments which foster tumour growth and invasiveness.

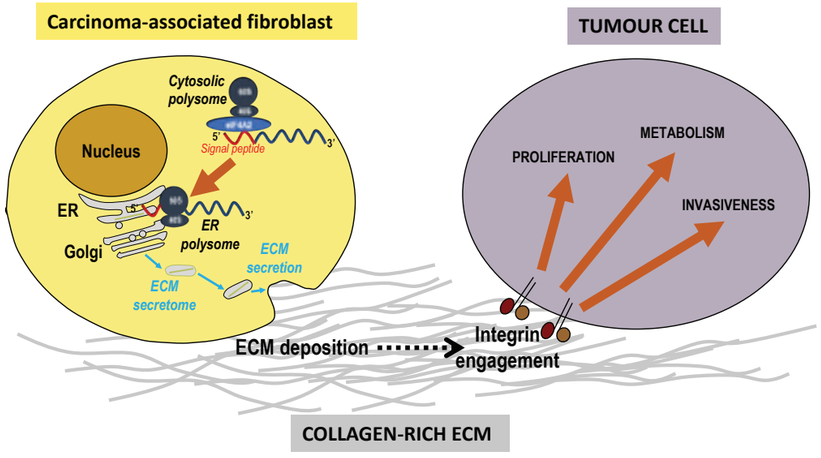

Figure 2
Regulation of the translation initiation machinery in synthesis of ECM-rich secretomes. In CAFs (left-hand cell) a component of the translation initiation machinery, eIF4A2 is required for the production of a secretome which is rich in ECM proteins. Preliminary data indicate that eiF4A2 achieves this by controlling the proper recruitment of a subset of mRNAs encoding ECM proteins to the ER. We are currently testing how the ECM secretomes commanded by eiF4A2 can drive tumour cell (right-hand cell) proliferation, metabolism and invasiveness.

www.beatson.gla.ac.uk/ed_roberts

IMMUNE PRIMING AND THE TUMOUR MICROENVIRONMENT

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In recent years tumour immunotherapy has led to dramatic patient benefits 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.

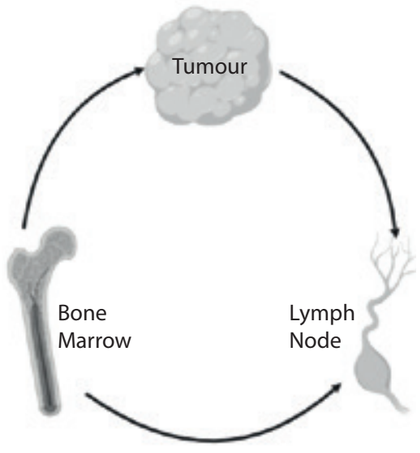


Figure 1
The DC lifecycle.
In CAFs DC precursors develop in the bone marrow and migrate to the tumour and the lymph node. Once within the tumour they sample proteins from the microenvironment and then mature and migrate to the lymph node. There the DC which migrated straight to the lymph node and those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

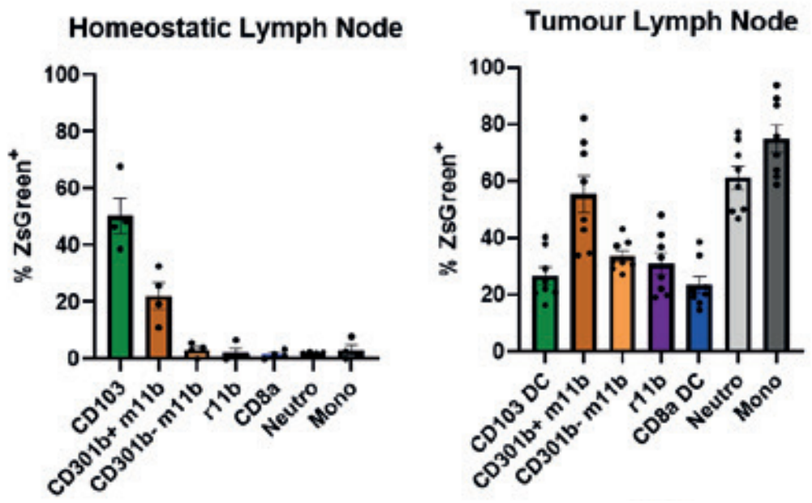
DC recruitment to the tumour

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

Antigen traffic to the lymph node

Beyond the number of DC at the tumour site, how DC carry tumour material to the lymph node, and how they distribute it, is also key to understanding how anti-tumour immune responses are generated. We have shown that the same protein, when expressed within a tumour cell, is handled differently than when expressed in normal tissue. Indeed, during normal development DC restrict these proteins and do noy 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 are investigating how protein is handled during an optimal anti-viral immune response and have generated numerous novel strains of virus to allow this to be investigated. Using these we will ask whether protein from a virus is treated like that from a tumour and whether the passing of that protein is accompanied by other unique or shared signals leading to lymph node activation. Furthermore, we have established systems to allow us to block migration of specific types of DC to investigate the roles of each subset during optimal and suboptimal settings with an aim to improving their functionality in the tumour.

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. In order to study this, 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 are now developing complementary approaches to allow identification of even more cell types within the lymph node microenvironment alongside building systems to allow robust analysis of tissue organisation. We aim to use these approaches to identify lymph node organisational defects which occur in the context of tumour development which we can further study.

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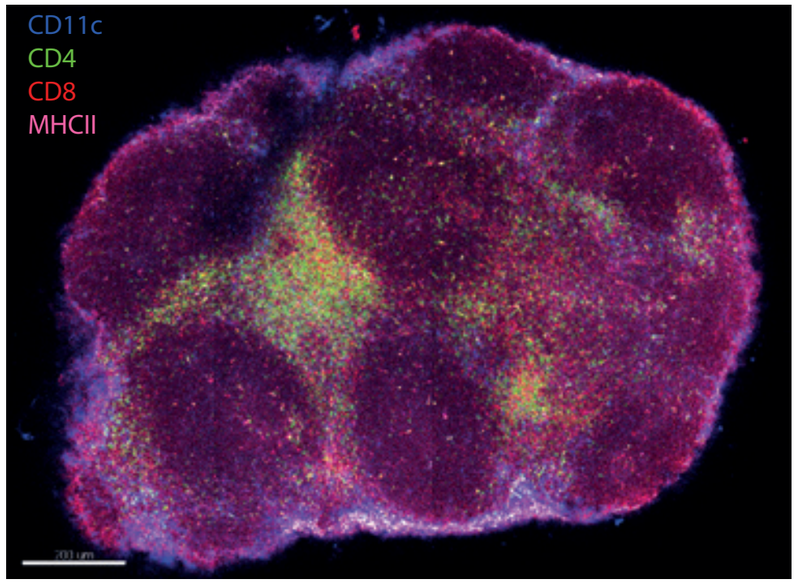
Figure 3
Lymph node organisation.
A whole cleared lymph node stained for T cell, B cell and DC markers shows the organisation of a lung tumour draining lymph node.



TUMOUR CELL DEATH

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The aim of our group is to understand the factors regulating cell viability in cancer. Since it is known that 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 both known cell death regulators as well as searching for novel proteins and pathways that control cell viability, tumour growth and chemosensitivity. We envisage that the knowledge gained from our studies will be translated and lead to the improvement of existing clinical regimens or new targets for therapeutic intervention.

Identification of new factors involving in nutrient sensing.

The ability to sense and respond to nutrients is a central facet of all living cells. Cancer cells are no different and in fact, they may rely even more heavily on nutrient sensing due to their high energetic and anabolic needs. A key component of nutrient sensing from yeast to humans is the serine/threonine kinase termed mTOR (mechanistic target of rapamycin – a natural compound that represses mTOR activity). mTOR is part of a complex called mTORC1 that responds to the presence of amino acids and growth factors. When activated mTORC1 localises to the lysosome membrane where it phosphorylates a number of substrates, which as a consequence promotes protein translation. At the same time, phosphorylation of other substrates leads to repression of a catabolic process termed macroautophagy (Figure 1). Overall, these events promote anabolism in cells and in the context of cancer tumour growth.

Macroautophagy (more often simply referred to as autophagy) is a membrane-trafficking process that delivers cellular constituents to lysosomes for degradation. The process serves to degrade cargoes such as damaged organelles or misfolded protein thereby preserving cellular integrity. As a result, autophagy is considered an important factor in cell viability, and in particular in cancer, as such there are currently multiple strategies to target autophagy to treat malignant disease.

In our previous work, we identified a factor activated by the tumour suppressor p53 that promotes autophagy. We termed this factor DRAM for the Damage-Regulated Autophagy Modulator. As we found that DRAM (now DRAM-1) is a lysosomal membrane protein, and since autophagy and mTORC1 activity are intricately linked, we questioned whether DRAM-1 may also be a regulator of mTORC1. Using DRAM-1-inducible cells or DRAM-null cells we indeed found that DRAM-1 is an important factor involved in the activation of mTORC1 in response to amino acids.

To better understand how DRAM-1 might be a contributing factor in the activation of mTORC1, we performed mass spectrometry to identify DRAM-1 interacting proteins. Amongst the proteins identified were 3 amino acid transporters – SLC1A5, SLC3A2 and SLC7A5, which had previously been implicated in the activation of mTORC1 (Nicklin et al. *Cell* 2006). Our studies further confirmed that DRAM-1 does indeed bind these transporters, leaving us with a dilemma since DRAM-1 is a lysosomal protein and the amino acid transporters are considered to be localised at the plasma membrane. Closer analysis revealed, however, that DRAM-1 facilitates the transport of a small proportion of these transporters to the lysosome membrane. Intriguingly, while we initially thought that the lysosomal localisation of these transporters might mediate amino influx into lysosomes, we found instead that they mediate amino acid efflux and that this efflux is important for the activation of mTORC1 (Beaumatin et al. 2019 *Molecular Cell*).

Ultimately our findings caused us to question how DRAM-1 facilitates localisation of amino acid transporters to the lysosome membrane. After finding that DRAM-1 brings newly synthesised transporters to lysosomes, we reasoned that DRAM-1 may affect a factor that is involved in transport from lysosomes/

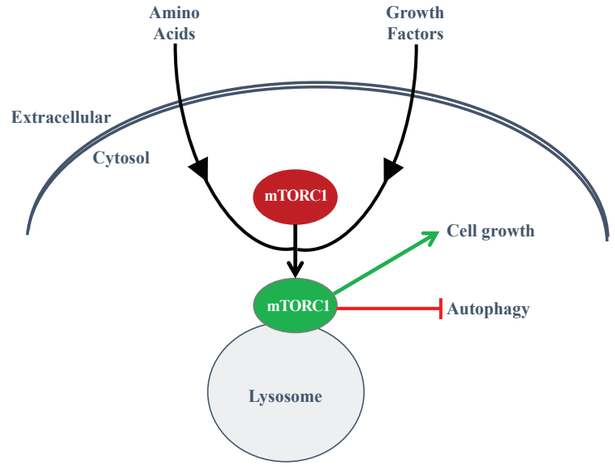


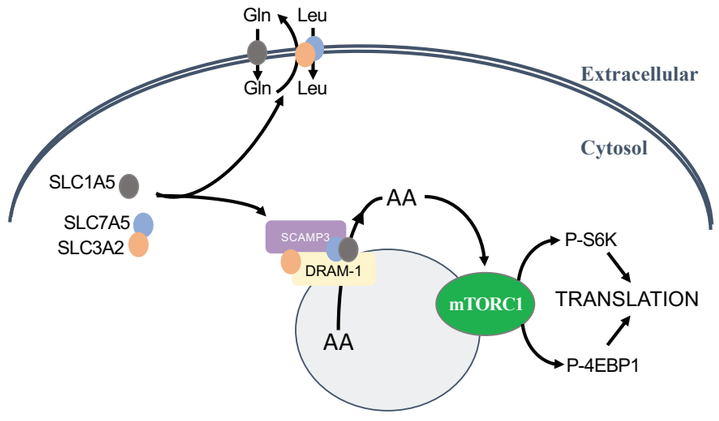
Figure 1
Activation of mTORC1 occurs at the lysosome membrane, promoting translation and repressing autophagy.
Following stimulation by growth factors and amino acids, mTORC1 localises to lysosome membranes. It is here that mTORC1 is active, causing phosphorylation events that promote protein translation and repress autophagy. mTORC1, mechanistic target of rapamycin complex 1.

endosomes to the plasma membrane. Through reassessment of the DRAM-1 binding factors identified by mass spectrometry, we noted that DRAM-1 interacts with SCAMP-3 (secretory carrier-associated membrane protein 3), which mediates many cellular trafficking events, including traffic to the plasma membrane. We confirmed that DRAM-1 binds to SCAMP3 and found that its depletion impairs DRAM1's ability to localise amino acid transporters at the lysosomes membrane (Figure 2). Moreover, in line with an involvement of this process in the sensing of amino acids, we also found that knockdown of SCAMP3 impaired activation of mTORC1 in response to amino acids. These studies therefore highlight two new components - DRAM-1 and SCAMP3 – of this important cellular response that is central to cellular growth.

DRAM-1 contributes to glucose tolerance.

Previous studies have shown that changes in mTORC1 activity can affect insulin signalling. This caused us to consider whether changes in DRAM-1 may also affect this process. To test this, we treated wild-type and DRAM1-null cells with insulin and found that loss of DRAM-1 caused increased and sustained AKT phosphorylation – a readout of insulin signalling. As a result, we were intrigued to know if loss of DRAM-1 affected glucose and insulin tolerance *in vivo*. As differences in glucose and insulin tolerance can become more acute in response to certain dietary states that affect metabolism, we fed wild-type and DRAM1-null mice a high-fat diet. Under these conditions, we found that mice lacking DRAM-1 were able to mitigate glycaemic stress at a faster rate that wild-type animals. Moreover, in line with the mechanistic observations we had made *in vitro*, we found that rapamycin caused insulin insensitivity in wild-type mice, whereas in contrast, DRAM-1 mice were unaffected by rapamycin treatment, indicating that mTORC1 activity is already impaired in these animals. A role in glucose and insulin tolerance therefore identifies DRAM-1 as a factor connecting cellular signalling from diet, insulin and obesity – factors which are becoming increasingly linked in populations around the world.

Figure 2
DRAM-1 and SCAMP3 facilitate the activation of mTORC1.
DRAM-1 binds to SCAMP3 and directs a proportion of SLC1A5, SLC3A2 and SLC7A5 to lysosome membranes. This facilitates amino acid efflux from lysosomes, which aids full activation of mTORC1. mTORC1, mechanistic target of rapamycin complex 1; AA, amino acids; DRAM-1, damage-regulated autophagy modulator-1; SCAMP-3, secretory carrier-associated membrane protein 3; P-S6K, phospho-ribosomal protein S6 kinase B1; P-4EBP1, phospho-eukaryotic translation initiation factor 4E binding protein 1; Gln, glutamine; Leu, leucine.



Identification of autophagy regulators in pancreatic cancer.

Pancreatic ductal adenocarcinoma (PDAC) is currently a cancer of significant unmet clinical need. There is now considerable evidence that certain pancreatic cancers are dependent on autophagy. So we were interested to search for cellular factors that might be required for autophagy and by association cell survival in this tumour type. To this end, a partnership was established by Cancer Research Technology (now Cancer Research UK Commercial Partnerships) that involved our lab, the lab headed by Dr. Sharon Tooze at the Francis Crick Institute and Astellas Pharma Inc. As part of this collaboration, a genome-wide RNAi screen was undertaken to identify promoters or at least factors required for successful autophagy. A number of hits were identified, and two were fully validated, MD1 and MPP7, which we found to be important for successful autophagy and PDAC growth/survival (New et al. *Can Res.* 2019). It is hoped that these and other factors of the screen may prove to be tractable new targets for the inhibition of autophagy in PDAC as well as other tumour types.

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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, 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, response to therapy, and metastasis. Our overarching goals are to identify early-stage diagnostic biomarkers and develop stage- and subtype-specific targeted therapies.

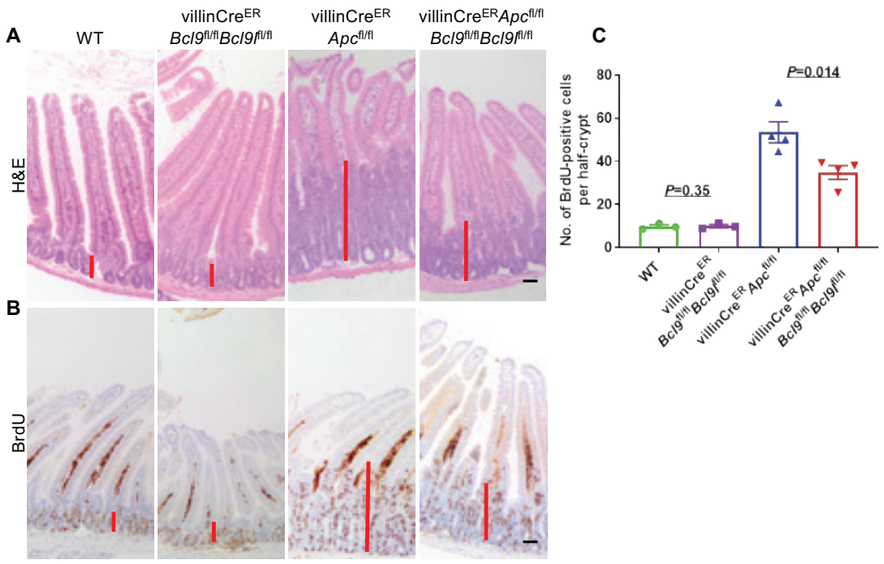

Figure 1
Bcl9/9l are required for acute intestinal transformation driven by *Apc* deficiency.
A) Small intestinal tissue from mice with indicated genotypes sampled 4 days post Cre-induction and stained with haematoxylin and eosin (H&E).
B) BrdU immunohistochemistry highlighting proliferating cells in small intestinal tissues from (A). Red bars indicate the proliferative crypt compartment. Scale bars, 50 µm.
C) Cell proliferation, quantified by BrdU incorporation, in small intestinal tissues from (A).

Inactivation of the APC tumour suppressor is a key initiating event in intestinal adenoma formation, with progression to carcinoma underpinned by the stepwise accumulation of mutations in oncogenes and tumour suppressors such as *KRAS*, *PIK3CA*, and *TP53*. Loss-of-function mutations in APC—an integral component of the destruction complex that targets β-catenin for degradation—lead to the aberrant accumulation of nuclear β-catenin and hyperactivation of pro-proliferative Wnt signalling, a hallmark of human CRC. However, targeting oncogenic Wnt signalling has proven controversial since dampening Wnt activity may allow tumours to form in regions of the intestine where the "just-right" sub-maximal threshold of Wnt signalling provides fertile ground for tumour growth. Moreover, on-target inhibition of Wnt/β-catenin signalling may incur collateral toxicity given the pivotal roles of this pathway in the regulation of stem cell self-renewal both in the intestine and other organs. With the aim of identifying a therapeutic window for inhibiting oncogenic Wnt-signalling, we have delineated key components of the pathway in normal and APC-deficient intestinal stem cells (ISCs) and ascertained differences in the transformed and homeostatic settings.

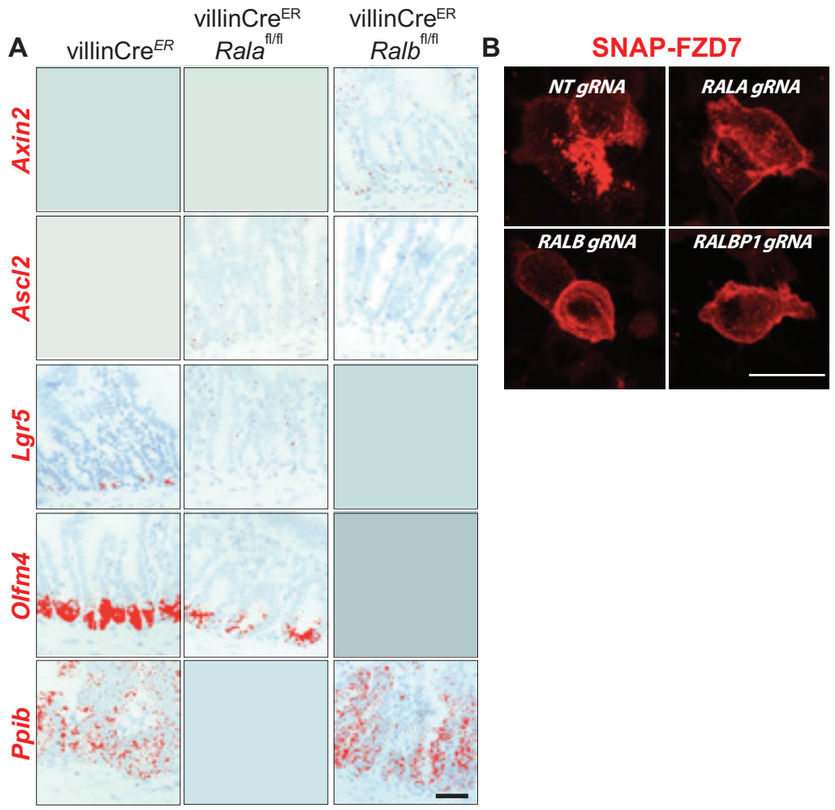

Figure 2
RAL GTPases are required for efficient activation of Wnt signalling.
A) RNA in situ hybridisation (RNAscope) for Wnt-target genes and/or ISC-markers (*Axin2, Ascl2, Lgr5, and Olfm4*), and the positive control *Ppib*, in villinCre^{ER}, villinCre^{ER} *Rala*$^{fl/fl}$, and villinCre^{ER} *Ralb*$^{fl/fl}$ intestinal tissue 4 days post Cre-induction. Scale bar, 50 µm.
B) Confocal imaging of fluorophore-labelled SNAP-tag Frizzled-7 (SNAP-FZD7; red) in HEK293T cells 10 minutes after serum stimulation. The subcellular localisation of Frizzled-7 was assessed in HEK293T cells, depleted of *RALA*, *RALB*, or their common effector *RALBP1* via CRISPR/Cas9 gene editing, and compared to cells expressing non-targeting (NT) gRNA. Scale bar, 5 µm.

Targeting Wnt-transcriptional machinery in APC-deficient cells

During homeostasis, the Wnt-signalling cascade is engaged by the binding of secreted Wnt ligands to their cognate receptors. However, the vast majority of CRCs harbour *APC* mutations and are, therefore, Wnt ligand-independent, with the corollary that strategies to interfere with this cell-autonomous Wnt signalling must perturb β-catenin transcriptional output. We, therefore, examined the requirement of BCL9/9L—two integral components of the Wnt enhanceosome—for intestinal homeostasis, regeneration, and transformation following *Apc* loss. While these genes are required for the maintenance of *Lgr5*$^{+}$ ISCs and post-injury regeneration, Bcl9/9l deletion does not impact intestinal homeostasis. Notably, *Bcl9/9l* deletion attenuates the proliferative burst associated with acute *APC* loss (Figure 1), suppresses the oncogenic Wnt-transcriptional output, and abrogates tumour growth both in the colon and liver, suggesting an opportunity for therapeutic intervention in Wnt ligand-independent tumours. Unexpectedly, however, *Bcl9/9l* deletion favours adenoma formation in the proximal small intestine (Gay *et al.*, *Nat Commun* 2019; 10: 723). Together, these findings support the notion that

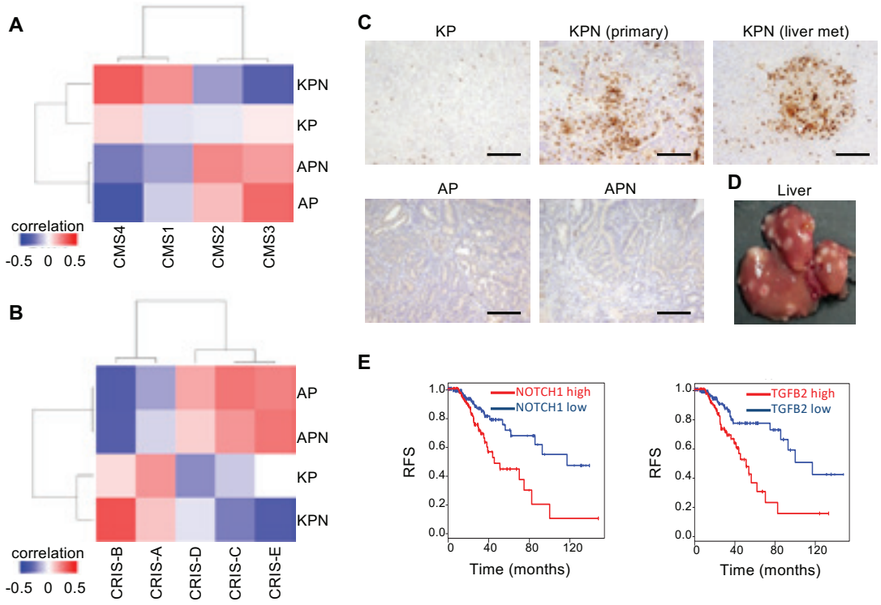

Figure 3
Epithelial NOTCH1 drives poor prognosis signatures in CRC and controls neutrophil recruitment to drive metastasis.
Heatmaps showing expression correlation of our intestinal cancer models with:
A) the consensus molecular subtypes (CMS), and
B) the CRC-intrinsic subtypes (CRIS). Blue: lower correlation; Red: higher correlation. KP: villinCreER KrasG12D/+ Trp53fl/fl; KPN: villinCreER KrasG12D/+ Trp53fl/fl Rosa26N1icd/+; AP: villinCreER Apcfl/+ Trp53fl/fl; APN: villinCreER Apcfl/+ Trp53fl/fl Rosa26N1icd/+ [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.
E) Recurrence-free survival (RFS) of CRC patients, stratified using the expression of canonical NOTCH1-target genes (left panel) or TGFB2 (right panel). Blue line: expression ≤ median score (low); Red line: expression > median score (high).

locoregional "just-right" levels of Wnt signalling influence tumour formation and distribution along the intestinal tract, and underscore the need to target Wnt-driven programs selectively activated in transformed cells.

Inhibiting Wnt signalling in a Wnt-proficient setting

Alongside *APC* loss, CRCs are commonly driven by oncogenic KRAS. In collaboration with Julia Cordero (University of Glasgow), we identified RAL GTPases—downstream KRAS effectors—as evolutionarily conserved regulators of Wnt signalling and ISC function in flies and mice. Deletion of *Drosophila Rala*, or either of the mouse orthologues (*Rala* or *Ralb*), compromised ISC function in homeostasis (Figure 2A) and drastically impaired regenerative capacity post-damage. Mechanistically, we found that RAL GTPases potentiate Wnt signalling by facilitating the internalisation of the Wnt-receptor, Frizzled-7 (Figure 2B), which suggests that Wnt-receptor endocytosis may represent a rational therapeutic target for Wnt ligand-dependent tumours harbouring *KRAS* mutations (Johansson *et al.*, *Cell Stem Cell* 2019; 24: 592–607.e7). Notably, however, targeting this node would be ineffective in tumours with cell-autonomous activation of the Wnt pathway. Our ongoing research is defining druggable nodes in the crosstalk between the RAS- and Wnt-signalling pathways.

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 (Figure 3E). 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). Going forward, we will assess how epithelial tumour cell-intrinsic signalling rewires the tumour microenvironment and identify key stromal determinants of immune evasion.

Collaborations— "more than the sum of their parts"

Our group is leading a Europe-wide, CRUK-funded consortium of basic, translational, and clinical scientists (ACRCelerate—Colorectal Cancer Stratified Medicine Network) to identify new therapeutic targets for the different CRC subtypes and deliver molecular insights with the potential to inform clinical decision-making and patient stratification. In November 2019, we were delighted to host the first ACRCelerate Workshop which provided an opportunity for researchers to present cutting-edge results, cement collaborative links and exchange ideas, while setting new milestones and forging a path for future directions.

Working within the "Rosetta" CRUK Grand Challenge consortium, we are examining how to therapeutically exploit the altered metabolic dependencies of oncogene-addicted CRCs, and how distinct tumour metabolic profiles can facilitate immune escape and confer resistance to standard-of-care therapies. We also continue to partake in the CRUK-funded "SpecifiCancer" Grand Challenge, wherein we are employing transcriptional, epigenetic, and proteomic approaches to understand tissue- and cell type-specific differences in the response to oncogenic Wnt-signalling, in order to decipher tumour-type specific vulnerabilities.

We are also partnering with leading industry innovators to accelerate the path from bench to bedside. As part of the CRT/Celgene Translational Alliance, we are investigating how changes in the translational control of gene expression impact oncogenic transformation and tumour progression. Using our suite of preclinical models, we are evaluating emerging therapeutics that target the selectivity and efficiency of translational machinery in transformed cells. We are also continuing to work closely with Novartis towards the development of novel KRAS inhibitors, which is especially important given that *KRAS* mutations confer resistance to anti-EGFR therapies currently used in the clinic.
Overall, these approaches will inform our understanding of CRC pathogenesis and metastatic competence, and provide a platform for the identification of early dissemination markers and the development of novel stage- and subtype-specific therapies.

Publications listed on page 101

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) 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 (Figure 1). 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. 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 begun exploring 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 (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. Ongoing work aims to understand how venetoclax mediates these effects and define whether these affect its cell killing ability.

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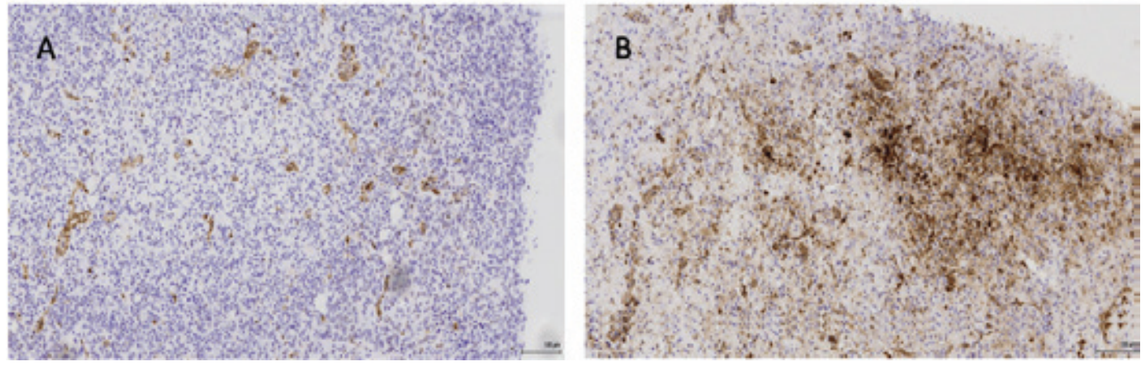

Figure 1
BH3-mimetic killing of glioblastoma in primary patient tumour samples
Consecutive 300µm thick slices of human glioblastoma tissue, cultured directly after surgical resection in neurobasal media were treated with either DMSO **(A)** or a combination treatment of BH3-mimetics (BCLxL inhibitor A1331852 and S-63845 at 2µM) **(B)** for 72 hours. To read out apoptosis, samples were stained for cleaved caspase-3 (brown stain). A, scattered cleaved caspase 3 positive areas, especially in the vicinity of intratumoural vessels B,Tissue after combination treatment with resident and vivid intratumoural vessels and immune cells. Dual inhibition of Bcl-xL and Mcl1 significantly reduces the amount of tumour cells by inducing apoptosis as indicated by the substantial increase in cleaved Caspase 3 (D).

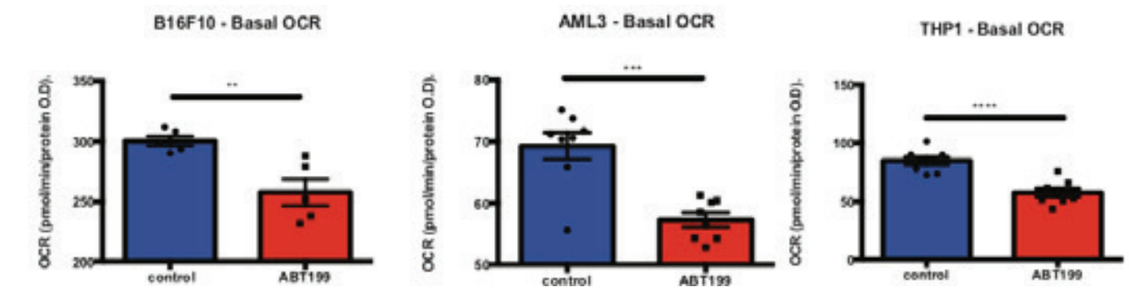

Figure 2
Bcl-2 targeting BH3-mimetic venetoclax supresses oxygen consumption.
Denoted cell lines were treated with venetoclax (ABT-199, 1µM) for 24 hours after which basal cellular oxygen consumption was measured by Seahorse analysis. In all cell types, venetoclax supressed cellular oxygen consumption. Error bars represent the SEM derived from three independent experiments.

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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. 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 a few rows of hepatocytes surrounding the central vein, which express high levels of glutamine synthetase. This enzyme has a high affinity for ammonia, and fixes it into the non-toxic glutamine, which is finally returned into circulation.

In liver tumours this metabolic zonation is disrupted. Liver tumours such as hepatoblastoma and hepatocellular carcinoma (HCC) with an overactive WNT/β-catenin signalling pathway show a widespread and sustained GS expression.

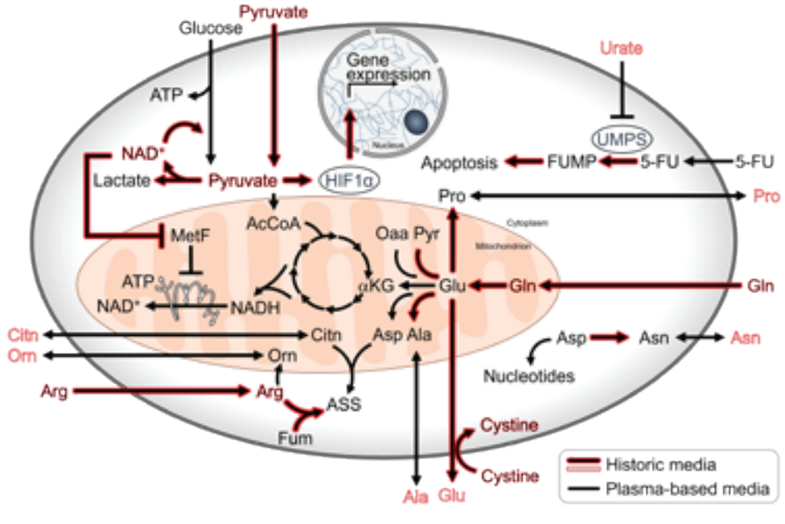

Figure 1
A schematic snapshot of the metabolism of cancer cells cultured in historic, or in physiological media. Red arrows and text indicate reactions or metabolite levels enhanced in historic media, e.g. DMEM. Clear text indicates nutrients and metabolites not present in DMEM.

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. In 2020, it will be available for biomedical research at https://ximbio.com/reagent/156371/plasmaxsuptm-sup-cell-culture-medium#datasheet

Figure 3
Human liver (HepG2) and brain-derived (LN18) cancer cells grown in DMEM and Plasmax™ both supplemented with 2.5% dialysed FBS. (A) At the indicated times live cells were stained with the fluorescent probe calcein-AM. Representative images are shown in pseudocolor, objective magnification 2.5x. (B) HepG2 and LN18 cells cultured in Plasmax™ grow at a faster rate than in DMEM.

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.

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, their response to environment, epigenotype and genotype, 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 far apart 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 (Figure 1).

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™ will be available at Ximbio.com, and 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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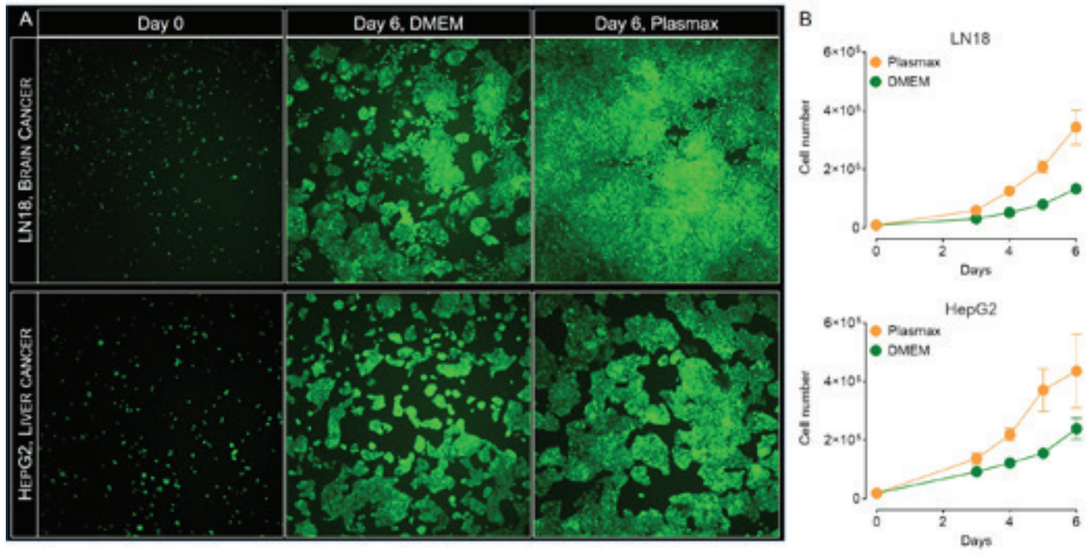

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MATHEMATICAL MODELS OF METABOLISM

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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 constrains, 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.

Is formate a cancer biomarker?

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. The inactivation of formate production in cancer cells inhibits their growth in cell cultures and *in vivo*. Pharmacological inhibitors of formate production are currently under investigation for cancer treatment.

We have demonstrated 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). We have observed formate overflow in mouse models of cancer (Meiser *et al.*, Nat Commun 2018, 9:1368). It manifested as an increase in the blood formate levels of tumour-bearing mice relative to healthy controls of the same strain.

This evidence brought us to hypothesise that blood formate levels should be higher in human cancer patients than in healthy controls. To test this, we established a collaboration with Javier Menendez (Catalan Institute of Oncology) and the METTEN study. We quantified blood formate levels in a group of breast cancer patients, lung cancer patients and healthy controls. To our surprise, blood formate levels where significantly different between the cancer patients and the healthy controls, but other than expected (Pietzke *et al.*, Cancer & metabolism 2019, 7:3). We observed lower blood formate levels in the human cancer patients in comparison to the healthy controls (Figure 1). Formate levels in the cancer patients were low to the extent that they could be used to separate them from healthy controls with high confidence.

The METTEN study was investigating the anti-diabetic drug metformin as an adjuvant to current breast cancer treatment. It also included samples from obese individuals, with or without a diagnosis of diabetes, as additional controls. We observed that obese individuals, similar to cancer patients, have low levels of blood formate relative to healthy controls (Figure 1). This means that obesity is a confounding factor when using formate to stratify cancer patients from non-cancer individuals. Fortunately, we can use glucose levels to separate obese individuals from non-obese individuals. We developed a classifier for cancer patients, obese individuals and healthy individuals using blood glucose and formate levels as input. In a cross-validation analysis the classifier exhibited a true positive rate of 87% and a false positive rate 8%, which are comparable rates for those reported for mammograms.

This investigation opens a number of questions: Can we say that human cancer patients have hypoforminaemia?

Should cancer patients receive formate supplementation?

Why are formate levels low in human cancer patients but high in tumour-bearing mice?

Do we need to include formate in the calculations of reference intake values?

Answering these questions will determine the relevance of formate in the context of early detection and cancer risk.

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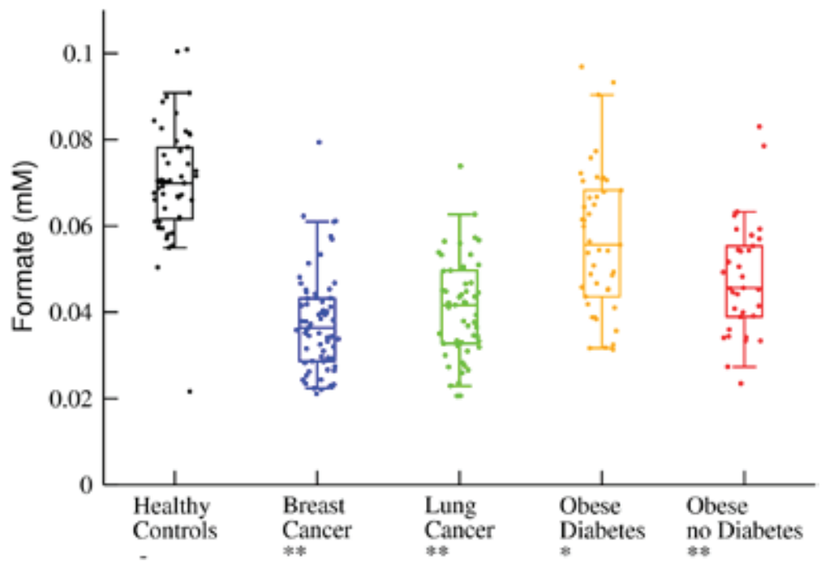

Figure 1
Blood formate levels across healthy humans, cancer patients and obese individuals. Obese individuals are further stratified as being diagnosed with diabetes (+) or not (-). An asterisk/double-asterisk denotes a significant difference of $10^{-3}/10^{-6}$ relative to healthy controls, two-tailed t test with unequal variance.

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TUMOUR MICROENVIRONMENT AND PROTEOMICS

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In solid tumours, cancer cells are embedded within a stroma populated by different cell types. Cancer associated fibroblasts (CAFs) are a major non-neoplastic stromal cell population, which our lab and other groups have shown play crucial roles in cancer progression. In fact, CAFs have a unique ability to establish crosstalk signalling with cancer cells and other stromal cells by secreting extracellular matrix (ECM) components, modifiers, soluble factors, and physically interacting with surrounding cells. Thus, our research focuses on CAFs; we envisage that targeting CAFs rather than, or in combination with, cancer cells is a promising strategy to hamper cancer growth and metastasis.

Our research primarily focuses on the role of CAFs (Santi *et al.*, Proteomics 2018) in breast and high-grade serous (HGS) ovarian cancers. These tumours contain a sizeable proportion of stroma, which is densely populated by CAFs, which have been shown to play active roles in the progression of both diseases. Importantly, ovarian cancer 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 this tumour type. We aim to decipher how CAFs contribute to tumour progression and metastasis; our overarching goal is to determine strategies to target these cells 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 contribute to cancer progression. CAF have also been shown to secrete extracellular vesicles (EVs) whereby their cargoes can support tumour progression by influencing cancer cell behaviour.

In order to understand how to target CAFs in tumours, it is crucial that we understand 1) the mechanisms that lead and sustain CAF activation, 2) how CAFs alter the tumour microenvironment and 3) how the surrounding stromal and cancer cells react to these changes. Our group has a strong expertise in mass spectrometry (MS)-based proteomics (van den Biggelaar *et al.* Blood 2014; Patella *et al.* Mol Cell Proteomics 2015; Diaz *et al.* J Cell Sci 2017, Hernandez-Fernaud, Ruengeler *et al.* Nat Commun 2017, van der Reest, Lilla *et al.* Nat Commun 2018), and we integrate this innovative technology in our research to tackle the above questions and provide new levels of understanding of CAF biology.

CAFs-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 (Figure 1). Our group has previously shown that CAFs secrete proteins, such as CLIC3, that influence endothelial cell behaviour and that this affects blood vessel growth and functionality (Hernandez-Fernaud, Ruengeler *et al.* Nat Commun 2017, Kugeratski *et al.* Science Signaling 2019, Reid *et al.* EMBO J. 2017). 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 on-going work has identified another way through which CAFs can influence EC behaviour, which is through the transfer of functional proteins mediated by extracellular vesicles. We are currently investigating this process and its impacts on breast cancer progression.

CAF & metabolism

It is well established that metabolic alterations are a hallmark of cancer. While a lot of effort has been devoted to understanding the metabolism of cancer cells, the knowledge of the metabolism of stromal cells is lagging behind. Several works have shown that cancer cells can hijack CAF metabolism by inducing CAFs to secrete metabolites that are necessary for their own growth, particularly under nutrient-limiting conditions. We asked whether CAF metabolism could regulate also other CAF functions important for tumours to progress. We have exploited our MS-phosphoproteomic platform to identify signalling pathways altered in CAFs upon activation and found strong regulation of key, rate limiting metabolic enzymes. On-going work shows that this metabolic regulation is important to support the increased production of ECM proteins, which is a hallmark of CAFs. We are investigating this mechanism further because the ECM produced by CAFs plays an active role in promoting tumour invasion and metastatic spread and we are assessing the potential of targeting CAF metabolism in this context.

News

This year, Doug Cartwright has joined our team to do his PhD. Doug is the first clinical research fellow to join our team and he is working on targetable tumour-stroma interactions in HGS ovarian cancer. Emily Kay has finished her PhD and successfully defended her thesis. This year Emily also presented her work as oral presentation at the Keystone Meeting "Cancer Metastasis: The Role of Metabolism, Immunity and the Microenvironment".

Publications listed on page 104

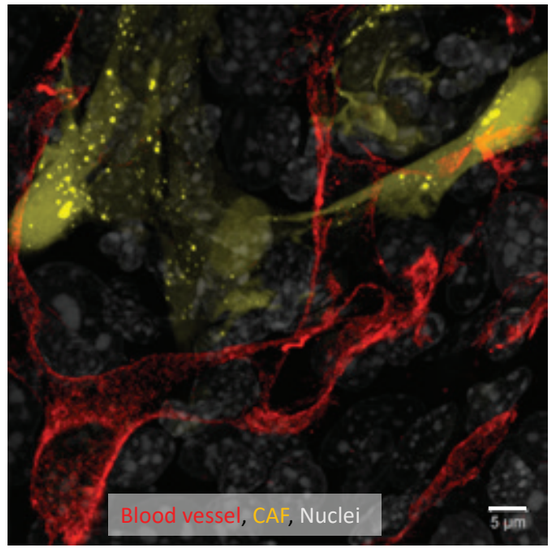

Figure 1
Lung metastasis generated by 4T1 breast cancer cells.
The image captures intra-tumoural blood vessels (red) and shows that CAFs (yellow) localise very closely to and interact with endothelial cells (red). In grey are shown the nuclei. Unstained cells are cancer cells.

Image by Alice Santi.

DRUG DISCOVERY

DRUG DISCOVERY UNIT

Co-Leads

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Heather McKinnon

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Biology
Anna Estevan Barber
Amy Bryson
Jonathan Clark
Daniel Croft
Sebastian Greenhough
Luke Hutchnison
Nicholas Johnson
Craig Mackay
Laura McDonald
Heather McKinnon
Mokdad Mezna
Francesca Pellicano
Nicholas Rooney
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Structural Sciences
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Marta Klejnot
Jennifer Konczal
Camila Pasquali
Katie Pollock
Alexander Schuettelkopf
Matja Zalar

Lab Support
Michael Kilday

The Drug Discovery Unit enables a critical link 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. We are particularly excited about our recently announced collaboration with Novartis on KRAS, a critical cancer-driving oncogene and we look forward to developing this project with them.

Drug Discovery is a data-driven endeavour and we are developing an Artificial Intelligence (AI) platform to help build predictive and generative models, enabling us to use data in an informative way to guide decision-making processes in a more efficient manner. This innovative approach to drug discovery provides a mechanism to capitalise on the continued advancements in the AI revolution that we are currently in.

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 LuAd. The most tractable approach to directly inhibit KRAS has been *via* covalent binding to $KRAS^{G12C}$ mutant protein, and some companies (Amgen with AMG 510 and Mirati with MRTX849) have recently managed to progress agents into early clinical studies. 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 BDDU. 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 BDDU take on all projects to ensure the best chance of success in delivering new treatments for patients, in the shortest possible timeframe. Within the CRUK-BI, we work closely with Owen Sansom's group who have 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 optimize our KRAS inhibitors

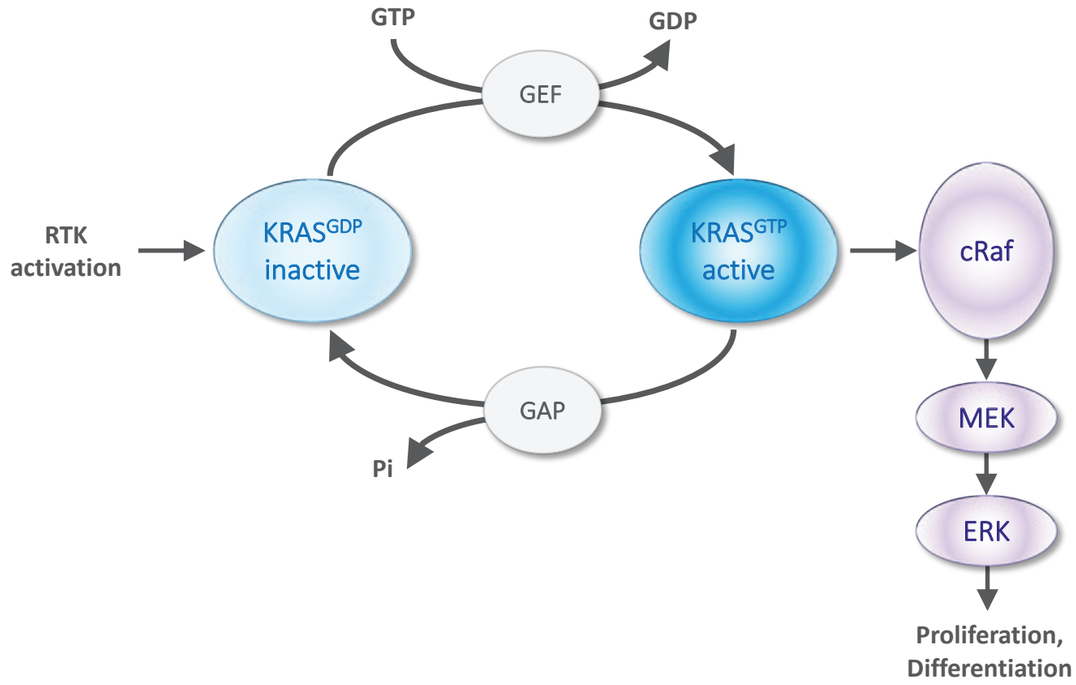


Figure 1
KRAS acts as a molecular switch rapidly converting to an active state upon receptor tyrosine kinase (RTK) stimulation, by exchanging GDP for GTP via a guanine nucleotide exchange factor (GEF). Active KRAS binds to effectors such as cRaf and activates downstream signalling through the MEK/ERK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

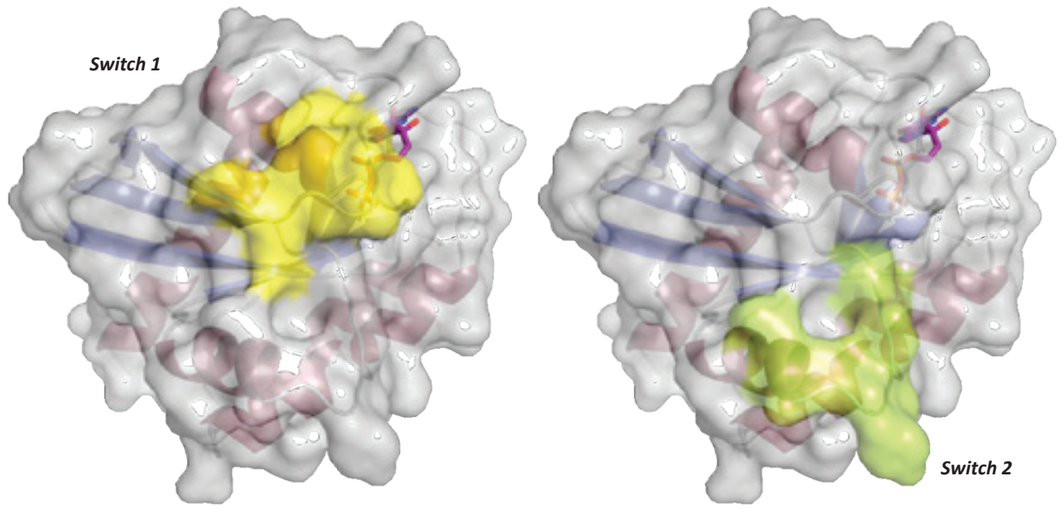


Figure 2
KRAS protein structure showing Switch 1 and Switch 2, which undergo conformational change upon activation to enable binding of effectors such as Raf1

and potentially identify a clinical development candidate.

Artificial intelligence (AI) is a rapidly growing technology and successful applications of AI in drug discovery are already numerous, from target identification to selection of populations for clinical trials. BDDU aims to be at the forefront of AI in drug design for CRUK as they look to expand into this evolving area of intelligent, predictive science. Within the BDDU Artificial Intelligence is being developed for both compound design and omics research. We are taking complementary approaches to explore this rapidly evolving field, collaborating with experts in order to provide a robust platform to progress projects through to deliver patient benefit.

A key aspect of the discovery process is the design of the drug itself (Figure 3) and deep generative or screening molecular design aims to craft novel chemical compounds with desirable chemical and pharmacological properties using deep neural networks. We aim to apply these ground-breaking deep learning techniques, combing AI-based to physics-based methods to our active drug discovery projects.

For omics research, we will also take a combination of in-house and external approaches, focusing on working closely with the Beatson Computational Biology group to develop an in-house capability whilst collaborating with companies that have promising new approaches, providing a platform to enable focus on the most promising oncology drug targets. We will develop inference AI models to predict potential novel disease targets and AI-led triage processes, ranking targets based on criteria such as chemical opportunity, safety and druggability.

Publications listed on page 92

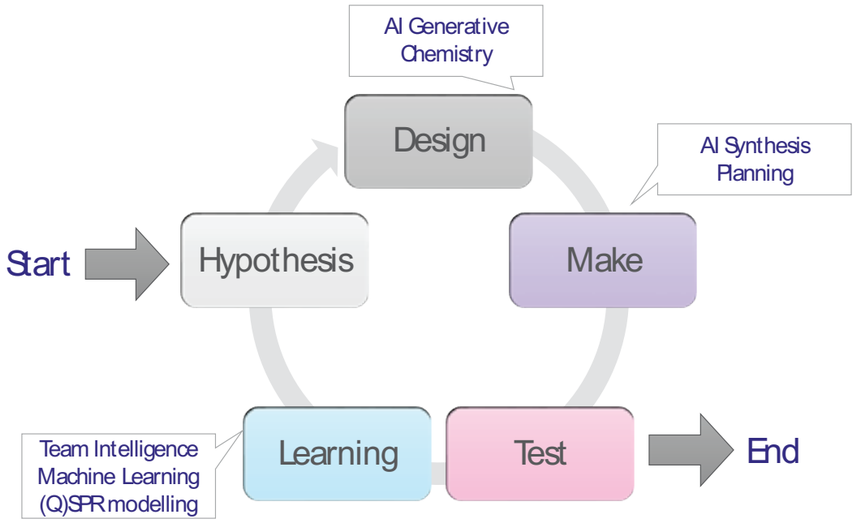


Figure 3
AI augmented Design-Make-Test cycle.

ADVANCED TECHNOLOGIES

www.beatson.gla.ac.uk/bair

BEATSON ADVANCED IMAGING RESOURCE

Head

Leo Carlin

Fellow of the Royal Microscopical Society (FRMS)

Scientific Officers
Tom Gilbey
John Halpin
Lynn McGarry
Ewan McGhee
Margaret O'Prey
David Strachan

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. 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:

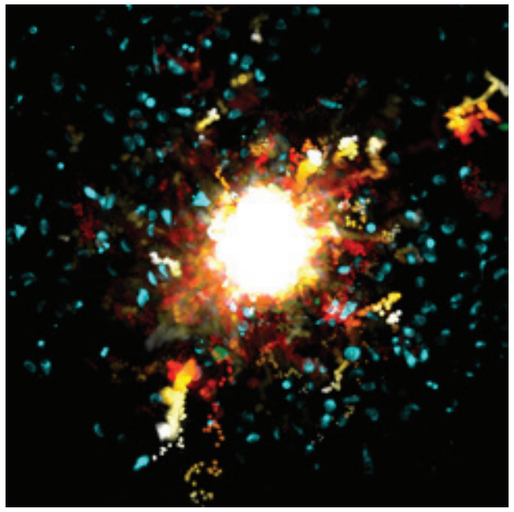
- Address multiplexed panels of up to 15 markers in liquid phase and dissociated tissue samples by **flow cytometry** and **sort cell populations** for downstream analysis (e.g. proteomics or transcriptomics using other advanced technology at the Institute).
- Perform automated liquid / multi-well plate 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 seven 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.
- 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.

For 2020, we are particularly excited to have Nikki Paul join our team. Nikki comes to us with a wealth of experience in imaging cancer cell biology. She will help to ensure that our researchers have access to the latest most promising imaging techniques by developing and transferring advanced imaging methodology.

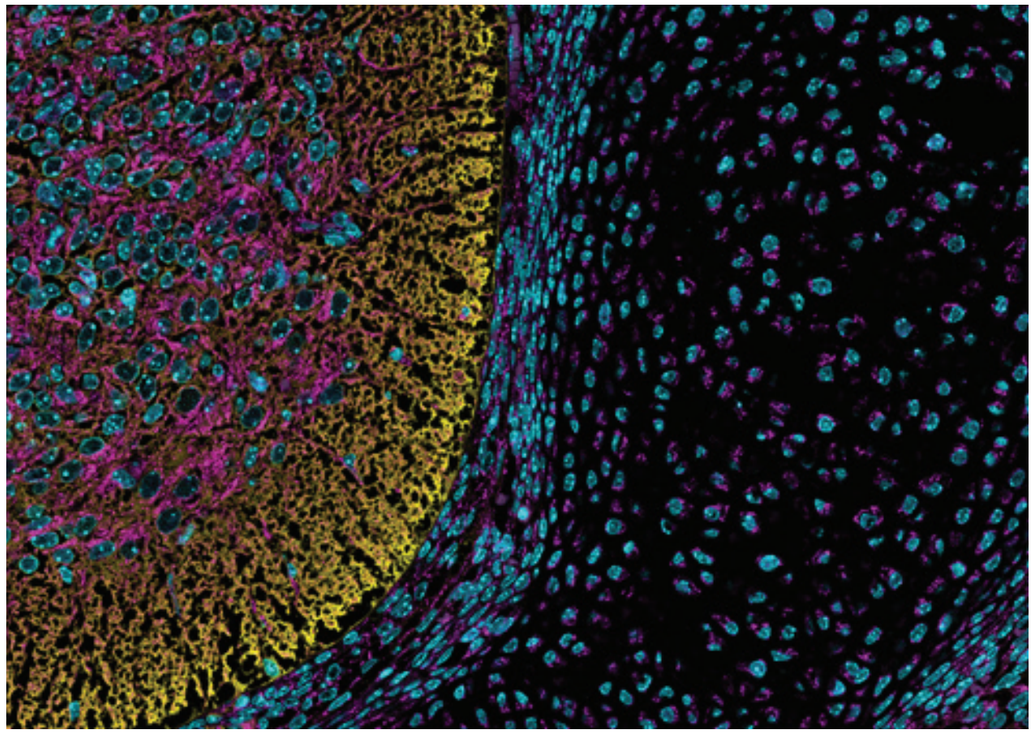
B16F1 melanoma cells expressing EGFP-WAVE 2 WT (in purple and green) or EGFPc1 (in yellow) and plated over laminin-coated glass bottom dish. Cells were stained for the actin cytoskeleton and imaged on Zeiss 880 with Airyscan. Cells were then arranged in a flower shape using Adobe Photoshop.

Image by Simona Buracco



The image is a time-lapse of a co-culture system with an invasive melanoma spheroid sitting at the centre, which represents the glowing sun. Surrounding the spheroid are non-invasive melanoma cells, which represents distant stars in cyan. Each shade of colour of the spheroid denotes a different time point.

The image was taken using the Nikon Timelapse system. Image by Anh Hoang Le



Mouse embryo section stained with TOM20 (mitochondria, magenta), integrin β5 (yellow) and DAPI (nucleus, cyan).

Acquired on Zeiss 880 with Airyscan as a tile scan composed of 10x10 regions stitched together. Image by Joel Riley

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 this year has been 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 software tools and packages from the Bioconductor project. We are particularly interested in establishing standardised workflows for initial pre-processing and up-stream analysis of single cell data sets in order to automate the more routine parts of the analysis.

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

The unit also provides support and guidance to graduate students and postdocs in other research groups who are using computational approaches to analyse their data. This includes advice on R scripting (by appointment), experimental design, and data presentation. We have established a bi-weekly 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.

INFORMATION SERVICES

Information Services provide a wide range of support services, including server provision, hardware cover, an on-site helpdesk providing both repair and software support as well as help with hardware selection and user training. There are over 350 users with over 400 PCs on site comprising a mixture of Windows computers, Apple Macs and Linux machines.

Peter McHardy

Iain White

All have central authentication, central file store and network printing. The servers have in the region of 2 Petabyte of online storage with daily backups, to provide support for microscopy, DNA sequencing and mass spectrometry data. Our central data store is a multi-headed Isilon storage system, offering fast access to data and the ability to expand the network file space easily and in a manageable way.

All PCs are built with a common desktop environment, around Windows or Mac OS X and Microsoft Office, and are actively managed and upgraded to ensure the best possible working environment. Mac OS X Mojave is being rolled out across the site and Windows computers upgraded to Windows 10 where appropriate. All email services run on Microsoft Exchange, which allows local client-based access and web access to email as well as delivering email, diaries and address books to mobile devices. Eduroam is available throughout the site.

Migration from physical servers to virtual servers using VMware® is complete. We offer access to virtualised servers for research groups allowing them greater flexibility for both test and production applications. This also allows us to provide virtual workstations for researchers with both high core counts (more 200) and large amounts of RAM, making them ideal for mass spectrometry analysis, machine learning applications or other computationally intense applications. We currently provide virtual desktops for OS X users requiring access to Windows-based packages. We have rolled out vApps for specific imaging, proteomics and metabolomics applications.

Significant investment has been put into creating documentation and procedures to allow us to run the service in a manner commensurate with ITIL. This is used as the foundation of our business continuity documentation and has led to the revision and ongoing improvement of many of our day-to-day working practices. Our intranet uses a content management system framework, allowing service managers and support departments the ability to easily upload forms and information for users.
A range of replacement hardware is stored on site to allow fast repairs. A good selection of loan IT hardware, from USB drives to digital projectors, is held centrally. We provide video conferencing facilities, enabling conference calls between the Institute and other Cancer Research UK sites as well as many other locations. Audio-visual support services for large conferences have been provided at a number of international venues, as well as overseeing the in-house 178-seat lecture theatre.

METABOLOMICS

Head
Gillian Mackay

Scientific Officers
David Sumpton
Giovanny Rodriguez-Blanco

Graduate Student
Rachel Harris

The Metabolomics facility supports many cancer metabolism research projects within the Institute. Our metabolomics platform is focused on the use of our two Thermo Scientific Q Exactive LC-MS systems, with their high resolution, accurate mass, Orbitrap technology, used for both targeted and untargeted analysis. We have well-established LC–MS methods using HILIC chromatography for polar metabolites and RP chromatography for lipids. Our Thermo Scientific Altis triple quad mass spectrometer offers increased sensitivity and specificity for known compounds. Our LC-MS systems are complemented with our Agilent Technologies GC-MS/MS triple quad instrument, for measuring compounds such as fatty acids, cholesterol and formate.

Our PhD project aims to identify metabolic targets in BRAF mutant melanoma using many of our metabolomics techniques. Drugs that selectively inhibit the activity of mutant BRAF are initially effective in melanoma patients, but drug resistance frequently develops. We are investigating which specific metabolic reactions are required by melanoma cells to proliferate upon BRAF inhibition, using both our targeted and untargeted approaches to metabolomics. As demonstrated in a previous collaborative study, melanoma cells resistant to BRAF inhibitors have enhanced glutamine dependency. We are studying the metabolic mechanism for this glutamine dependency using a glutaminase inhibitor and ^{13}C glutamine stable isotope tracing. The therapeutic potential of the metabolic targets identified will be assessed in *in vivo* models of melanoma.

The Metabolomics facility's untargeted approach to metabolomics is supporting Jim Norman's group to understand how metabolic changes drive breast cancer progression and metastatic disease. The MMTV-PyMT model is a well characterised murine model of metastatic breast cancer in which Polyoma Middle T-Antigen is expressed specifically in the mammary epithelium leading to the development of primary mammary tumours which can consequently metastasise to the lungs. We have performed our untargeted method for metabolomics on the serum and tumours of tumour bearing and non-tumour bearing mice and the serum of breast cancer patients compared to healthy volunteers. This dataset gives us a comprehensive analysis of the metabolic landscape of these *in vivo* models in relation to human disease and is now enabling us to identify pathways that may be important in driving metastasis in breast cancer.

The Metabolomics facility work with Alexei Vasquez' group using the GC-MS to quantify formaldehyde and formate in cells, cell culture media and tissue samples to investigate mitochondrial one-carbon metabolism in cancer and endogenous pathways of formaldehyde metabolism.

The Metabolomics facility developed a more sensitive method for accurately measuring acetyl coenzyme A (coA), using the Q Exactive mass spectrometer in SIM (Selected Ion Monitoring) mode, specifically for a project in Sara Zanivan's group. Sara's group showed that cancer associated fibroblasts (CAFs) remodel their metabolism to produce more acetyl coA. Our SIM method for acetyl coA was adapted to measure ^{13}C labelled acetyl coA for experiments where ^{13}C labelled glucose was added in the media. The tricarboxylic acid (TCA) cycle metabolism was also investigated in this project with ^{13}C stable isotope tracing.

Our untargeted and targeted approaches are being used for a project in Saverio Tardito's group aiming at identifying how glucocorticoids (i.e. dexamethasone) are able to rewire glioma cell metabolism, using LC-MS metabolomics. Glucocorticoids-based therapy for the management of the vasogenic oedema forming in brain tumour patients has been shown to reduce mortality and morbidity of brain tumour patients worldwide. However, many scientific papers have highlighted the controversial effects that they can exert on glioma cells, for instance in terms of enhanced proliferation and interference with chemotherapeutic drugs. Using several patient-derived cell lines grown in physiologically relevant culture conditions, the metabolites differentially produced when the cells are treated with dexamethasone are being investigated.

With Owen Sansom's group, our targeted metabolomics analysis of samples from genetically engineered mouse models (GEMMs) for colorectal cancer (CRC) has identified that concomitant mutation of APC and KRAS within the intestinal epithelium profoundly rewires metabolism *in vivo*, driving essential amino acid import via specific upregulation of amino acid transporters to fuel the proliferative demands of the cancer cells. For another project, we are measuring low abundant metabolites in tissues derived from a GEMM of CRC, optimising our methods to detect these metabolites either using the Altis triple quad LC-MS or a SIM method on the Q Exactive.

We are involved in a CRUK's Grand Challenge (Rosetta) project, led at the Beatson Institute by Owen Sansom. The lack of understanding of the spatial distribution of metabolic processes in the tumour or in the individual tumour cells is being addressed. Using the facility's LC-MS based metabolomics approaches, we can help to validate the data produced from the novel advanced mass spectrometric imaging where the molecular chemistry can be visualised in 2D and 3D. The Metabolomics facility is capable of providing the baseline metabolic signatures for each model system that the high-resolution imaging analysis aims to segment within tumour tissue.

Saverio Tardito's group is investigating the role of glutamine synthetase in β-catenin-driven hepatocellular carcinoma. Using the facility's new lipidomics approach with *in vitro* and *in vivo* models, interesting and novel results linking glutamine synthetase and the metabolism of lipids have been obtained. This interesting metabolic connection led Saverio's group, to be directly involved in the Metabolomics facility's development and validation of new protocols to extract lipid from cell lines and tissue.

For the Prostate Cancer Group, led by Hing Leung, the Metabolomics facility is investigating untargeted LC-MS lipidomic approaches to better understand the role of specific enzymes related to lipid metabolism which are highly dysregulated in advanced prostate cancer. Hing's group are interested in identifying the molecular mechanisms associated with castration-resistant prostate cancer (CRPC), a lethal form of the disease. Proteomic analyses of multiple preclinical models have suggested profound changes in the metabolism of cancer cells metabolism following resistance to treatment. Metabolomics (steady state as well as metabolite tracing using ^{13}C stable isotopes) is being used to understand how these metabolic adaptations sustain CRPC progression.

Publications listed on page 97

PROTEOMICS

Head
Sara Zanivan

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

[1]CRUK Multidisciplinary Award

Proteins constitute 50% of the cell (dry) mass and are key functional units that actively contribute to tumour initiation, progression and metastatic spread. Mass spectrometry (MS)-based proteomics is fundamental to unravel the identity and function of each protein in the cell. 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. 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 post-translational modifications, including cysteine oxidation. For the latter, we have recently developed SICyLIA, a method that enables the quantitative assessment of cysteine oxidation levels at global scale with no enrichment steps required (van der Reest, Lilla *et al.*, Nat Commun 2018).

This year, we have recruited Maja Bailey as Senior Scientific Officer. Maja is funded through a CRUK Early Detection Primer Award and is working with the rest of the team to establish an innovative platform for plasma redox proteomics for early detection of cancer.

During 2019, 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.

TRANSGENIC MODELS OF CANCER

Head
Karen Blyth

Scientific Officers
Dimitris Athineos
Sandeep Dhayade
Susan Mason

The Transgenic Models of Cancer lab uses *in vivo* models to recapitulate human cancer and interrogate all aspects of disease progression within a biological context (from early disease through to metastasis and recurrence). Validating *in vitro* discoveries in physiologically relevant models in this way will expedite novel therapeutic approaches for patient benefit. The group has expertise in modelling different cancer types using state-of-the art genetic and refined transplantation models to interrogate how oncogenic pathways, altered metabolism and the tumour microenvironment contribute to cancer.

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, 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.

Research collaborations

This year, the lab has worked on projects straddling the key themes of the Institute with studies centred on cancer metabolism, metastasis and cancer immunology. We have a long-standing collaboration between our lab and Oliver Maddocks (University of Glasgow) on projects involving amino acid vulnerabilities in cancer therapy. Using metabolic tracing to better understand how cancer cells use alternative metabolic pathways, and dietary intervention as a means to exploit certain sensitivities, we have been able to show the biological relevance of targeting cancer cell metabolism. Other metabolism-related projects in the lab are focusing on the role of one-carbon metabolism in cancer progression with Alexei Vazquez demonstrating the relevance of this as a therapeutic option. Profiling metabolic pathways in early and advanced disease states of breast cancer is a project we have been working closely with Jim Norman's lab where we have elegantly shown (in collaboration with Iain MacPherson) that circulating levels of certain metabolites correlate with metastasis both in mouse models and human patients. This has led to a new and exciting project funded by Breast Cancer Now (awarded to Iain MacPherson, Karen Blyth, Jim Norman) to use preclinical mouse models as a means to study Glutamate Metatropic Receptor 3 as a therapeutic target in breast cancer.

We continue to work closely with Seth Coffelt's lab and with Sara Zanivan's lab on models of breast and ovarian cancer to interrogate the role of the tumour microenvironment, and in particular the interactions with the immune system and cancer associated fibroblasts. This has also involved applying advanced imaging techniques in exciting collaborations with Leo Carlin's lab to study the dynamics of metastatic seeding of breast cancer cells and the interactions with the tumour microenvironment. In orthotopic xenograft models of prostate cancer, we have been working with David Bryant and Rachana Patel (Leung lab) to test the effects of Podocalyxin in metastasis and will start a new project following on from the work of a PhD student in David's lab that IQSEC1 is required for cancer cell invasion. With the recent procurement of a small animal radiation research platform (SARRP) to permit targeted radiotherapy treatment we are excited to be part of the CRUK RadNet Radiation Research Centre of Excellence in collaboration with Anthony Chalmers and the RadNet consortium adopting our world-leading preclinical models to explore the potential of radiotherapy to improve outcomes for cancers of unmet need.

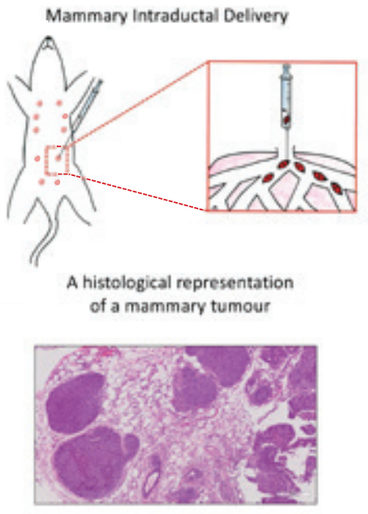


Figure 1
Improved models for breast cancer research
The lab has been adopting the mammary intraductal delivery system for a more physiologically relevant model to study breast cancer. Here, cancer cells are injected directly into the ductal system of the mammary gland. A representative tumour is shown (stained by H&E). Sandeep in the lab has combined this technology with imaging on the PEARL near-infrared fluorescence detector by using iRFP-labelled cells so that these developing tumours can be imaged, and metastasis followed *in vivo*. The images in the hatched boxes are magnified to show the increased tumour burden over the 4-week period post-injection.

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, TSE PhenoMaster metabolic chamber system and the IDEXX ProCyte Dx haematology analyser. As a lab, we continue to focus on innovative technologies to refine and improve cancer models for the benefit of the Institute. In particular, our lab provides expertise in surgical procedures such as renal capsule delivery, orthotopic prostate delivery, and primary tumour removal. Mammary models is one area where our lab has particular affiliation (see page 14) and to this end, we have been developing better genetic models for recurrent breast cancer as well as exploiting the mammary intraductal delivery route to improve the specificity of breast cancer models (Figure 1). In all our approaches we continually promote the 3Rs refining our models and exploring replacement models such as mammary organoids.

TRANSGENIC TECHNOLOGY

Head
Douglas Strathdee

Research Scientist
Eve Anderson

Scientific Officers
Cecilia Langhorne
Farah Naz Ghaffar
Cristina Rigau Granes

The Transgenic Technology Laboratory uses molecular genetic approaches to study the role of genes in the development and progression of cancer. By using stem cells, we can introduce precise genetic modifications into endogenous genes by using methods such as gene targeting and genome editing. This allows us to generate accurate models of human cancers by introducing identical changes that have been discovered in human disease directly into stem cells. Furthermore, recent advances allow us to introduce multiple genetic alterations into stem cells simultaneously allowing us to study how these combinations of mutations interact in the development of human cancers.

Developing better reagents for cancer modelling

Stem cells have a number of useful properties, which allow us to use them to study the role of gene mutations in cancer. Firstly, homologous recombination works relatively efficiently in these cells, which enables the generation of precise genetic modifications to endogenous genes. It is this property that allows us to copy the exact genetic changes, which have been uncovered in human tumours, into genes in stem cells. Introducing these exact mutations found in human disease allows us to study how these can alter protein function in intact cells and tissues. Secondly, stem cells can also differentiate to produce a wide variety of cell types found in a range of different tissues. Consequently, once a genetic change has been introduced into the stem cells, we can subsequently differentiate the cells to allow us to study the role of these mutations in cells from the tissue of origin of the tumour in which the mutation was discovered.

During the course of this year, we have collaborated with a number of other groups at the Institute to generate a variety of different allele types in stem cells. These include conditional knockouts, point mutations and fluorescent protein tags. Furthermore, advances in molecular technology have equipped us to be able to replace endogenous genes in stem cells with their direct human genomic equivalents. This means that mutations that we generate in genes in the stem cells can be done so in the appropriate genetic context, ensuring that these alterations more accurately reflect the outcomes of the mutations associated with disease.

Controlled expression of introduced sequences

One important tool for analysing gene function is the ability to engineer expression of novel sequences. These include a wide variety of different sequences like protein tags, fluorescent proteins or other modified cDNAs. Fluorescent proteins can, for example, be tagged onto endogenous genes to act as a reporter of the location of the endogenous protein. Alternatively, we can express biologically active cDNAs, such as oncogenes. We also routinely use these approaches to allow us to control the location, timing and level of gene expression, which can be a powerful way of assessing gene function in tumourigenesis.

One modification of this method we use routinely is to target transgene expression to a specific location in the genome choosing a locus, which is permissive for gene expression. This has a number of advantages compared to conventional transgenesis, normally carried out by microinjection of DNA. As a single copy of a transgene can be inserted into a defined location in a specific chromosome this avoids the unpredictable effects caused as a consequence of the integration site, or by integration of the transgene DNA in large multicopy arrays, both of which can have a substantial impact on the expression of the introduced transgenes. Furthermore, targeting transgenes to a specific location ensures that the transgenes do not disrupt endogenous genes when integrating into the genome, which can be a source of unwanted mutations.

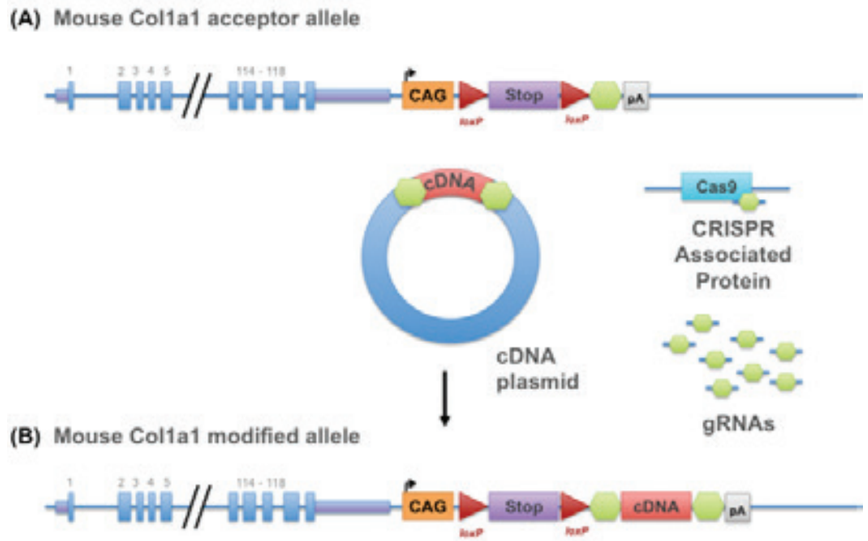


Figure 1
Using genome editing to insert a cDNA into a specific site downstream of the Co1a1 locus.
Each diagram represents a different adjacent to the Col1a1 locus. Blue numbered boxes represent exons. Red triangles are loxP sites. Green hexagons are the gRNA target site. (A) A targeted allele of the Col1a1 locus. A promoter followed by a stop cassette and a poly A signal sequence are inserted downstream of the endogenous Col1a1 gene. A cDNA can be inserted directly into the target site (green hexagon) using genome editing. This requires the CRISPR associated protein Cas9, an RNA guided DNA endonuclease, along with a gRNA designed to the target site and a plasmid containing a donor cDNA. (B) The cDNA is inserted directly into the target site. In this location, the cDNA can be expressed by the CAG promoter following removal of the stop cassette as a consequence of Cre recombinase expression.

Although this approach is very successful it is relatively laborious, as it requires generation of complex DNA targeting vectors followed by transfection into cells. We are now optimising a new method of rapidly engineering expression of exogenous proteins using genome editing. Using this approach, an individual cDNA can be inserted directly into a specific target location using genome editing, rather than gene targeting. The advantage of this approach is that it does not require construction of gene targeting vectors, or transfection of stem cells but allows the transgene sequence to be introduced directly using microinjection. Instead of a targeting vector this method simply requires that the cDNA is introduced along with Cas9, a RNA-guided DNA endonuclease enzyme and the guide RNA (gRNA) molecules which direct the Cas9 to its specific target site (Figure 1). Cas9, guided by the gRNA, cleaves the target site in the specific genomic location and also on both sides of the introduced cDNA. This allows the linearised cDNA to be directly inserted into the cleaved target site. In the example shown the acceptor allele is placed adjacent to the Col1a1, but we already have a number of other locations in the genome such as Gt(ROSA)26Sor and Hprt. This would allow the insertion of multiple different DNA sequences at different locations in the genome, which can help in generating some of the more complex models requiring the expression of multiple genes simultaneously.

By using these methods and the ones described above we can increasingly generate more complex and accurate models of human cancer. This should allow us to determine the roles that different genes can play in the progression of tumours.

Publications listed on page 103

TRANSLATIONAL MOLECULAR IMAGING

Head
David Lewis

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

Staff Scientist
Gaurav Malviya

Medical Physicist
Caroline Findlay[1]

Scientific Officers
Emma Johnson
Agata Mrowinska

[1]Beatson Cancer Charity

Translational Molecular Imaging (TMI) advances novel imaging technologies and serves as a hub for emerging imaging research. Operating over two sites: the CRUK Beatson Institute and the West of Scotland PET Centre at Beatson Cancer Centre, our facility houses state-of-the-art radiochemistry and imaging equipment. Within the TMI there is expertise in several key areas of imaging which is further supported by a wide network of expert collaborators. The TMI drives collaborative imaging research across this network. The focus is on developing and applying innovative imaging technologies such as new PET radiotracers and MRI methodology to aid in the visualisation and understanding of cancer biology.

PET radiochemistry

In 2019, we finished upgrading the research radiochemistry laboratory at the PET Radiopharmaceutical Unit in the West of Scotland PET Centre at Gartnavel General Hospital with radiolabelling and analytical equipment. The R&D radiochemistry platform is now well equipped for development of novel carbon-11 and fluorine-18 labelled PET probes. To ensure reliability and sustainability of radiotracer development we recruited a second experienced radiochemist - Gavin Brown, who brings in expertise in amino-acid labelling with carbon-11.

Throughout the year the radiochemistry team has supported extensive imaging programmes across the Glasgow Cancer Centre network. The list of available radiotracers includes: [^{11}C]acetate (ACE), [^{18}F]fluoro-ethyl-tyrosine (FET), [^{11}C]methionine, [^{18}F]fluoro-thymidine (FLT), [^{18}F]terafluoroborate (TFB), [^{18}F] fluorodeoxyglucose (FDG) and [^{18}F]LW223 (TSPO).

To facilitate clinical research programmes at the West Of Scotland Glasgow PET Centre we are training in quality control procedures of [^{18}F]FMISO – the first clinical non-FDG tracer in Glasgow. We have also started preparation for [^{11}C]acetate GMP production for a translational imaging trial at the PET Radiopharmaceutical Unit.

Preclinical and translational imaginig

Projects in the TMI range from standard imaging studies where we facilitate access to technology to much wider scale projects where the TMI acts as a collaborative partner in the production and development of novel imaging agents or *in vivo* molecular phenotyping. The unique opportunity at the Beatson is to guide collaborative research using the world-class cancer models at the Institute to develop clinical imaging biomarkers for new applications such as tumour classification and personalised cancer therapy.

In 2019 and in collaboration with Owen Sansom's group, we performed the first stratification of colorectal cancer (CRC) using a panel of PET and MRI imaging biomarkers. We classified five colon cancer organoid models derived from genetically engineered mice with multiple modified alleles representing a spectrum of human colon cancer: *Apc*$^{Min/+}$ *Kras*$^{G12D/+}$ (AK), *Apc*$^{Min/+}$ *Kras*$^{G12D/+}$ *Tp53*$^{-/-}$ *Tgfbr2*$^{-/-}$ (AKPT), *Apc*$^{Min/+}$ *Kras*$^{G12D/+}$ *Tp53*$^{-/-}$ *Rosa26*$^{N1icd/+}$ (AKPN), *Kras*$^{G12D/+}$ *Tp53*$^{-/-}$ *Rosa26*$^{N1icd/+}$ (KPN) and liver-homing *Kras*$^{G12D/+}$ *Tp53*$^{-/-}$ *Rosa26*$^{N1icd/+}$ (KPN$_{LIVMET}$).

Reprogramming of energy metabolism has been recognised as an emerging hallmark of cancer cells, and metabolic biomarkers have potential for stratification of colon cancer subtypes. We initiated a novel approach employing PET/MRI imaging based metabolic probes. These biomarkers could identify co-existing subtypes and subtype switching following therapy. The goal of molecular stratification is to aid the development of subtype-specific therapies with improved efficacy for particular patient subgroups.

Our data demonstrated that [^{18}F]FDG ([^{18}F] fluorodeoxyglucose) and [^{18}F]FET ([^{18}F] fluoroethyltyrosine) are more effective than [^{18}F]FLT (3'-deoxy-3'[^{18}F]fluorothymidine) and [^{11}C]ACE ([^{11}C]acetate) for stratifying models of CRC subtypes (Figure 1). In collaboration with Bioinformatics, we are validating a machine-learning algorithm using support vector machines (SVM) and testing it against Monte-Carlo permutations to determine the distinguishing features of each subtype.

In 2019, we also finished a study with the Transgenic Technology group to investigate *in vivo* metabolic changes in a mouse model of Barth's syndrome using PET/MR imaging. Our results demonstrate that [^{11}C]ACE PET identifies an oxidative deficiency in the hearts of Taz KO mice and a corresponding increase [^{18}F]FDG indicating a switch to higher myocardial glucose consumption. Now validated, these biomarkers can be developed for monitoring the effectiveness of novel therapies in Barth Syndrome.

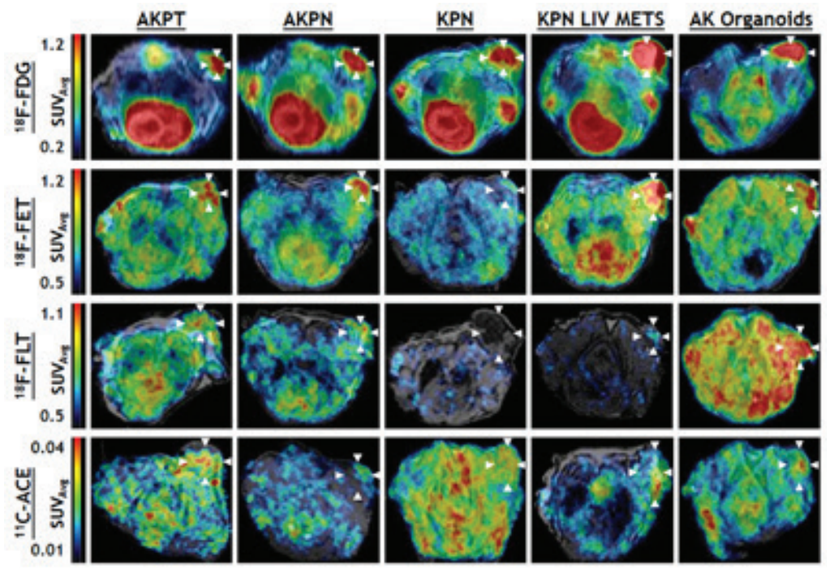


Figure 1
Colon cancer organoids derived from five genetically engineered mouse models have different metabolic features on PET/MR imaging. [^{18}F]FDG and [^{18}F]FET PET uptake ($SUV_{average}$) differentiate colon cancer subtypes more readily than [^{18}F] FLT and [^{11}C]ACE.

HISTOLOGY

Colin Nixon

Barbara Cadden
Denise McPhee
Fiona McGregor
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 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, specialised processing cycles. We have four large capacity automated tissue processors allowing large scale consistent processing, but when required specialised processing cycles can be designed. Other material such as organotypic assays, cell pellets, spheroids and agar plugs can also be processed to provide a wax block allowing sectioning and further investigation. All paraffin wax blocks sectioned are stained with haematoxylin and eosin in order to allow general analysis of cell morphology and structure. After initial analysis, more specialised 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 or immuno-histochemical or immunofluorescence staining methods.

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.

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 make sure we keep pace with researchers' demands and up-to-date with relevant wider areas of interest. New antibodies can be optimised to produce a working protocol that allows the antibody to be used either on an autostainer or for hand staining by the researcher. Training can be provided in order that an individual scientist can understand the rationale and techniques available to allow them to perform the staining to an acceptable and consistent standard.

Where there is no antibody available for immunohistochemistry analysis or a more specific conclusive technique is required, the service can provide an *in situ* hybridisation technique using a reagent system designed to visualise cellular RNA targets in formalin-fixed paraffin-embedded tissue sections using bright-field microscopy. This technique can be performed for single probe staining or dual staining of probes on a 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 now means that when a probe is available or one can be specifically designed to the meet the researcher's needs, we can label and visualise much smaller targets, around 50 base pairs in size. This technique is now automated, allowing for improved quality and reproducibility of the results.

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

The histology service provides a fully automated large capacity Leica SCN400F slide scanner which is capable of capturing bright-field or fluorescent images. This allows high-quality digital images to be scanned and stored, and if required automated quantitative interpretation can be performed. For digital analysis we are able to offer access to Indica HALO™ image analysis software. This allows staining techniques to be scored using algorithms designed specifically for that staining result using the researcher's input to designate which specific areas are to be scored. This produces accurate and reproducible scoring. The service provides full training regarding the software and modules available in order for the researcher to be able to use the image analysis software.

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 with the remit to ensure the smooth operation of building facilities and to support research services. We, thereby, provide support to all the research groups housed within the institute, giving them the freedom to focus on delivering their world class research.

Building Facilities

Alistair Wilson, Andrew Hosie, John Trivett

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.

Our department has seen changes in personnel with a new Head of Department joining us in November plus two new maintenance technicians for our in-house maintenance team. These additions not only replace retired staff (Sue Fowler & Alex Kernahan) but will enhance our ability to support the needs of the institute going forward.

This year we have completed structural works to prepare laboratory areas for specialist equipment, management of space continues to present challenges, and we continue to implement solutions to meet the needs of our research staff.

Laboratory Management

Laura Bence, Richard Selkirk, Karen Thomas

The Laboratory Management team are 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. Any training needs are met and deficiencies rectified and adequate provision is made to fulfil the Institute's legal obligations to staff. All staff and students are required to attend a safety update seminar once a year and new starts attend a series of safety induction talks. In addition, a number of training sessions are arranged for new PhD students.

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 the latest state-of-the art technology first-hand. As a result, we purchased several new pieces of equipment. A number of training seminars were also organised to enable researchers to gain knowledge of new and improved techniques using the equipment and technologies already present within the Institute. Regular training sessions were also arranged for some of the key core equipment.

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

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

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 investigating having 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 liaises with Stores to acquire free samples of new products to ensure the best and most appropriate products are used by the researchers.

Laboratory Support Services & Stores

Laboratory Support Services and Stores have welcomed a new manager, Angela Miller, to their team who now oversees both services which support the Institute

Laboratory Support Services

Angela Miller, **Tracy Shields**, Elizabeth Cheetham, James Dyball Dilhani Kahawela, Nicky McCarter, Kirstie McPherson, Jonny Sawers, Linda Scott, Lauren Ure

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

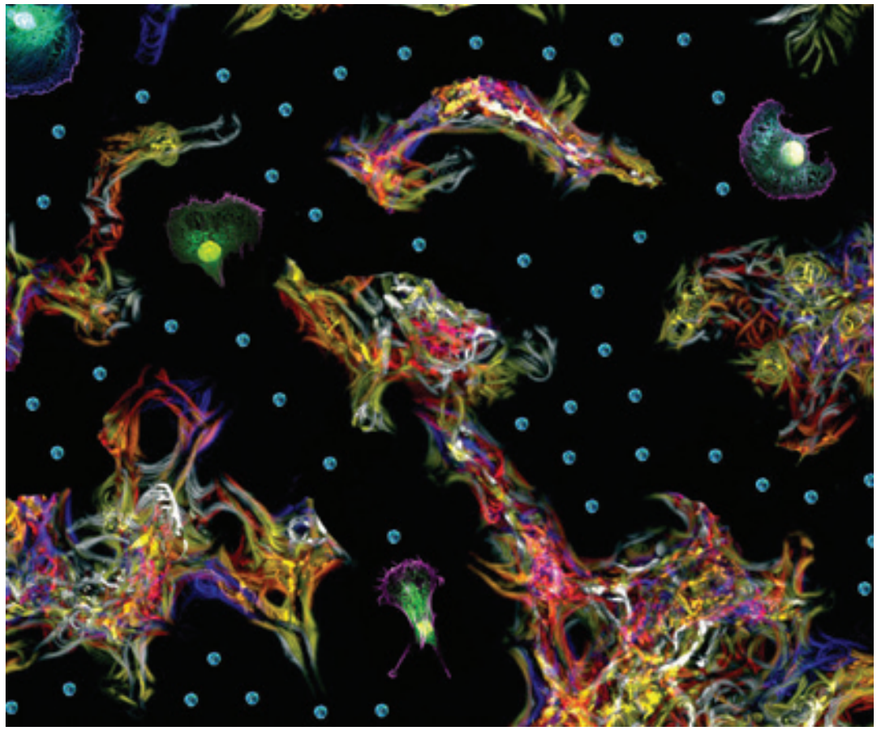
Angela Miller, Michael McTaggart, George Monteith

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.

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 shipment of UK based packages to an alternative courier, and this will be implemented for world-wide packages in the near future, without impacting on the service provided for the current users. 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.

PacMan.
Pancreatic cancer cells randomly migrating on collagen matrix used as maze. Videos taken by Nikon Long Term Timelapse microscope and every time frame coloured differently. Phagocytic beads taken up by cells were used. Pancreatic cells and beads imaged using Zeiss 880 LSM with Airyscan.

Image by Savvas Nikolaou



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-ITTM (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 15,000–17,000 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. Cell lines are mainly tested using a luciferase assay (Lonza) that detects mycoplasmal enzymes. 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. Stocks of commonly used tissue culture medium are ordered and the batch testing of serum is coordinated. 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

Tom Bird (page 12)
Liver Disease and Regeneration

Primary Research Papers
Amoros R, King R, Toyoda H, Kumada T, Johnson PJ, Bird TG.
A continuous-time hidden Markov model for cancer surveillance using serum biomarkers with application to hepatocellular carcinoma. ***Metron*** 2019; 77: 67-86

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Liko D, Mitchell L, Campbell KJ, Ridgway RA, Jones C, Dudek K, King A, Bryson S, Stevenson D, Blyth K, Strathdee D, Morton JP, Bird TG, Knight JRP, Willis AE, Sansom OJ.
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Karen Blyth (page 14)
In Vivo Cancer Biology

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Justin Bower & Heather McKinnon (page 66)
Drug Discovery

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Hayatigolkhatmi K, Padroni G, Su W, Fang L, Gomez-Castaneda E, Hsieh YC, Jackson L, Pellicano F, Burley GA, Jorgensen HG.
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David Bryant (page 16)
Molecular Control of Epithelial Polarity

Primary Research Papers
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Hypoxic cancer-associated fibroblasts increase NCBP2-AS2/HIAR to promote endothelial sprouting through enhanced VEGF signaling. ***Sci Signal*** 2019; 12: eaan8247

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

Primary Research Papers
Meijer HA, Schmidt T, Gillen SL, Langlais C, Jukes-Jones R, de Moor CH, Cain K, Wilczynska A, Bushell M
DEAD-box helicase eIF4A2 inhibits CNOT7 deadenylation activity. Nucleic Acids Res. 2019; 47: 8224-8238

Skalka G, Hall H, Somers J, Bushell M, Willis A, Malewicz M
Leucine zipper and ICAT domain containing (LZIC) protein regulates cell cycle transitions in response to ionizing radiation. ***Cell cycle***. 2019; 18: 963-975

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Leo Carlin (page 20)
Leukocyte Dynamics

Primary Research Papers
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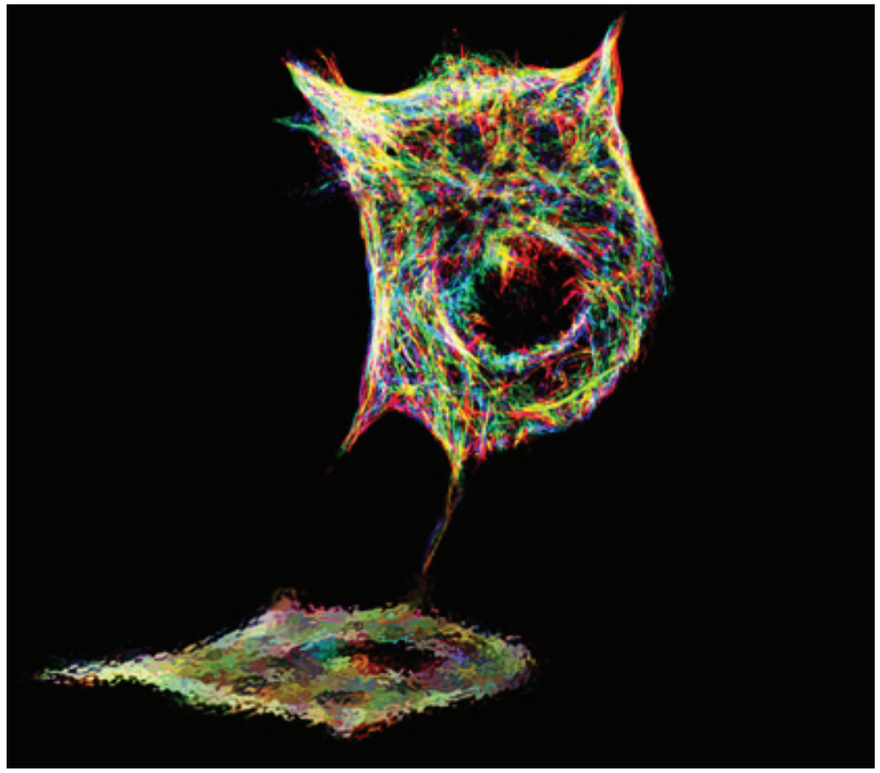
Image by Savvas Nikolaou



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Payam Gammage (page 24)
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Crispin Miller (page 40 & 72)

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Jennifer Morton (page 42)

Precision-Panc Preclinical Lab

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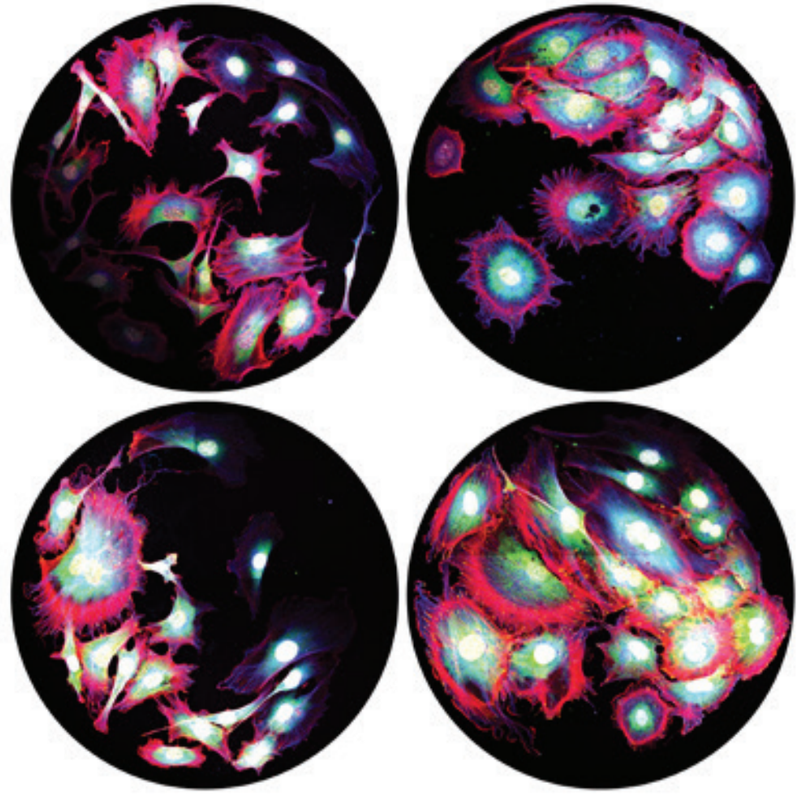

Mouse embryonic fibroblasts were grown in circular micropatterns on the PRIMO system and imaged on Zeiss 880. Actin cytoskeleton (Red), Tubulin (Blue), Vinculin (Green) and DAPI (White).

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Daniel Murphy (page 44)

Oncogene-induced Vulnerabilities/ Thoracic Cancer

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Ed Roberts (page 48)

Immune Priming and Tumour Microenvironment

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Kevin Ryan (page 50)

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Owen Sansom (page 52)

Colorectal Cancer and Wnt Signalling

Primary Research Papers

Allen-Petersen BL, Risom T, Feng Z, Wang Z, Jenny ZP, Thoma MC, Pelz KR, Morton JP, Sansom OJ, Lopez CD, Sheppard B, Christensen DJ, Ohlmeyer M, Narla G, Sears RC.
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AKT/mTORC2 Inhibition Activates FOXO1 Function in CLL Cells Reducing B-Cell Receptor-Mediated Survival. ***Clinical Cancer Res***. 2019; 25: 1574-1587

Gay DM, Ridgway RA, Muller M, Hodder MC, Hedley A, Clark W, Leach JD, Jackstadt R, Nixon C, Huels DJ, Campbell AD, Bird TG, Sansom OJ.
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Brf1 loss and not overexpression disrupts tissues homeostasis in the intestine, liver and pancreas. ***Cell Death Differ*** 2019; 26: 2535-50

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Reader CS, Vallath S, Steele CW, Haider S, Brentnall A, Desai A, Moore KM, Jamieson NB, Chang D, Bailey P, Scarpa A, Lawlor R, Chelala C, Keyse SM, Biankin A, Morton JP, Evans TJ, Barry ST, Sansom OJ, Kocher HM, Marshall JF.
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A MYC-GCN2-eIF2alpha negative feedback loop limits protein synthesis to prevent MYC-dependent apoptosis in colorectal cancer. Nat ***Cell Biol***. 2019; 21: 1413-1424

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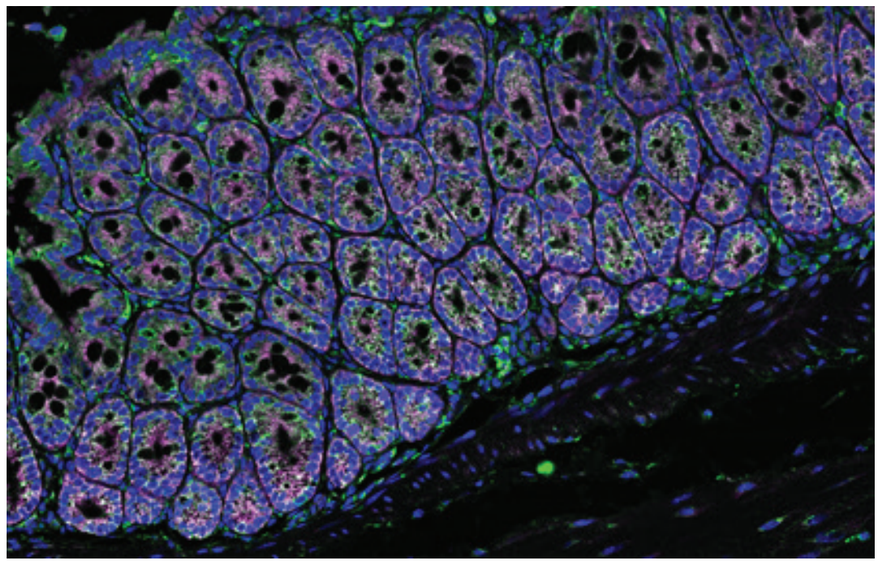

Mouse intestine stained with TOM20 (mitochondria, magenta), cytochrome c (inner mitochondria, cyan) and DAPI (nucleus, blue). Acquired on Zeiss 880 with Airyscan as a tile scan composed of 10x10 regions stitched together.

Image by Joel Riley

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Douglas Strathdee (page 80)
Transgenic Technology

Primary Research Papers

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A MYC-GCN2-eIF2alpha negative feedback loop limits protein synthesis to prevent MYC-dependent apoptosis in colorectal cancer. ***Nat Cell Biol***. 2019; 21: 1413-1424

Stephen Tait (page 54)
Mitochondria and Cell Death

Primary Research Papers

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Mitochondria and inflammation: Cell death heats up. ***Front Cell Dev Biol*** 2019; 7: 100

Saverio Tardito (page 58)
Oncometabolism

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Clerc I, Abba Moussa D, Vahlas Z, Tardito S, Oburoglu L, Hope TJ, Sitbon M, Dardalhon V, Mongellaz C, Taylor N.
Entry of glucose- and glutamine-derived carbons into the citric acid cycle supports early steps of HIV-1 infection in CD4 T cells. ***Nat Metab*** 2019; 1: 717-30

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Ackermann T, Tardito S.
Cell culture medium formulation and its implications in cancer metabolism. ***Trends Cancer*** 2019; 5: 329-32

Alexei Vazquez (page 60)
Mathematical Models of Metabolism

Primary Research Papers

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METTEN study group. Stratification of cancer and diabetes based on circulating levels of formate and glucose. ***Cancer Metab*** 2019; 7: 3

Sara Zanivan (page 62)
Tumour Microenvironment and Proteomics

Primary Research Papers

Ferraro DA, Patella F, Zanivan S, Donato C, Aceto N, Giannotta M, Dejana E, Diepenbruck M, Christofori G, Buess M.
Endothelial cell-derived nidogen-1 inhibits migration of SK-BR-3 breast cancer cells. ***BMC Cancer*** 2019; 19: 312

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Disrupting MLV integrase:BET protein interaction biases integration into quiescent chromatin and delays but does not eliminate tumor activation in a MYC/Runx2 mouse model. ***PLoS Pathog***. 2019; 15: e1008154.

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The antimicrobial peptide defensin cooperates with tumour necrosis factor to drive tumour cell death in Drosophila. ***eLife***. 2019; 8.

Pucino V, Certo M, Bulusu V, Cucchi D, Goldmann K, Pontarini E, Haas R, Smith J, Headland SE, Blighe K, Ruscica M, Humby F, Lewis MJ, Kamphorst JJ, Bombardieri M, Pitzalis C, Mauro C.
Lactate Buildup at the Site of Chronic Inflammation Promotes Disease by Inducing CD4(+) T Cell Metabolic Rewiring. ***Cell Metab***. 2019; 30: 1055-1074

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Gorgoulis V, Adams PD, Alimonti A, Bennett DC, Bischof O, Bishop C, Campisi J, Collado M, Evangelou K, Ferbeyre G, Gil J, Hara E, Krizhanovsky V, Jurk D, Maier AB, Narita M, Niedernhofer L, Passos JF, Robbins PD, Schmitt CA, Sedivy J, Vougas K, von Zglinicki T, Zhou D, Serrano M, Demaria M.
Cellular Senescence: Defining a Path Forward. ***Cell***. 2019; 179:813-827.

Humpton T, Vousden KH.
Taking up the reins of power: metabolic functions of p53. ***J Mol Cell Biol***. 2019; 11: 610-614.

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Adhesion and growth factor receptor crosstalk mechanisms controlling cell migration. ***Essays Biochem***. 2019; 63: 553-567.

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 Valentin Barthet from Kevin Ryan's group. He has been investigating the tumor suppressive role of autophagy in liver cancer.

Theses

Däbritz, Henry (2019) Genetically engineered mouse models reveal a tumourigenic collaboration between Sdhb deficiency and oncogenic Hras. [PhD thesis, University of Glasgow, Beatson Institute]

El Maghloob, Yasmin (2019) Trafficking of lipid-modified proteins to specialised membrane domains [PhD thesis, University of Glasgow, Beatson Institute]

Hodder, Michael (2019) Investigating the impact of intestinal stem cell dynamics on tumour initiation. [PhD thesis, University of Glasgow, Beatson Institute]

Kay, Emily (2019) An epigenetic switch links pyruvate dehydrogenase activation to extracellular matrix production via proline synthesis [PhD thesis, University of Glasgow, Beatson Institute]

Mackey, John (2019) Identification, Confirmation and Functional Relevance of

Neutrophil Maturity in Homeostasis, Inflammation and Disease [PhD thesis, Imperial College London, Beatson Institute]

McGregor, Grace (2019) The role of the mevalonate pathway in cancer and how it can be exploited to improve cancer therapy [PhD thesis, University of Glasgow, Beatson Institute]

Michalopoulou, Evdokia (2019) Regulation of micropinocytosis in pancreatic cancer and implication on drug effectiveness. [PhD thesis, University of Glasgow, Beatson Institute]

Nacke, Marisa (2019) Investigating the role of the ARF GEF IQSEC1 in prostate cancer cell invasion [PhD thesis, University of Glasgow, Beatson Institute]

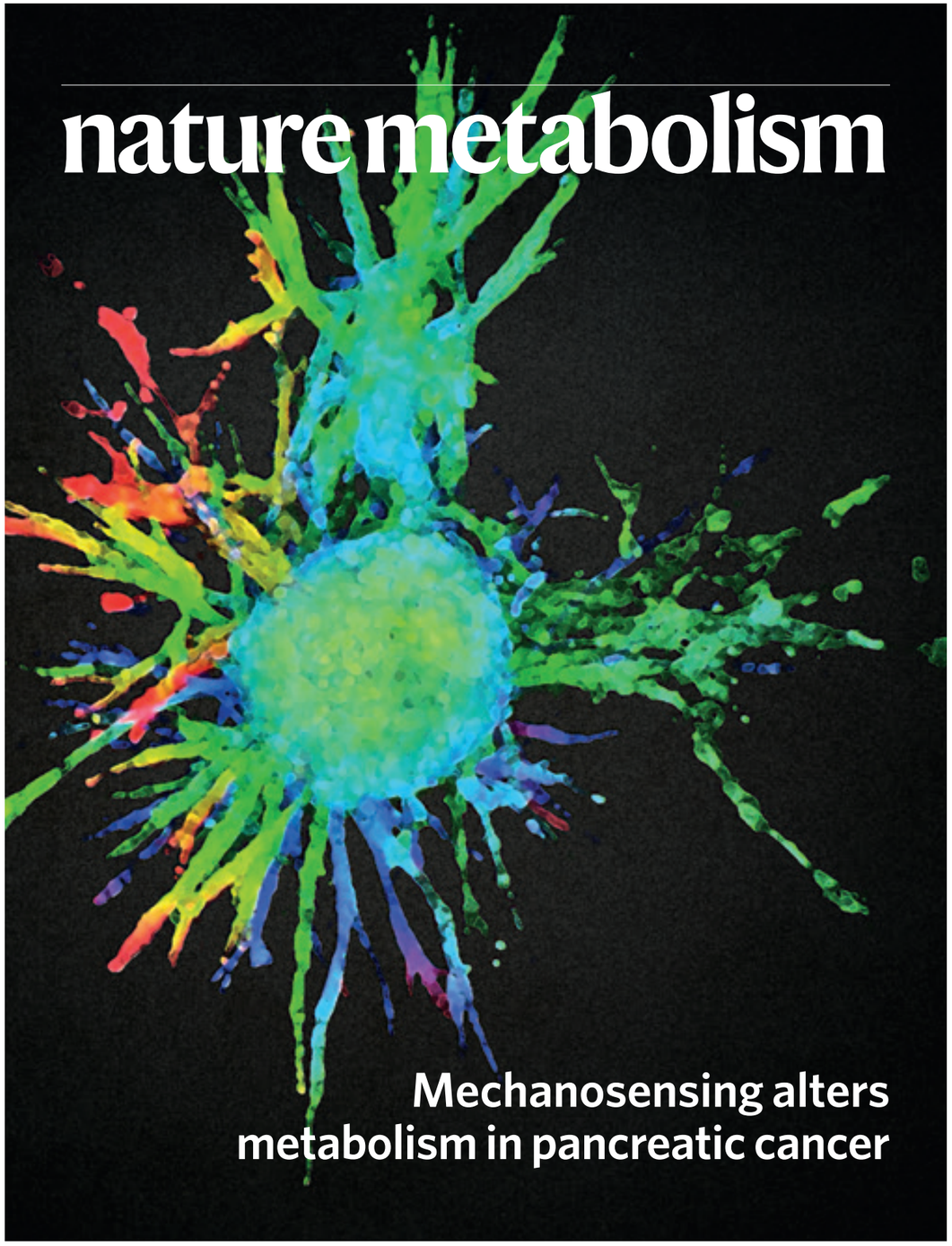
Nomura, Koji (2019) Regulation of p53 by catalytically-inactive MDM2 mutants [PhD thesis, University of Glasgow, Beatson Institute]

Papalazarou, Vasileios (2019) ECM mechanics and spatiotemporal energetics in pancreatic cancer [PhD thesis, University of Glasgow, Beatson Institute]

Rabas, Nicolas (2019) An investigation into the role of extracellular glutamate in cancer cell invasion [PhD thesis, University of Glasgow, Beatson Institute]

Secklehner, Judith (2019) Interrelationships between Natural Killer cells and neutrophils in the pulmonary vasculature [PhD thesis, Imperial College London, Beatson Institute]

Skalka, George (2019) Molecular characterisation of putative WNT signalling protein, Leucine Zipper and ICAT domain containing (LZIC) [PhD Thesis, University of Leicester, Beatson Institute]



PhD student Vassilis Papalazarou, and colleagues from the Machesky lab and the University of Glasgow published their findings on how mechanosensing alters metabolism in pancreatic cancer online in December 2019. Nature Metabolism featured the work on its cover.

Reference: Papalazarou et al. (2020) The creatine–phosphagen system is mechanoresponsive in pancreatic adenocarcinoma and fuels invasion and metastasis. Nature Metabolism. 2: 62-80.

CONFERENCES AND WORKSHOPS

Cancer Research UK Beatson International Cancer Conference

Protein Dynamics in Cancer

30th June-3rd July 2019
Bute Hall, University of Glasgow
Scientific Committee: Kevin Ryan, Martin Bushell, Vignir Helgason, Danny Huang

For many decades, genomic stability had been the key focal point for cancer prevention. For our 2019 meeting, we put the spotlight on protein integrity as a mechanism to avert malignant disease. As mutated genes ultimately encode for dysfunctional proteins or alter protein turnover rates, we aimed to detail different pathways in which proteins are involved to protect against cancer and contribute to tumour maintenance. Our intention was to understand the therapeutic potential of the involved pathways.

Poul Sorensen (University of British Columbia) opened the conference with the Colin Thomson Memorial Lecture sponsored by Worldwide Cancer Research where he described links between cellular stress adaptation, and tumour growth and metastasis. We were grateful for the support from Multiple Digital Publishing Institute sponsoring the keynote lecture given by David Ron (Cambridge Institute of Medical Research). David shared insights into the unfolded protein response in the endoplasmic reticulum and stimulated thoughts on its relevance in cancer.

Throughout the conference we heard from a number of accomplished speakers John Le Quesne, Nicolas Rabas, Vincenzo d'Angiolella, Koji Nomura, Arnaud Blomme, Nicola Manfrini, John Knight, Francesca Rapino, Marta Crespi-Sallan and Angela Ianniciello who gave selected short talks.

Over 50 posters invited to direct engagement and discussion with the researchers during the poster session. Our particular thanks go to AMSBIO for their continued sponsorship of the poster prize. We congratulated the winner George Skalka for showcasing the role of LZIC in cell cycle regulation in response to ionising radiation.

The Beatson International Conference is held in the magnificent Bute Hall at the University of Glasgow.

Image by Mark Nakasone

The meeting was generously co-sponsored by Cancer Research UK. Our gratitude also goes to Transnetyx Inc, the Company of Biologists and the Glasgow Convention Bureau for their contribution to the Beatson International Cancer Conference 2019.

Cartography of Cancer: Mapping Tumours in 3D 2020

12th -17th July 2020
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 2020 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 key note lectures by Greg Hannon and Josephine Bunch.

Once again, the conference will be held at the beautiful University of Glasgow – a successful venue for many previous Beatson conferences. The scientific content of the conference is guaranteed to stimulate, and there are some events arranged to give you a taste of the culture and warmth of Glasgow and the West of Scotland.

Upon going to press, the Beatson International Cancer Conference 2020 had been postponed in light of the circumstances arising from the COVID-19 pandemic. The meeting will be rescheduled for July 2021 with the same topic. We are looking forward to confirming dates and location on our website in the near future.

3rd Glasgow Imaging Network (GiN)

19th August 2019
For the first time the Beatson Institute hosted the Glasgow-wide initiative to bring together researchers across disciplines to share capabilities and needs in microscopy.

Keynote: Prof Paul French from Imperial College London – FLIM FRET

Special thanks go to Nikon UK and the Royal Microscopy Society for sponsoring prizes that have been awarded to Frederic Fercoq for best poster and Gavin Meehan for best oral presentation, respectively.

Glasgow Immuno-Oncology Colloquium

16th September 2019
Jointly with the University of Glasgow's Institute of Infection, Immunity and Inflammation the colloquium encouraged researchers to learn about cancer treatment specifically immunotherapy. Postdocs and PhD students from Glasgow were given the opportunity to present their work on cancer immunology in flash talks. The meeting came to a close with a fantastic talk by Drew Pardoll from John Hopkins University detailing the patients' response to the immune-stimulating, anti-PD1 treatment – in particular giving molecular insights into non-responders.

ACRCelerate Workshop

24th-26th November 2019
The European-wide ACRCelerate Colorectal Cancer Stratified Medicine Network invited to it's inaugural Workshop and Training Day. The aim of the meeting was to exchange knowledge on colorectal cancer focusing on state-of-the-art preclinical models, to strengthen relationships and to forge new collaborations. The workshop was attended by over 150 delegates from the UK and Europe.

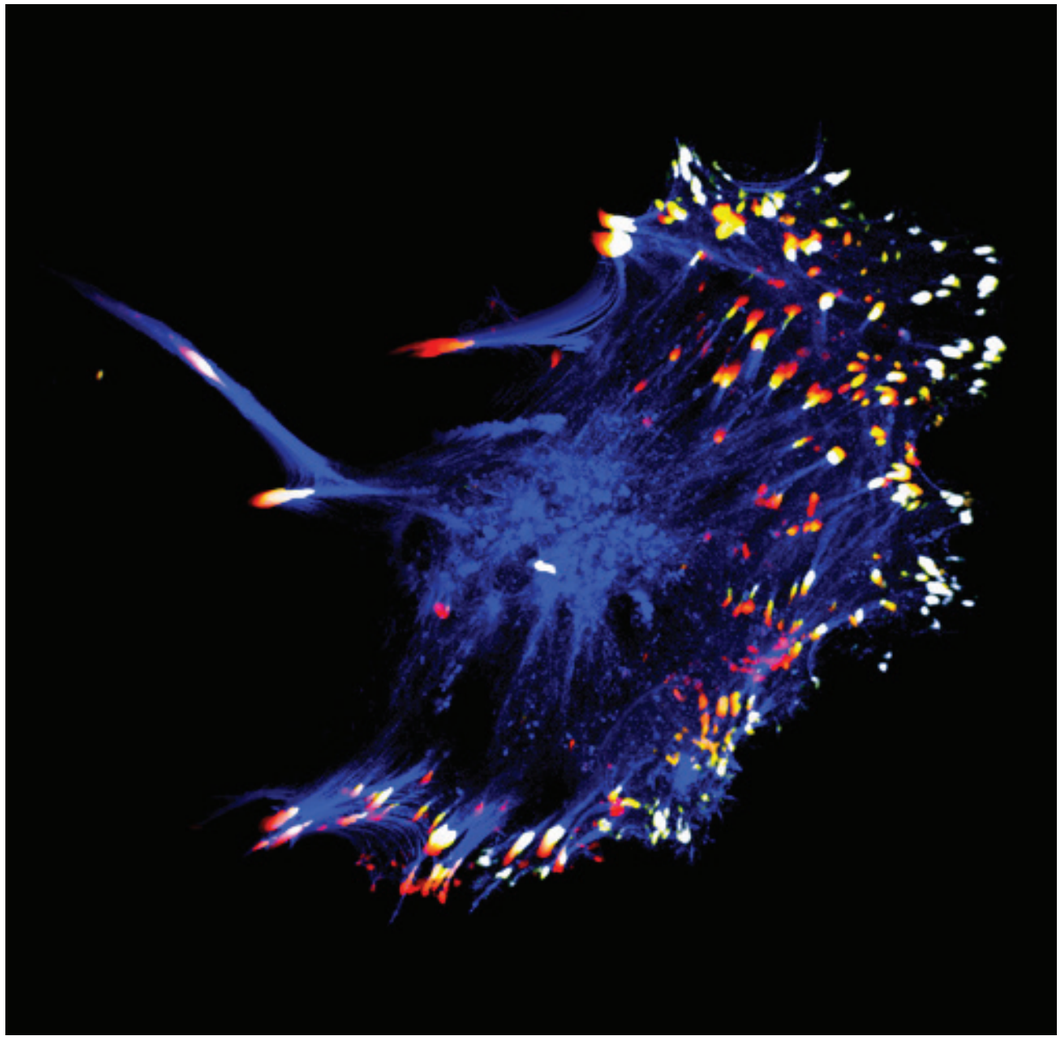
Two days of talks showcased the work of the main collaborating sites including guest speaker Julia Cordero (University of Glasgow), Doug Winton (University of Cambridge) and Sabine Tejpar (UZ Leuven, Belgium).

The meeting was rounded up by a training day on "Preclinical Modelling Systems" featuring presentations by Nicola Valeri (ICR, London), Steve Pollard (University of Edinburgh), Tamsin Lannagan (CRUK Beatson Institute, Glasgow), Lars Vereecke (UZ Ghent, Belgium), Henner Farin (Georg-Speyer-Haus Institute for Tumor Biology and Experimental Therapy, Frankfurt), Chris Tape (UCL Cancer Institute) and Danny Allen (University of Oxford).

The ACRCelerate consortium will celebrate this meeting's success by bringing together both scientists and clinician at the forefront of colorectal cancer research at future workshops across the collaborating sites.

We would like to express our gratitude to Advanced Cell Diagnostics, AMSBIO, Cell Signaling Technology, Merck, Life technologies (Thermo Fisher Scientific) and Transnetyx for their invaluable sponsorship towards this workshop.

Poul Sorensen (middle) gave the Colin Thomson Memorial Lecture at the Beatson International Conference 2019; pictured with Venya, representative from Worldwide Cancer Research and Kevin, Conference organiser



Mouse embryonic fibroblasts expressing LifeAct-TagRed and pEGFP-Paxillin and imaged every minute for 30 minutes. Focal adhesion turnover is shown is shown over time from red to yellow. Actin cytoskeleton (Blue), Paxillin (Red to Yellow). Imaged on Zeiss 880.
Image by Jamie Whitelaw

SEMINARS

The following seminars were held at the Cancer Research UK Beatson Institute throughout 2019.

January

Fatima Mechta-Grigoriou, Institut Curie, Paris, France

February

Tom McKerrall, Cambridge University Hospitals NHS Foundation Trust, University of Cambridge, UK

Steve Pollard, MRC Centre for Regenerative Medicine, University of Edinburgh, UK

Sarah-Maria Fendt, Center for Cancer Biology, VIB-KU Leuven, Belgium

Allison Bardin, Institut Curie, Paris, France

March

Michael Wakelam, Babraham Institute, Cambridge, UK

Alasdair Russell, Cancer Research UK Cambridge Institute, University of Cambridge, UK

Adrian Saurin, Division of Cancer Sciences, University of Dundee, UK

Graham Pavitt, Division of Molecular and Cellular Function, University of Manchester, UK

April

Andrés Cano Galiano, Department of Oncology, Luxembourg Institute of Health, Luxembourg

Colin Goding, Ludwig Institute for Cancer Research, University of Oxford, UK

Ainhoa Mielgo, Institute of Translational Medicine, University of Liverpool, UK

May

Miguel Martins, MRC Toxicology Unit, University of Cambridge, UK

Bart Vanhaesebroeck, Cancer Institute, University College London, UK

Harald Stenmark, Institute for Cancer Research, Oslo University Hospital, Norway

Mondira Kundu, St. Jude Children's Research Hospital, Memphis, USA

June

Christoph Borner, Institute for Molecular Medicine, University of Freiburg, Germany

Conchita Vens, Netherlands Cancer Institute, Amsterdam, Netherlands

Tony Green, Wellcome /MRC Cambridge Stem Cell Institute, University of Cambridge UK

July

Gail Risbridger, Biomedicine Discovery Institute, Monash University, Australia

Ross Cagan, Icahn School of Medicine at Mount Sinai, New York, USA

August

Duncan Graham, Technology and Innovation Centre, University of Strathclyde, UK

Susan Ozanne, Department of Clinical Biochemistry, University of Cambridge, UK

Bin Tean Teh, Duke-NUS Medical School, Singapore, Singapore

Anders H Lund, Biotech Research & Innovation Centre, University of Copenhagen, Denmark

Daniela Krause, Institute for Tumor Biology and Experimental Therapy, Frankfurt, Germany

Eileen White, Rutgers Cancer Institute of New Jersey, New Brunswick, USA

September

Valerie Speirs, Institute of Medical Sciences, University of Aberdeen, UK

Melissa Junttila, Genentech, San Francisco, USA

Richard Youle, National Institutes of Health, Bethesda, USA

October

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

James Kirkland, Robert and Arlene Kogod Center in Aging, Mayo Clinic, Rochester, USA

November

Frederic de Sauvage, Genentech, San Francisco, USA

Jacqui Shields, MRC Cancer Unit, University of Cambridge, UK

Jean-Charles Nault, French Institute of Health and Medical Research, Paris, France

Cristina Lo Celso, Imperial College London/ Francis Crick Institute, UK

December

Helen Weavers, University of Bristol, UK

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.

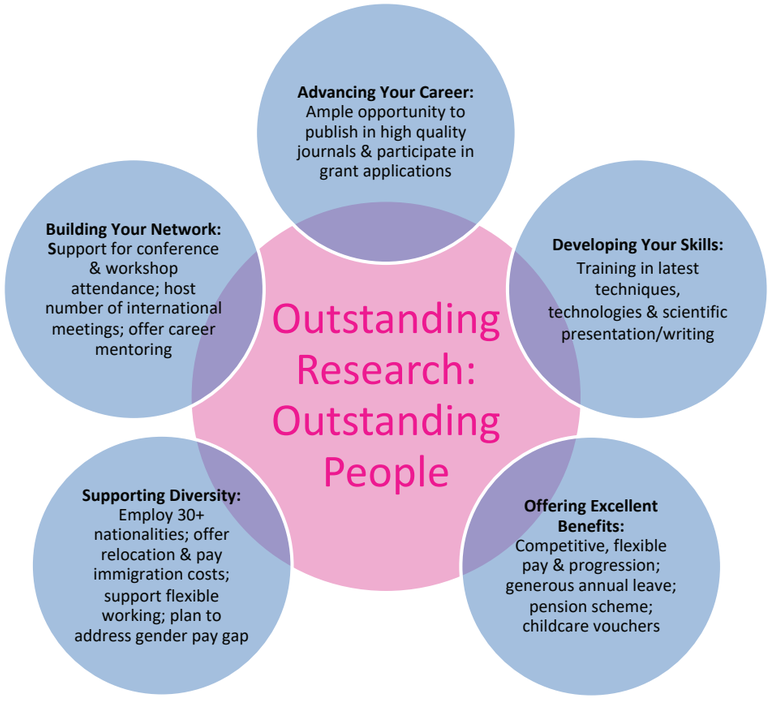
Our students are fully integrated with University of Glasgow graduate school (www.gla.ac.uk/colleges/mvls/graduateschool) and are allocated primary and secondary supervisors who are jointly responsible for supporting and monitoring their progress. The primary supervisor is responsible for developing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

Human Resources

Angela Stuart FCIPD, Elaine Marshall ACIPD, Selina Mungall GCIPD

Our mission is to enable cancer discovery for patient benefit by providing a professional finance and human resources service to our stakeholders to allow them to efficiently and effectively manage the Institute's resources. Our vision is to be a Finance and Human Resources team that is professional, open, inclusive and collaborative.

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 costing and management information required by operational managers. During the year, the team completely rebuilt the annual budget model, with automated links into the new HR system, and introduced a five-year forecasting and scenario-planning model.

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 2019, the team continued to develop the new HR system by introducing employee self-service and online performance management. HR also rolled out a mental health wellbeing initiative with awareness sessions for staff and training for Mental Health First Aiders. The recruitment of a new HR Administrator has led to an improved focus on recruitment, onboarding and induction. As we enter 2020, we will be working with CRUK on a branding project and we will also introduce improved performance and development reviews for the postdocs at the Institute with a focus on developing key leadership, collaboration and personal effectiveness skills.

In addition, the Finance and Human Resources 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.

Administration

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

The Administration team provides an extensive range of secretarial 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 submission; providing external communications for the Institute such as an up-to-date website and reports on the Institute's research outputs; and setting agendas and taking minutes at scientific meetings and reviews.

In 2019, the team took a leading role in organising a joint retreat with the other CRUK Institutes and a review of our Advanced Technologies groups. We also supported a number of grant applications, including successful Accelerator Award, Clinical Academic Training Programme and Radiotherapy Research Network bids, and the arrival in October of 18 new PhD students and clinical research fellows. Fiona Paulin-Ali joined the team as Project Coordinator for the Colorectal Accelerator Award, while Kate Schraut joined as maternity cover for Angela Kelsey. Jackie Beesley and Catherine Winchester also presented the Institute's approach to research integrity training at the 'World Conference on Research Integrity' in Hong Kong in May.

Cancer Research UK's Commercial Partnerships

Natasha Tian PhD

Cancer Research UK's Commercial Partnerships Team helps to develop promising research into new cancer therapeutics, diagnostics and enabling technologies. The team helps to develop and commercialise exciting new CRUK-funded discoveries. 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 manages intellectual property and commercialisation through CRUK's subsidiary Cancer Research Technology (CRT), an oncology-focused technology transfer and development company wholly owned by CRUK. CRT has exclusive rights to £350 million of cancer research per year and leveraged £20 million in industry funding to progress promising cancer research last year. This includes the recent major 3-year collaboration between the Beatson 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 Beatson Institute comes from CRUK, CRT manages all intellectual property–related matters on behalf of the Institute and the Charity. To facilitate this, there is a CRUK Commercial Partnerships Translation Executive based full-time at the Institute helping to assist with the translation and commercialisation of new CRUK-funded research.

CANCER RESEARCH UK'S PUBLIC ENGAGEMENT

Research Engagement Manager
Kirsteen Campbell
Cancer Research UK

Public engagement with research is increasing in importance as charities and institutions realise that transparency, trust and two-way communication with the public, and people affected by cancer, can be of great benefit and deliver impactful interactions.

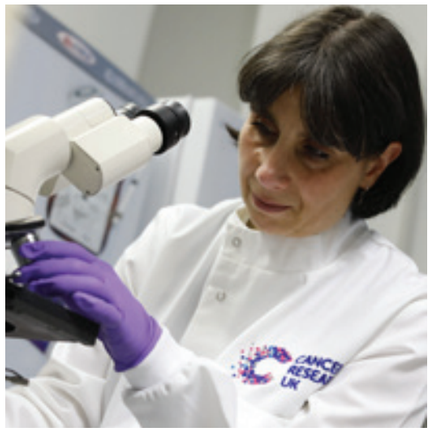
There is a long-established culture of participation in public engagement at the CRUK Beatson Institute, from supporting Cancer Research UK's events by providing speakers to scientists participating in STEM events at local schools. In 2019, over 120 researchers were involved in our public engagement programme.

Karen Blyth was selected to be part of CRUK's Legacy Pledge campaign which has seen supporters and scientists coming together to achieve incredible things and make advances in research that will last forever.

The 2019 Beatson Institute Open Evening, hosted by **Rob Insall**, welcomed 170 people to hear about the multi-disciplinary and collaborative approach to cancer research in Glasgow. Our scientists delivered lay talks to highlight progress being made in our labs and provided the opportunity for visitors to discuss research more informally through engaging activities, tours and one to one discussion.

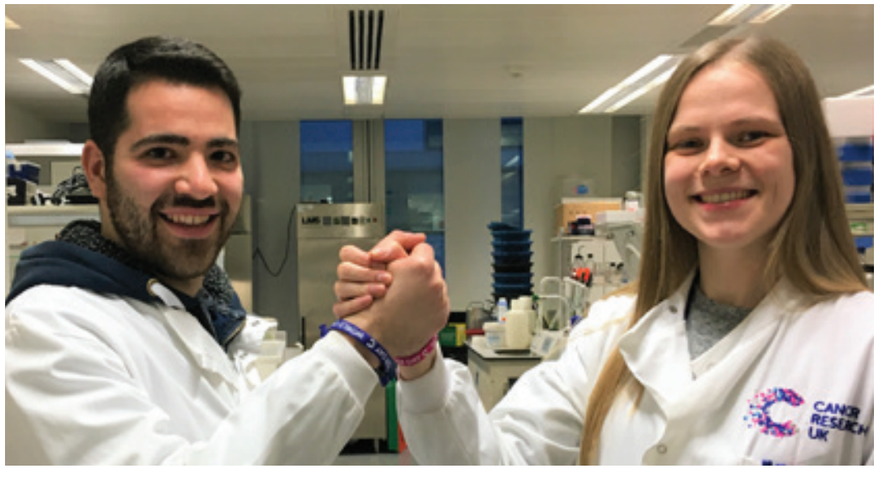
Throughout the year, staff supported a range of scheduled lab tours for CRUK charity partners, local politicians, supporters and volunteers. A core team of staff support the lab tours, and their expertise in speaking about research in lay terms is invaluable. The Beatson Institute is a flagship for CRUK in Scotland showcasing our work to charity partners such as ScottishPower, TK Maxx and the Business Beats Cancer Board. This is critical to ensure supporters trust and value the pioneering work we undertake.

Work is ongoing to develop our digital engagement strategy, as increasingly individuals seek out more ways to connect with research beyond the traditional face to face interaction. Our aim is to broaden awareness of the innovative research being led by the Institute including profiling our involvement in CRUK's Grand Challenge, Accelerator and Pioneer Awards. We are offering the public ways to engage with our staff and better understand the impact that research has on the wider population. A wide variety of Institute staff have been included in both Scottish and national CRUK social media feeds for events such as Ada Lovelace Day, World Cancer Day and Pride Month.



As part of our commitment to equality, diversity and inclusion last year, we saw the Beatson host the first panel discussion specifically talking about LGBTQIA+ in academia. Hosted by **David Bryant**, over 80 staff and students attended and the panel members' willingness to openly discuss their experiences of being part of the LGBTQIA+ community allowed for an open, supportive and inclusive event.

Public engagement is thriving at the Institute and it has been rewarding to see an increase in staff driving their own initiatives to invite colleagues and the public into their world and highlight our vision to beat cancer.



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, University of Glasgow Paterson Endowment Fund, Wellcome Trust

Karen Blyth
Breast Cancer Now

Martin Bushell
AstraZeneca, BBSRC, Celgene, Medical Research Council

Leo Carlin
Imperial College London, National Heart & Lung Institute Foundation

Drug Discovery Unit
Celgene, Daphne Jackson Trust

Danny Huang
European Research Council, Nuevolution

Gareth Inman
DEBRA

Robert Insall
EPSRC Physics of Life, UKRI

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

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

Jennifer Morton
Medical Research Council, Pancreatic Cancer UK

Jim Norman
West of Scotland Women's Bowling Association

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

Alexei Vazquez
European Community

Institute of Cancer Sciences, University of Glasgow

David Bryant
EssenBio, Royal Society

Seth Coffelt
Breast Cancer Now, European Community, Medical Research Council, Naito Foundation, Pancreatic Cancer UK, Tenovus, Wellcome Trust, William Forrest Charitable Trust

West of Scotland Women's Bowling Association marking their continued support over the last 65 years together with Beatson Scientists.

David Lewis
Beatson Cancer Charity

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

Saverio Tardito
Cancer Research Technology
EMBO

Stephen Tait
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

PATRONS AND BOARD OF DIRECTORS

Patrons

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

Board of Directors

The Beatson Institute is an autonomous charity, constituted as a company limited by guarantee, registered in Scotland. The Institute is governed by its Board of Directors who are the directors of the company and 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.

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

Ms Rita Akushie
Chief Financial Officer, CRUK

Mr Craig Anderson
Former Senior Partner, KPMG

Ms Rosalie Chadwick
Partner, Pinsent Masons

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

Prof Anton Muscatelli
Principal of the University of Glasgow

Company Secretary
Mr Gary Niven
The Beatson Institute for Cancer Research

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Website: www.beatson.gla.ac.uk

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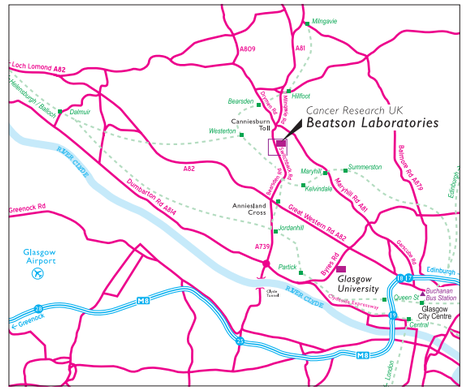
Switchback Road, Bearsden,
Glasgow G61 1BD

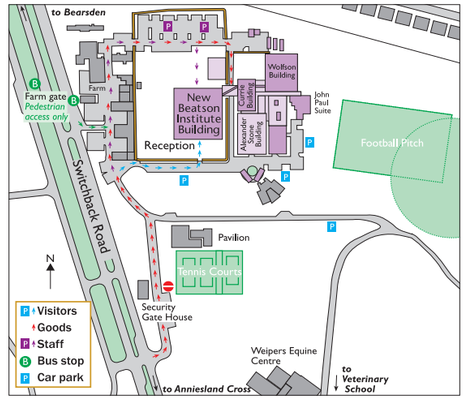
Tel +44(0) 141 330 3953
www.beatson.gla.ac.uk

Electronic version of this report can be found at:
www.beatson.gla.ac.uk/annual_report

Cancer Research UK
Cancer Research UK is a registered charity in England and Wales (1089464), Scotland (SC041666) and the Isle of Man (1103).
Registered address: Angel Building, 407 St John Street, London EC1V 4AD

Tel +44(0) 20 1234 5678
www.cruk.org





NOTES

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