SCIENTIFIC REPORT 2018

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BEATSON INSTITUTE

University of Glasgow

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Beatson Institute building. Photo credit: Laura Machesky

CONTENTS

DIRECTOR'S INTRODUCTION

Director of the Cancer Research UK Beatson Institute

Professor Owen Sansom FRSE, FMedSci

2018 was a particularly busy and productive year for the Institute, and I would like to thank everyone who contributed to making it such a success. We have published extremely well in all of our research theme areas and our group leaders have been active in taking our findings to international audiences.

At the start of the year, we held an off-site retreat to present our future research strategy to all members of staff. This was an extremely positive event as it gave us ample opportunity to interact and share ideas, and to welcome and integrate our new recruits. We welcome Crispin Miller (from the CRUK Manchester Institute) as a Senior Staff Scientist, and Head of Bioinformatics and Data Science. Crispin will be starting early in the New Year and, with his interest in machine learning techniques and the regulation of gene expression, he will be an excellent fit for us. In addition, our new Director of Research Strategy, Gareth Inman (from University of Dundee) started in September. He will oversee operations within the CRUK Glasgow Centre and help coordinate a number of large strategic bids from Glasgow in the coming months, as well as running his research group focused on TGF beta and skin cancer. Finally, Senior Group Leader, Martin Bushell and his team joined us from the MRC Toxicology Unit in June. Martin brings a wealth of expertise in RNA biology and translation to the Institute, which will not only contribute to his own research programme but also to collaborative work with the Drug Discovery Unit (DDU) and others. We said farewell to Senior Group Leaders, Mike Olson (Ryerson Institute, Canada), Eyal Gottlieb (Technion Institute, Israel) and Peter Adams (Sanford Burnham Prebys Medical Discovery Institute, USA), and thank them for their contributions to the Institute over a number of years and wish them all every success in their new positions.

Our DDU is an important component of our strategy and mission to apply discovery science to the clinic, and we were pleased to be able to appoint internal candidates Justin Bower and Heather McKinnon as its joint heads in February. Each brings complementary skills to the leadership of the DDU; Heather with her considerable experience of pharmacology and the pharmaceutical industry, and Justin with his particular expertise in chemistry and fragment-based drug design.

To further facilitate interactions with the University of Glasgow, I became Director of the Institute of Cancer Sciences in August, with Jeff Evans acting as my clinical deputy and Laura Machesky as my non-clinical deputy. This year, we worked extensively with the University on the recruitment of senior clinical academics and have been able to attract Richard Wilson from Belfast as Professor of Gastro-Intestinal Oncology. His role will support our translational research in colorectal cancer. Glasgow was also successful in securing a CRUK Accelerator Award application focused on colorectal cancer, which I am delighted to be leading. Importantly, this will consolidate models systems across the UK (Glasgow, Edinburgh, Birmingham, Oxford and Manchester), Barcelona and Turin, with the aim of driving a new era of precision medicine trials in colorectal cancer. In addition, Tom Bird led the Glasgow component of Newcastle's successful liver cancer Accelerator Award bid.

Our Scientific Advisory Board (SAB) met for the first time in October and was very positive about our strategy, key research themes and collaborative ethos as well as the DDU's role in challenging drug discovery projects. The SAB suggested that our third, emerging research theme of precision-prevention will require further development through external funding and additional recruitment of a senior academic with expertise in early detection technologies. We are extremely grateful to our SAB members for their input and advice, as we are to a number of other external reviewers who helped us throughout this year with reviews of our junior group leaders and intermediate clinician scientists.

This year, the NCRI Cancer Conference was held for the first time in Glasgow. Margaret Frame (Edinburgh) and I co-chaired this and were very pleased with the quality of the programme and the level of participation. One of our junior group leaders, Seth Coffelt was awarded the BACR AstraZeneca - Young Scientist Frank Rose Award 2018 at the meeting, which was very well deserved. In addition, our annual Beatson International Cancer Conference was again a resounding success and I would like to take this opportunity to thank Jim Norman, who has chaired the meeting for a number of years, for making it such an excellent event, especially as it enables us to showcase the Institute and its research. In addition, the Institute's approach to good practice in research was highlighted by an article in Nature describing the role of our research integrity adviser, Catherine Winchester.

Nathiya Muthalagu (second from left) was awarded the prestigious l'Oréal UNESCO Women in Science award.

We continue to engage with CRUK fundraisers, donors and members of the public here at the Beatson and also at events elsewhere, and are always delighted to be able to tell people about the cancer research we are doing and its potential impact for cancer patients. In March, we held a well-attended open evening for high school students and teachers, which included excellent, interactive talks by Anna Koessinger, Jiska van der Reest, David McGarry and Karen Blyth. Our researchers also conducted a large number of lab tours throughout the year and we are particularly grateful to Margaret O'Prey (BAIR) and Colin Nixon (Histology) for their efforts in this regard as the feedback we get from visitors about their demonstrations is always so positive.

Finally, a number of our early career researchers were successful in 2018, including Toshi Suzuki who was awarded an ERC Marie Sklodowska Curie Actions Individual Fellowship, Nathiya Muthalagu who received a L'Oréal Foundation UK & Ireland Fellowship for Women in Science and Evangelos Giampazolias who won the Pontecorvo Prize for the best CRUK PhD thesis of 2017. Two of our postdocs were also awarded independent fellowships to set up their own labs - Simone Cardaci won an AIRC Starter Grant to work at the San Raffaele Research Institute in Milan, while Johannes Meiser received a FNR Luxembourg ATTRACT Programme - and we wish them both every success for the future.

RESEARCH HIGHLIGHTS

In 2018 our scientists have made a number of key discoveries in the following strategically focused areas:

Energetic Stress and Metabolism

This year, we published key papers highlighting the rewiring of cancer metabolism in complex tumours (Maddocks et al. Nature 2018), the need for in vitro conditions to more precisely recapitulate in vivo conditions (Vande Voorde et al. Science Advances 2019), and the requirement to measure these in vivo (Meiser et al. Nature Communications 2018). We also expanded our work in lipid metabolism (Ackerman et al. Cell Reports 2018).

Given our focus on modelling pancreatic and colorectal cancers, we have shown that depletion of certain key kinases and provision of sugars such as mannose expose new metabolic dependencies in colorectal cancer (Port et al. Cancer Discovery 2018, Gonzalez et al. Nature 2018, Scopelliti et al. Cell Metabolism 2018), and we have used novel imaging techniques to unravel the role of hypoxia in resistance to metabolically targeted drugs (Conway et al. Cell Reports 2018).

Microenvironment and Metastasis

We have focused our invasion and metastasis research theme on the metastatic niche and the requirements for metastases to disseminate and grow, which include immune suppression. We have shown that macrophages are highly immunosuppressive in pancreatic cancer (Candido et al. Cell Reports 2018), that exosomes from mutant p53 driven pancreatic cancers shape the pro-invasive niche in metastatic target organs (Novo et al. Nature Communications 2018). We have described a number of new pathways leading to loss of polarity and invasiveness in cancer (Dornier et al. Nature Communications 2017, Fort et al. Nature Cell Biology 2018, Roman-Fernandez et al. Nature Communications 2018) and elucidated new mechanistic details of the functioning of the T cell immune synapse (Stephen et al. Developmental Cell 2018).

Early Disease Biology

We have generated new models of early colorectal cancer, mesothelioma, lung, pancreatic and hepatocellular cancers (Sansom, Murphy and Bird laboratories) and we believe these will be important for early detection of cancers in the future. We have made fundamental observations that early events in carcinogenesis, such as reduced stem cell competition, may increase tumour initiation (Huels et al. Nature Communications 2018), that inhibiting Wnt signalling and transcriptional programmes (but not the Wnt ligand levels) potently suppresses colorectal tumourigenesis (Gay et al. Nature Communications 2019) and that targeting TGFbeta can restore regeneration in acute liver injury by suppressing senescence (Bird et al., Science Translational Medicine 2018). We have also helped highlight the importance of cancer prevention in the press. Tom Bird wrote an opinion piece in the Herald on obesity and liver cancer in Scotland, in support of CRUK's Scale Down Cancer campaign, and Michael Hodder's work from the Sansom laboratory on prevention was featured in the Scotsman and the Herald.

PRECISION-Panc network

We are dedicated to enabling the delivery of precision medicine clinical trials for cancers of unmet need, in particular pancreatic cancer. PRECISION-Panc, which is led by scientists and clinicians in Glasgow, started this year. Eleven sites opened for recruitment to Master Protocol; 60 pancreatic cancer patients were screened, 43 were registered and 17 were randomised to the PRIMUS-001 trial (first line, metastatic). Moreover, we are using the information from the genetic analysis of these human tumours to generate new pre-clinical models of PDAC in mice that, in turn, will enable the rationale and structure of clinical trials for pancreatic cancer to be supported mechanistically.

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 GROWTH AND METABOLISM

www.beatson.gla.ac.uk/imran_ahmad

MODELS OF ADVANCED PROSTATE CANCER

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

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

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

In addition, to our knowledge, we are the first to demonstrate the strength of the Sleeping Beauty transposon model system in successfully determining low-frequency somatic mutations that may drive prostate tumourigenesis. We are further investigating and validating other novel and clinically relevant 'hits' from this screen.

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.

Publications listed on page 82

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

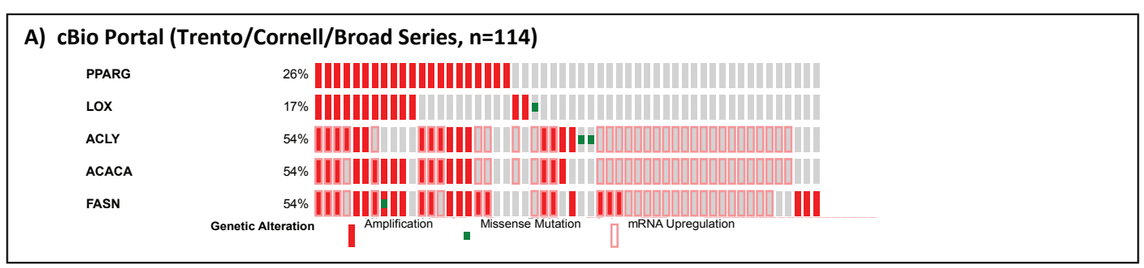


Figure 2
Genetic modifications of the PiggyBac mice.

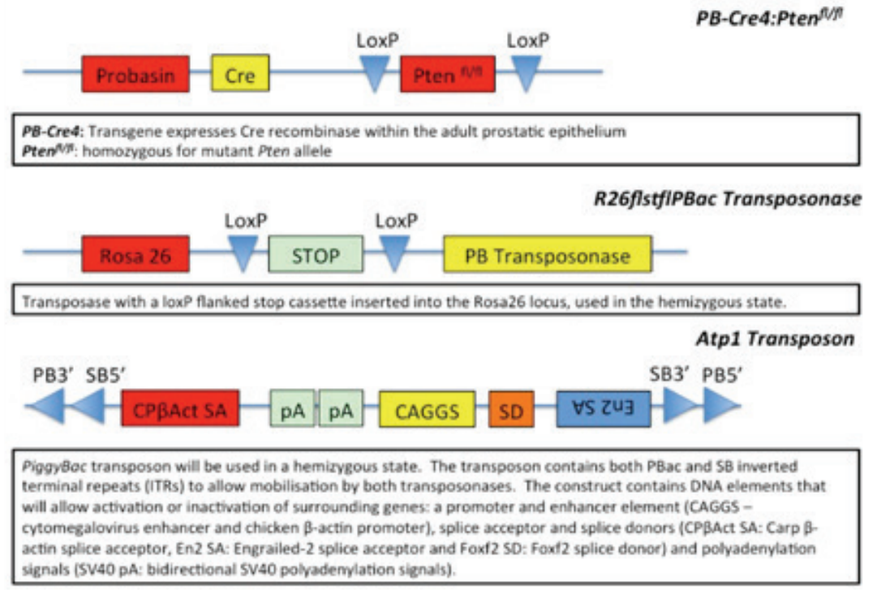
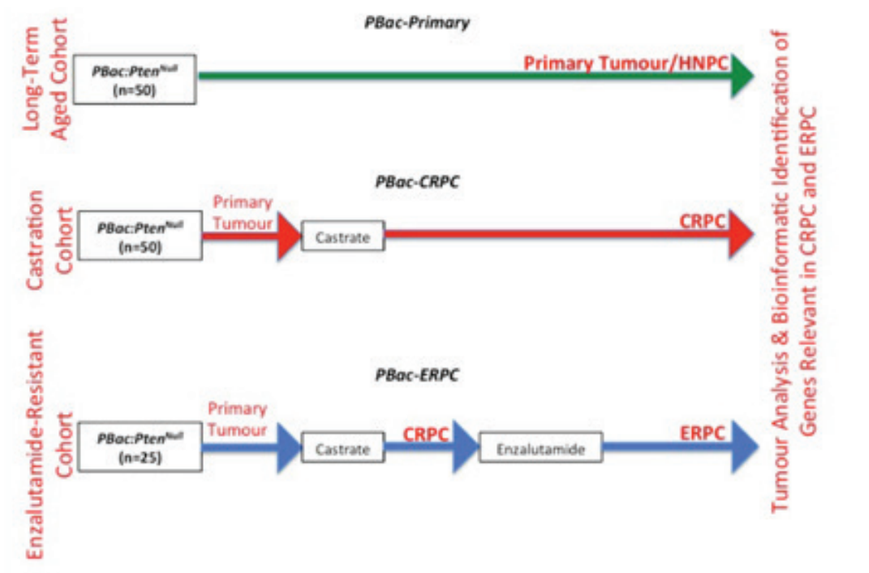


Figure 3
Experimental design for the ageing, castration and enzalutamide-treatment of the PiggyBac (PBac) mice.



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RNA AND TRANSLATIONAL CONTROL IN CANCER

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The dysregulation of protein synthesis in the tumour clone and the supporting stroma is essential for the delivery 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 required to create the microenvironment essential to support the growth of the tumour clone. These distinct programmes of gene expression dictate the capacity of the tumour to grow 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 to decoding to mRNA decay. One such critical RNA helicase is eIF4A1 which, as part of the eIF4F complex, is responsible for the initial steps leading to the decoding of mRNAs, a key rate-limiting event in gene expression. eIF4A1 is specifically required for selection of mRNAs encoding oncogenic proteins within the highly proliferating tumour cell clone, due to their high degree of secondary structure within the 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 only functions to unwind RNA when in a trimeric state, with different eIF4A1 subunits performing distinct enzymatic activities within this complex (Fig 1). This insight opens up new avenues for drug discovery activities targeting this central oncogenic driver and is currently being investigated in close collaboration with the Institute's Drug Discovery unit.

eIF4A1 has a closely related paralogue, eIF4A2, which shares over 90% identity at the amino acid level. This helicase has previously been believed to function interchangeably with eIF4A1 within the eIF4F complex. However, our studies have highlighted that eIF4A2 is mainly expressed in the supporting stromal compartment of the tumour, unlike eIF4A1 which is predominately expressed in the tumour clone (Fig 2). We are currently investigating the role of eIF4A2 within the stromal compartment; however, it is clear that eIF4A2 does not interact with the eIF4F complex but rather with an RNA binding complex central to mRNA decay, the CCR4-NOT complex (Meijer *et al.*, Science 2013; 340: 82–5). Understanding the role of these two helicases in these 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.

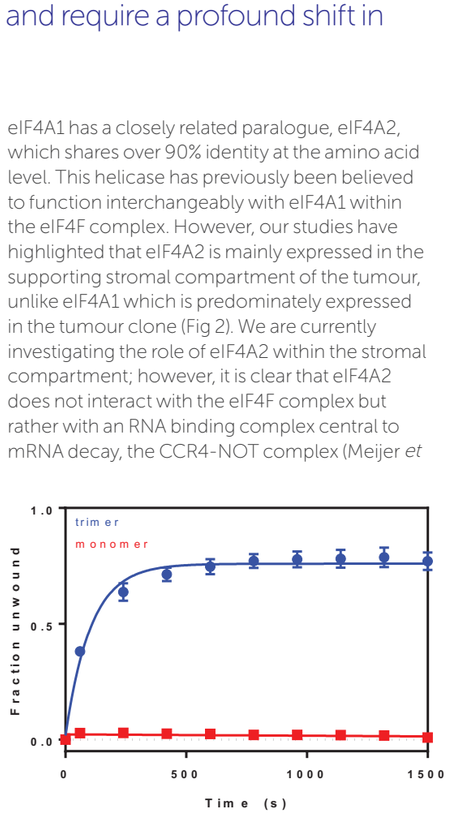
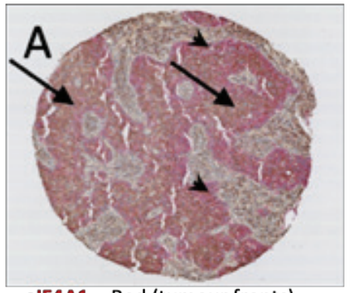
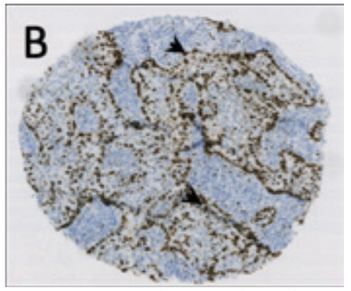


Figure 1
Real-time unwinding assay shows only high-order eIF4A1 complexes separate an RNA duplex, while monomeric eIF4A1 is completely inactive.





eIF4A1 – Red (tumour fronts)
eIF4A2 – Brown (central tumour areas & stroma)

Ki67 - Brown (tumour fronts)

Figure 2
eIF4A1 and eIF4A2 exhibit inverse patterns of expression in tumour tissue, which is related to proliferation.
Duplex immunostained TMA core of lung adenocarcinoma showing expression of eIF4A2 at the centre of solid tumour islands (brown areas, arrows) and eIF4A1 expression at invasive fronts (red areas, arrowheads). eIF4A2 expression is also high in desmoplastic stroma (Dr John Le Quesne, University of Leicester).

Oncogenic gene programmes have distinct codon patterns and require modulated tRNA expression

The degeneracy of the genetic triplet code means that multiple codons and cognate tRNAs can 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. In yeast, mRNAs encoding proteins with the same amino acid sequence, but with distinct synonymous codon usage, have dramatically different mRNA half-lives. Those with a codon composition less compatible with the cognate tRNA pool are very poorly translated and more rapidly degraded (Radhakrishnan *et al.*, Cell 2016; 167: 122–32).

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.

In yeast, the helicase Dhh1 (DDX6 human homolog), which also interacts with the CCR4-NOT complex, much like eIF4A2, has been shown to 'sense' imbalances between the frequency of synonymous codons and tRNA availability and cause destabilisation of mRNAs with non-optimal codon composition.

We have been investigating three major mRNA binding complexes which regulate different phases of the mRNA lifecycle (Fig 3). eIF4A1, as part of the cap binding complex eIF4F, interacts with mRNAs displaying a synonymous codon profile similar to that for proteins promoting differentiation. At the opposite end of the mRNA lifecycle lies a complex central to miRNA-mediated repression and mRNA decay – the CCR4-NOT complex, which interacts with two helicases: DDX6 and eIF4A2. We find that the mRNA interactome of this complex has a distinct codon profile from that of eIF4A1 in the eIF4F complex, in keeping with both its described 'sensing' of non-optimal codons in yeast and its role in mRNA repression and decay. This is the first evidence that human DDX6 functions to determine mRNA fate by interrogating tRNA/codon utilisation within a given mRNA.

We are now continuing our studies to understand how codon optimality and tRNA abundance dictate mRNA stability and protein output in model systems developed by different groups within the Institute. 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.

Publications listed on page 83

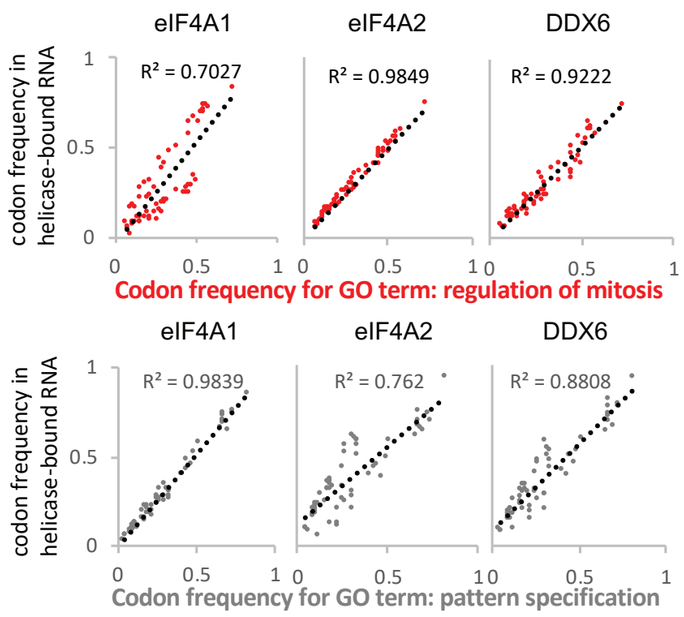


Figure 3
mRNAs bound by the Ccr4-NOT complex (eIF4A2 and DDX6) have a distinct synonymous codon signature to mRNAs bound by eIF4A.
This signature conforms to the codon signature identified in Gingold *et. al.* Cell (2014), showing that mRNAs encoding pro-mitotic or pro-differentiation genes have distinct synonymous codon frequencies. Synonymous codon frequency in the coding sequence of mRNAs bound by the indicated proteins plotted against codon frequency in mRNAs representing the GO term 'regulation of mitosis' or 'pattern specification'.

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 (Fig. 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 functions, 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 develop leukemia. More recently, inhibition of CBL-B in immune cells has been shown to activate their anti-metastatic activity. Over the past year we have investigated the mechanism by which CBL mutants exert oncogenesis and 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 inhibition of the 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 the MDM2–p53 axis.

Targeting RING E3s

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 (Fig. 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 domains often contain pockets or grooves to recognise certain amino acid motifs or post-translational modifications on the substrate and therefore can be targeted by small molecules or peptides.

Figure 1

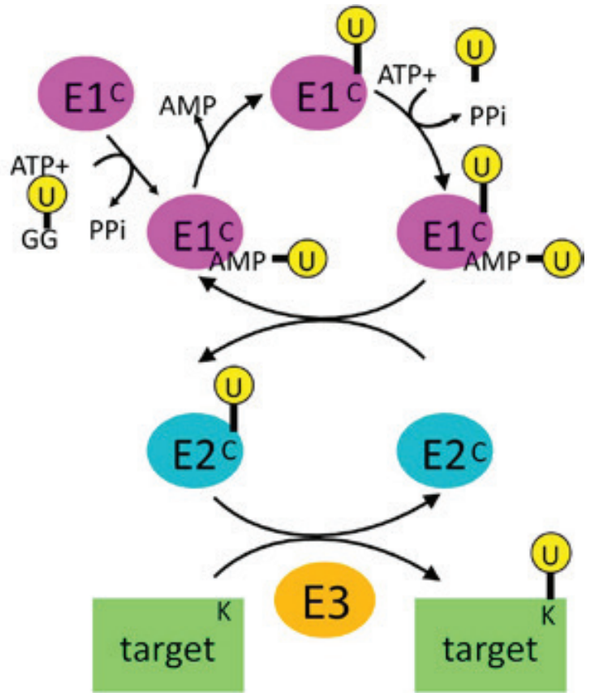

Figure 1
Enzymatic cascade for Ub modifications

Figure 2

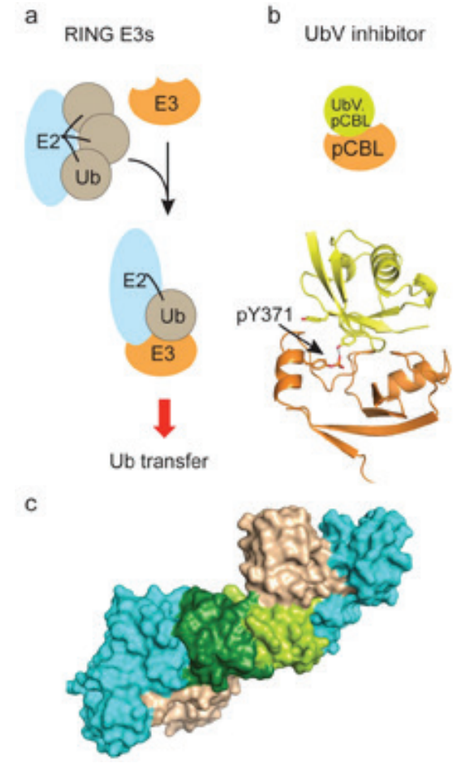

Figure 2
Mechanism and targeting of RING E3s
(a) Activation of the E2~Ub complex by the RING domain. The RING domain binds the E2~Ub complex and promotes the formation of the closed E2~Ub complex to facilitate Ub transfer.
(b) UbV.pCBL functions as an inhibitor. The crystal structure of the pCBL RING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

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.* Molecular Cell 2017; 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 (Fig. 2b). We have now applied both phage-displayed ubiquitin variant (UbV) and peptide libraries to identify UbVs or peptides that are selective for MDM2 and CBL and are currently characterising their actions and investigating the relevance of inhibition of these RING E3s in cancers.

Ligase-independent function of MDM2 in limiting p53 activity

We have determined the crystal structure of the MDM2–MDMX RING dimer bound to an E2, UbcH5B, covalently linked to Ub (Nomura *et al.* Nat Struct Mol Biol 2017; 24: 578–87). 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 p53 regulation and suggests that inhibition of the MDM2 E3 ligase activity may be a more gentle approach to activate p53. More recently, we have determined the crystal structure of the MDM2 RING homodimer bound to UbcH5B covalently linked to Ub (Fig. 2c). This structure reveals how homodimeric MDM2 recruits and activates E2~Ub for catalysis and suggests a potential strategy to selectively target the MDM2 homodimer and MDM2–MDMX heterodimer.

Publications listed on page 85

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.

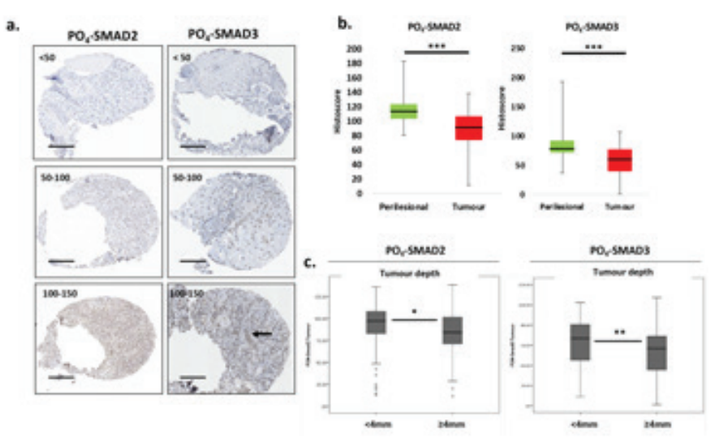

Figure 1 Active endogenous TGF-β signalling is significantly reduced in invasive cSCC compared to perilesional tissue.
A) Representative images of tumour cores from tissue microarray (TMA) at 10-fold magnification, demonstrating varying degrees of histoscores from low (<50) to high (100–150) for PO_4–SMAD2 and PO_4–SMAD3 immunostaining as labelled. Tumour–stroma heterogeneity labelled by arrow.
B) Graphical representative of mean histoscores for perilesional tissue (green) and tumour (red) across the whole TMA for PO_4–SMAD2 and PO_4–SMAD3 immunostaining as labelled. *** p = <0.001 (Wilcoxon matched pairs test). Scale bar = 200μm.
C) TMA histoscores identify a significant association between mean nuclear R-Smad expression and cSCC tumour depth. Nuclear PO_4–SMAD2 and PO_4–SMAD3 activity by tumour depth, defined as <4mm and ≥4mm.

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 frequently mutationally inactivated in human cutaneous squamous cell carcinoma (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). To definitively determine canonical TGFβ signalling activity in human skin and cSCC, we developed robust immunohistochemical techniques to measure PO_4–SMAD2 and PO4–SMAD3 levels in formalin-fixed, paraffin-embedded tissues and constructed a tissue microarray containing 230 primary human cSCC tumour cores with 150 matched perilesional tissue cores. PO4–Smad3 activity was found to be very low in the interfollicular epidermis (IFE) and concentrated in telogen hair follicle bulge stem cells in normal human skin, consistent with our previous studies indicating that cSCC may originate from this stem cell compartment (Cammareri et al., 2016). PO_4–SMAD2 activity was higher than that of PO_4-SMAD3 in the IFE and comparable to that found in peri-lesional tissue. Overall, a reduction in active nuclear PO_4–SMAD2 and PO_4–SMAD3 staining was detected in invasive tumours when compared to site-matched peri-lesional skin (Fig. 1), indicating that impaired canonical TGFβ signalling activity may represent a common feature of primary invasive human cSCC. There was a significant reduction in mean PO_4–SMAD2 histoscores seen in larger diameter tumours (≥20mm versus <20mm), a significant reduction in mean PO_4–SMAD3 histoscores in tumours invading beyond subcutaneous fat, and a significant reduction in both mean PO_4–SMAD2 and PO_4–SMAD3 histoscores seen in thick tumours (≥4mm versus <4mm) (Fig. 1). This suggests that the loss of TGFβ-mediated tumour suppression provides a significant growth advantage for cSCC tumour cells.

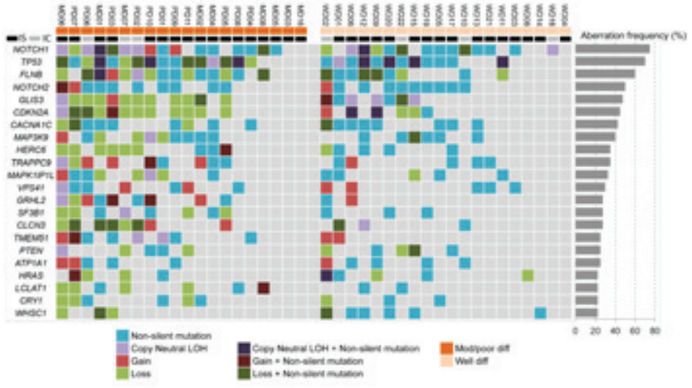

Figure 2
Twenty-two driver genes identified in 40 cSCC samples.
Mutation OncoPrint of the 22 driver genes identified by WES and at least two of the three methods (MutsigCV, OncodriveFM and OncodriveClust) with their mutational frequencies also indicated with percentage bars. IS = immunosuppressed, IC = immunocompetent patients from which tumours were isolated.

Adapted from Inman et al. 2018

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 Dr 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.

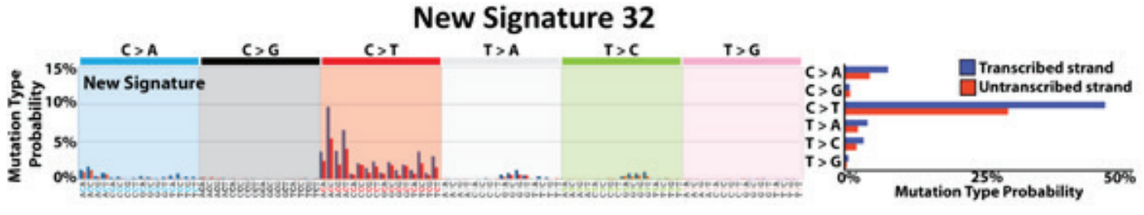

Figure 3
Novel mutational signature 32 identified in cSCC primary tumours from immunosuppressed patients
Signature 32 is predominately C>T mutations (75%) in combination with C>A, T>A and T>C mutations. There seems to be a clear presence for C>X at ApCpN and T>X at GpTpN. This novel mutational signature exhibits a very strong transcriptional strand bias, potentially indicating an interplay with transcription-coupled nucleotide excision repair due to adducts on guanine and adenine, and correlates with exposure to azathioprine.

Adapted from Inman et al. 2018

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 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, and initial whole exome sequencing (WES) analyses have revealed a high mutation rate with gross chromosomal aberrations and numerous copy number alterations indicative of genetic instability contributing to a complex molecular landscape (South et al., J Invest Dermatol. 2014; 134: 2630-8). In collaboration with Professors Irene Leigh, Catherine Harwood, Claude Chelala (Barts Cancer Institute), Charlotte Proby (University of Dundee) and Mike Stratton and Ludmil Alexandrov (Sanger Institute), we have performed WES of 40 primary cSCC tumours and integrated this analysis with separate gene expression data sets. These analyses have 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, including *NOTCH1*, *NOTCH2*, *TP53* and *CDKN2A* (Fig. 2). 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, Fig. 3). 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. In collaboration with Peter Bailey (University of Glasgow), we are expanding our cSCC molecular landscaping studies, deciphering the similarities with other squamous tumour subtypes and determining the biological roles of the genes, pathways and processes identified in these studies.

Publications listed on page 85

PROSTATE CANCER BIOLOGY

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Prostate cancer affects one in eight men in the developed world, and now accounts for more cancer-related deaths in men than breast cancer does in women. Despite improvement in patient survival with novel androgen receptor inhibitors and taxane chemotherapy, patients with advanced disease typically die within five years. We have a highly comprehensive cross-disciplinary programme of translational research aimed at tackling treatment (hormonal and/or taxane chemotherapy) resistance. We have at our disposal a wide range of preclinical models and clinical resources to help us discover new treatment targets and understand the molecular mechanisms of how aggressive prostate cancer can resist current treatment.

Our group applies cutting-edge technologies and innovative laboratory model systems to enhance our knowledge in treatment resistance. We ultimately aim to discover new therapeutic targets and develop better treatment strategies and improved clinical biomarkers to support personalised medicine in patients with advanced prostate cancer (Figure 1).

Mebendazole, an anthelmintic drug, is identified as a clinical candidate to enhance the efficacy of docetaxel to treat advanced prostate cancer

Docetaxel chemotherapy in prostate cancer has a modest impact on survival. To date, efforts to develop a combination therapy have not translated into new treatment regimes. It is increasingly appreciated that drugs previously developed for a specific indication may be successfully 're-purposed' to treat conditions that were not intended. To this end, we performed a drug repurposing screen using our treatment-resistant prostate cancer cell model. Cells were treated with docetaxel alone, or in combination with drugs. We assayed the viability of cells by nuclear counting using high-content imaging analysis.

Mebendazole, an anthelmintic drug that inhibits microtubule assembly, was selected from hit compounds for mechanistic and functional analyses (*in vitro and in vivo*) and shown to potently synergise docetaxel-mediated cell kill. Interestingly, both docetaxel and mebendazole bind to distinct aspects of the microtubule structure.

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 collaboration with Dr Christine Dufès (University of Strathclyde), we engineered liposomes to entrap docetaxel and mebendazole at a pre-determined ratio to study evidence of *in vivo* synergism. Combined treatment indeed drastically suppressed *in vivo* prostate tumour growth and extended progression-free survival when compared to singleton drug treatment. Collectively, our data suggests targeting microtubules with more than one agent may improve tumour response. We propose that the concept of combined mebendazole and docetaxel treatment therefore warrants formal clinical evaluation.

AR-mediated rewiring of cancer cell metabolism supports resistance to AR inhibitors in CRPC

Besides chemo-resistance, we have a major interest in understanding the molecular basis of tumour resistance to hormonal therapy (or androgen deprivation therapy), referred to as castration-resistant prostate cancer, CRPC.

Over the last decades, specific inhibitors targeting the androgen receptor (AR inhibitors or ARI) have shown promising effects in the clinic, significantly improving patient outcomes. However, despite the clinical success of AR-targeted therapies, reactivation of AR signalling remains the main driver of CRPC progression.

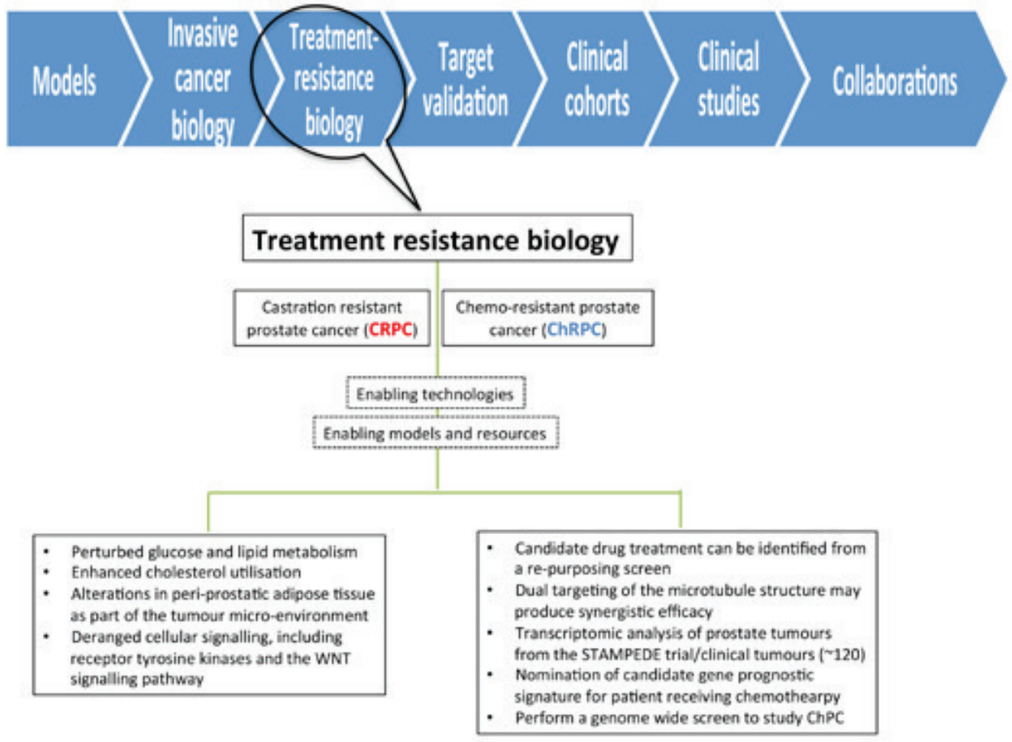

Figure 1
The research programme in our group comprehensively spans multiple aspects of translational cancer research, including basic discovery cancer biology studies, validation of novel druggable targets, collaborative drug discovery campaigns, clinical cohort studies and clinical trials of novel therapeutic agents. In this report, we highlight recent research milestones in our studies of treatment-resistant prostate cancer, namely castration-resistant and chemo-resistant disease. *Enabling technologies*: e.g. transcriptomic, proteomic and metabolomic analyses; *Enabling models and resources*: e.g. genetically engineered mouse models, orthotopic xenograft models, primary cultures, tissue microarray of clinically resected prostate tumours, bio-repository from clinical trials and biobanks.

To decipher the molecular mechanisms associated with treatment resistance, we performed a comprehensive unbiased characterisation of LNCaP cells chronically exposed to first- and second-generation ARI, namely bicalutamide, apalutamide and enzalutamide. Combined proteomics and metabolomics analyses implicated an acquired metabolic phenotype common in CRPC cells regardless of the inhibitor used or the culture conditions (2D or 3D). This metabolic reprogramming was associated with perturbed glucose and lipid metabolism and was dependent on AR signalling reactivation. In order to exploit this phenotype, we delineated a subset of proteins consistently associated with ARI resistance. We are currently performing experiments to formally validate the value of the protein panel as biomarkers of CRPC and to investigate the impact of candidate proteins in supporting CRPC progression through altered cell metabolism.

BRF1 accelerates prostate tumourigenesis and perturbs immune infiltration

Aberrant translation is an important molecular feature of carcinogenesis. RNA Polymerase III (Pol III)–mediated transcription controls the expression of transfer RNAs and other short non-coding RNAs such as the ribosomal component 5S RNA to support protein synthesis through translation. A rate-limiting factor for Pol III function is BRF1 RNA Polymerase III Transcription Initiation Factor Subunit (or BRF1). Using a cohort of 652 resected prostate tumours, we found that elevated levels of BRF1 protein expression was significantly associated with poor patient survival.

BRF1 is also reported to be linked to PTEN function, which is closely implicated in prostate cancer. We therefore developed a novel genetically engineered mouse model with BRF1 overexpression and Pten deficiency, which showed accelerated prostate tumourigenesis and shortened survival. Transcriptomic and proteomic analyses of the prostate tumours revealed altered immune and inflammatory processes, which were associated with decreased levels of factor D and C7 components of the complement cascade and reduced infiltration of neutrophils and CD4-positive T cells within the tumours. Furthermore, expression of C7 significantly correlates with expression of CD4, and low levels of C7 associate with poorer patient prognosis. Based on these data, we plan to further investigate the role of C7 and other components of the complement system as potential therapeutic targets in prostate cancer.

Publications listed on page 87

MOLECULAR IMAGING

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Our lab develops new ways to visualise cancer – we use state-of-the-art imaging methods such as PET/MRI to non-invasively detect and characterise tumour development. We create novel molecular imaging agents targeting metabolic reprogramming, a hallmark of cancer growth. These tools have a role in clinical imaging for diagnosing cancer and directing treatment. This year we developed novel vectors for tomographic imaging of tumour initiation and treatment response in cell lines and transgenic mouse models. By identifying heterogeneity in drug response, we aim to design better combination therapies.

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

The key advantage of using radionuclide reporter genes is their high sensitivity, penetration depth and ability to provide tomographic images. The application of radionuclide imaging reporters in cancer research has been principally in targeted radiotherapy, cell tracking and assessment of tumour growth. As the clinical application of drugs targeting metabolic pathways becomes reality, preclinical tools providing rapid readout of on-target effect could become useful tools for translational drug development.

We have explored the use of mNIS (mouse sodium iodide symporter protein) as a radionuclide imaging reporter gene to provide a direct and rapid readout of tumour cell response to therapy specifically targeting glycolysis and oxidative phosphorylation, the key pathways utilised by tumour cells to regenerate ATP. As a proof of concept, we produced clonal cell lines stably expressing mNIS protein by infecting the HEK293T cells with a 3rd generation lentiviral vector carrying the mNIS sequence under the control of the EF1alpha promoter. Using radioactive mNIS substrates used for PET or SPECT imaging ([^{18}F]tetrafluoroborate (TFB) or [^{99m}Tc]pertechnetate, respectively) and established treatments targeting singly, or in combination, glycolysis and oxidative phosphorylation (2-deoxyglucose and oligomycin A, respectively), we were able to demonstrate a rapid (within 30 minutes) decrease in uptake by the treated mNIS-expressing cells when compared to untreated 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 mNIS activity on the sodium gradient across the plasma membrane, generated by Na^+/K^+ ATPase in an ATP-dependent manner.

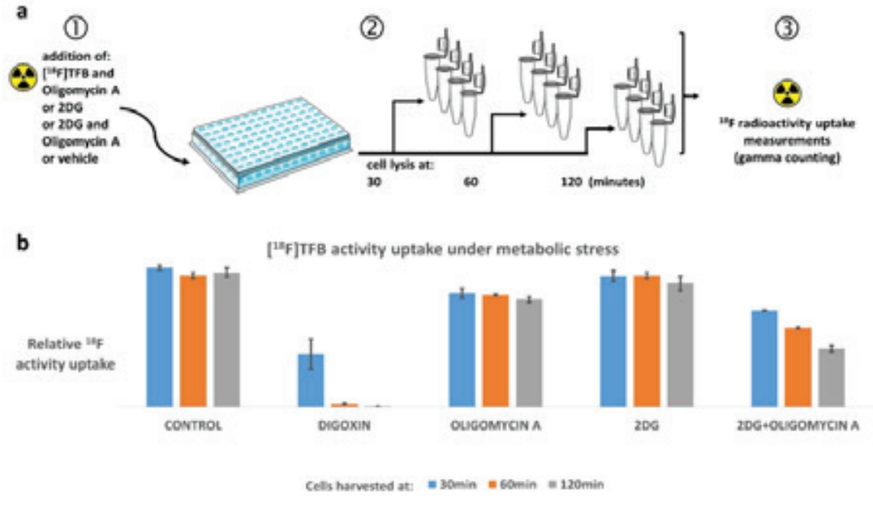

Figure 1
Sodium iodide reporter provides a rapid read-out of metabolic response
(a) HEK293T cells infected with a 3rd generation lentiviral vector PGK-Cre-EF1a-Luc-P2A-NIS. [^{18}F]TFB, and 1μM oligomycin A or 3mM 2DG, or a combination were added. 100nM of the Na/K ATPase inhibitor (Digoxin), was used as a positive control and DMSO was used as negative control. The cells were harvested at 30, 60 or 120 minutes and radioactivity counted.
(b) Modest reduction in ATP-dependent uptake of [^{18}F]TFB was observed following inhibition of glycolysis or oxidative phosphorylation. However, combination therapy produced a significant decrease. Na+/K+ ATPase dependence was demonstrated.

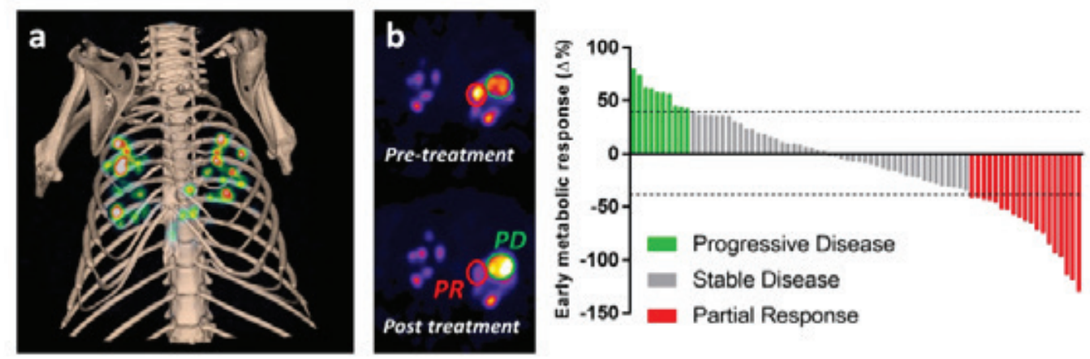

Figure 2
Non-invasive identification of responding and resistant tumour clones using radionuclide imaging
(a) Lung tumour imaging in the KRAS$^{G12D/+}$; p53$^{-/-}$ (KP) mouse with [^{99m}Tc]TcO_4^- SPECT/CT imaging after intranasal lentiviral LV-PGKCre-EF1NIS infection.
(b) Following 1 mg/kg bortezomib, 24% of tumours have early (24 hour) response (PR) while 13% have progressive disease (PD).

Non-invasive identification of clonal resistance using a multi-transgenic vector and tomographic reporter genes

We are entering a new era for some 'hard-to-treat' tumours like lung cancer. Recent clinical trials have shown a proportion of patients with sustained responses to new treatments. Typically only a subset of patients respond; therefore, we need to develop approaches to stratify patients prior to therapy and identify rare responders versus those with innate resistance. We have developed novel vectors for *in vivo* transduction of somatic cells, allowing imaging of spontaneous lung tumourigenesis and single lesion detection of drug responders to investigate the mechanisms underlying heterogeneity in drug response.

We developed a mouse model where we could identify and isolate responders with nanolitre resolution. To be relevant, a mouse model should share several clinical features of lung cancer, including genetic, phenotypic and response heterogeneity. To address these aims in collaboration with Scott Lyons, Cold Spring Harbor Laboratory, we developed two novel lentiviral vectors (LV-PGKCre-EF1SN and LV-PGKCre-EF1LS) to deliver multiple transgenic elements to somatic cells of adult mice with conditional (floxed) oncogenic *Kras* (LSL-Kras$^{G12D/+}$) and *p53$^{fl/fl}$* alleles (KP mice) by intranasal administration. Mice were longitudinally imaged using bioluminescence, radionuclides ([^{18}F]TFB PET and $^{99m}TcO_4^-$ SPECT) and CT. Drug responses were determined using a double baseline and imaging pre- and post treatment. Lesions down to tens of nanolitres could be repeatedly imaged with a coefficient of variation of 11.8%. Drug therapy identified single initiating lesions with significantly reduced $^{99m}TcO_4^-$ SPECT uptake and high CC3 staining not identified by bioluminescence or CT imaging (Fig. 2).

We are exploiting the tomography of radionuclide imaging to track single lesions at nanolitre resolution during cancer therapy and identifying inter- and intratumoral heterogeneity in drug response. These vectors comprise a new platform technology and provide a quantitative, 4D readout of cell viability, critical for monitoring therapeutic efficacy and identifying responders within a heterogeneous tumour.

Publications listed on page 88

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ONCOGENE-INDUCED VULNERABILITIES

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

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.

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 mean 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.

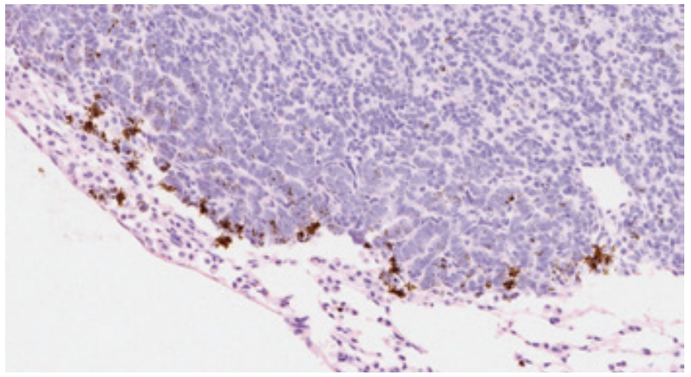

Figure 1
ERBB signalling during progression of KRAS mutant lung cancer
The image shows in situ hybridisation of the ERBB ligand Amphiregulin (*Areg*; black dots) at the interface between tumour and normal tissue. High expression of *Areg* may reflect progression from benign to invasive lung cancer but also may predict responsiveness to broad-spectrum ERBB inhibitors.

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.

Oncogene cooperation in pancreatic cancer

Activating mutations in KRAS initiate almost all cases of pancreatic ductal adenocarcinoma (PDAC), the deadliest form of pancreatic cancer. MYC is an obligate effector of RAS's oncogenic output, and genetic ablation of even one copy of MYC can dramatically extend the lifespan of KPC mice. In collaboration with Rosalie Sears (Oregon Health Sciences University) and Jennifer Morton, we are examining the role of MYC during pancreatic development to explore potential MYC-induced vulnerabilities that might reveal new therapeutic opportunities. We have shown that a modest elevation of MYC above physiological expression dramatically accelerates onset of PDAC and drives lineage plasticity that is strongly implicated in the severity of this debilitating disease.

Major developments in 2018

We published two primary papers in leading cancer research journals along with six related datasets. Our work identifying a druggable requirement for ERBB activity in KRAS mutant lung cancer was widely reported in the press and highlighted in two *Nature Reviews* family editorials. The work has spurred substantial interest from industry and plans are progressing for a Phase 2 clinical trial in KRAS mutant lung cancer, to be led from Glasgow. Continued work on the same project has revealed a novel signalling activity between tumour and infiltrating immune populations that may be exploitable for early detection.

Another major scientific development saw the successful generation of a new model for malignant pleural mesothelioma. We additionally published a review on mesothelioma (in collaboration with Dr Kevin Blyth) and an editorial on MYC and oxidative stress, and lab members contributed to a publication led by the lab of Dr Julia Cordero (University of Glasgow). External funding was awarded by the British Lung Foundation to work on mesothelioma (in collaboration with Mick Knighton).

Outreach activities included hosting a student delegation for Southwest Community College, Tennessee; interviews for television, newspaper and online media outlets; participation in a PCUK fundraiser; and invited lectures at MRC Hammersmith, RCS Ireland, Sick Kids Hospital in Toronto and the annual AMPK conference at Niagara-on-the-Lake, Canada. Former lab members Dr Jennifer Port and Dr Tiziana Monteverde received their official degrees.

Publications listed on page 90

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TUMOUR CELL DEATH

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[1]Worldwide Cancer Research
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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.

Mannose affects glucose metabolism and cancer cell growth

A common feature of many cancers is that they exhibit enhanced uptake and a greater dependency on glucose. So marked is this characteristic that the uptake and retention of glucose by tumours is widely used for diagnosis, and particularly for the detection of disseminated disease. As a result, we became interested in how tumour cells may respond to other forms of sugar and found that the monosaccharide mannose can profoundly affect the growth of some, but not all, cancer cell types (Gonzalez *et al.*, Nature 2018; 563: 719–23).

Mannose is very closely related to glucose in terms of molecular structure and it is taken into cells via the same transporters. We therefore reasoned that mannose may be affecting the growth of cancer cells by interfering with glucose uptake. However, in a comparison of glucose uptake in mannose-sensitive versus insensitive cells, there was no correlation with mannose sensitivity. We also considered that the sensitivity to mannose may be related to relative mannose uptake, but again there was no correlation between the uptake and sensitivity to the sugar in different cell lines. In light of these results and previous studies which showed that mannose can inhibit three key enzymes involved in glucose metabolism (Figure 1), we considered instead that mannose may be interfering with the intracellular metabolism of glucose. To test this, we first examined the impact of mannose on glycolysis, which revealed that mannose markedly reduces the amount of glucose converted to lactate in mannose-sensitive cells. Further analysis showed that mannose also impacted other pathways downstream of glucose, including those involved in anabolism such as the pentose phosphate pathway, global transcription and translation and glycan synthesis.

Mannose affects chemotherapeutic responses

Previous studies have shown that glucose deprivation can enhance cell death responses and so we were interested to know if mannose had a similar effect. To test this, cells were treated with cisplatin and doxorubicin – two widely used chemotherapeutic drugs – and cell death was analysed in either the absence or presence of mannose. This revealed that in cells in which mannose impairs cell growth, the levels of cleaved poly-ADP ribose polymerase and cleaved caspase 3 were higher when treated with chemotherapeutic drug and mannose when compared to either mannose or drug alone. This indicated that mannose may be enhancing cell death by caspase-dependent apoptosis and we subsequently found that the entire cell death response could be blocked by treating with the pan-caspase inhibitor zVAD-fmk.

Cell death by apoptosis can proceed by two main pathways – the extrinsic pathway, where initiating signals originate from receptors on the surface of the cell, and the intrinsic pathway, which is controlled by changes in the outer mitochondrial membrane and does not involve cell surface receptors. The extrinsic pathway depends on factors such as caspase-8 and FADD (Fas-associated death domain) and deletion of these factors using CRSPR/Cas9 had no impact on the cell death induced by chemotherapy plus mannose. In contrast, CRISPR/Cas9-mediated deletion of Bax and Bak – key factors in the intrinsic pathway – completely blocked the cell death response.

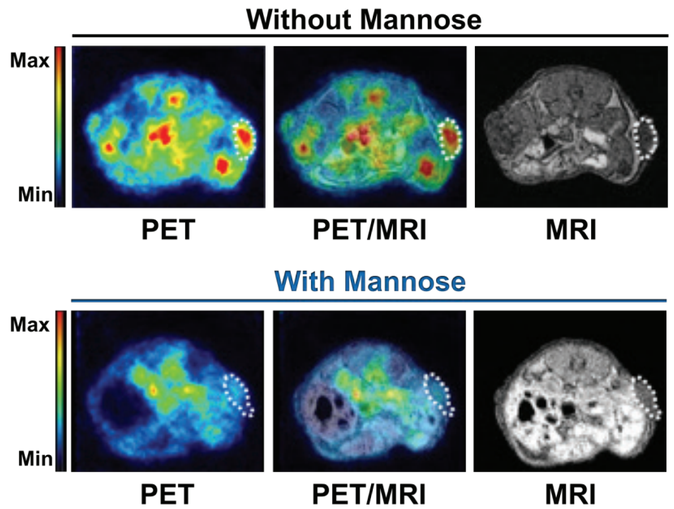
Figure 1
Mannose impairs glucose metabolism
Mannose is taken into cells by the same transporters as glucose and impairs three key enzymes involved in glucose metabolism. Glu, glucose; Man, mannose; HK, hexokinase; Glu-6P, glucose-6-phosphate; Man-6P, mannose-6-phosphate; G6PDH, glucose-6-phosphate dehydrogenase; PGI, phosphoglucose isomerase; Pyr, pyruvate; Lac, lactate.

The intrinsic cell death pathway and permeabilisation of the outer mitochondrial membrane is regulated by members of the Bcl-2 family of proteins. Profiling of these proteins during cell death induced by chemotherapy and mannose showed that Bcl-x_L and Mcl-1 (two anti-apoptotic members) were down-regulated and that Noxa (a pro-apoptotic member) was up-regulated. We were able to show that these changes were causally associated with cell death responses as over-expression of Bcl-x_L or Mcl-1, or CRISPR/Cas9-mediated deletion of Noxa, all impaired the extent of cell death observed.

Mannose affects tumour growth

Inspired by our results *in vitro*, we were keen to understand if mannose could affect tumour growth *in vivo*. Previous studies had shown that mice tolerate the administration of mannose over a period of time, and we also found this to be the case. Tumours were therefore generated in several mouse models and we found that mannose could reduce glucose uptake and retention in these models (Figure 2) and impair tumour growth, without any weight loss or obvious signs of ill health in the mice. Importantly, our *in vitro* studies had shown that sensitivity to mannose was dependent on the levels of phospho-mannose isomerase (PMI) – low levels of PMI indicate sensitivity, and high levels resistance. Building on these findings, we were also able to show *in vivo* that mice bearing tumours with high levels of PMI could be made sensitive to sugar upon PMI knockdown.

Figure 2
Mannose reduces glucose uptake and retention in tumours in mice
Mice were given either a solution of mannose or drinking water as control and injected with ^{18}F-deoxyglucose (FDG). The uptake and retention of FDG by tumours was assessed by positron emission tomography (PET) and magnetic resonance imaging (MRI). Image is abridged from Gonzalez *et al.*, Nature 2018; 563: 719–23

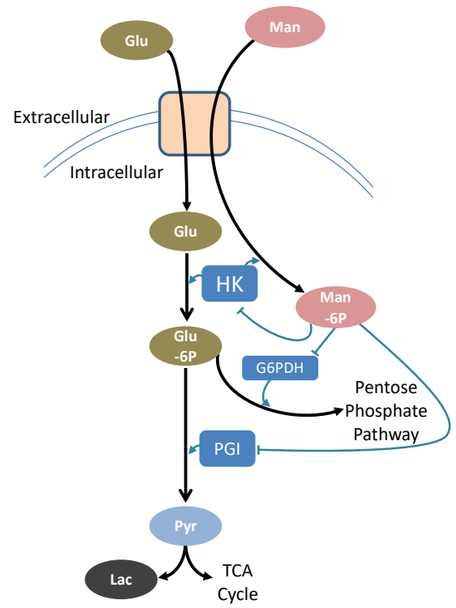


Colorectal cancers are sensitive to mannose

As sensitivity to mannose was connected to the levels of PMI, we examined human tissue microarrays containing samples from breast, ovarian, prostate, kidney and colorectal cancers. Similar to our cell lines, this showed that the levels of PMI vary not only within each tumour type, but also between different tumour types, indicating that the levels of PMI could potentially be used to stratify patients with regard to the likelihood of their tumours responding to the sugar. Furthermore, we found colorectal cancers, when compared to other cancers, often have low levels of PMI, indicating that these tumours may be generally sensitive to mannose treatment. To test this experimentally, we examined two different mouse models of colorectal cancer and in both cases, the administration of mannose in drinking water had a significant impact on tumour formation. Once again, this did not have any negative effects on the weight or overall health of the animals. When taken together, these results show that mannose may be a simple and effective way to improve therapeutic responses in multiple tumour types, and we are currently pressing forward to test this possibility in clinical trials.

Publications listed on page 90

Figure 1



MITOCHONDRIA AND CANCER CELL DEATH

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[1]Breast Cancer Now project grant
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[3]BBSRC project grant
[4]CRUK Glasgow Centre
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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.

Mitochondria drive immunogenic cell death

Irrespective of caspase activity, widespread MOMP commits a cell to die and is therefore a point-of-no-return. As long as a cancer cell dies, should we care how it dies? Our findings argue a resounding yes. We have found that under caspase-inhibited conditions following MOMP, cells still die through caspase-independent cell death (CICD) but produce a variety of pro-inflammatory cytokines; these can stimulate an immune response towards the dying cell. As such, unlike apoptosis, CICD can be considered an immunogenic form of cell death. Importantly, we have found that CICD can elicit anti-tumour immunity. Using an in vivo model that mimics partial therapeutic responses, we have found that CICD is much more effective than apoptosis at reducing tumour growth – often CICD led to complete regression. These beneficial effects are entirely dependent on intact immunity, consistent with CICD being an immunogenic cell death.

Investigating how CICD could be immunogenic, we focused on the role of mitochondria. Interestingly, we find that, under caspase-inhibited conditions, mitochondrial permeabilisation leads to activation of the NF-κB transcription factor pathway. This, in turn, is required for inflammatory signalling during CICD. Mechanistically, mitochondria activate NF-κB by releasing proteins that downregulate cIAP1/2, resulting in NIK and NF-κB activation. As such, while mitochondrial apoptosis is largely viewed as a non-inflammatory type of cell death, the central event that initiates it – MOMP – is in itself highly pro-inflammatory.

Inner membrane permeabilisation enables mtDNA release leading to cGAS-STING signalling

Similar to others, we have found that permeabilised mitochondria, by releasing mtDNA, can also activate cGAS/STING, triggering an interferon response. mtDNA resides in the mitochondrial matrix, therefore how can it activate cytosolic cGAS-STING signalling? We investigated this key question using high-resolution light microscopy. Unexpectedly, we find that during apoptosis, the mitochondrial innermembrane becomes permeabilised, enabling mtDNA release into the cytoplasm (Fig. 1). Applying live-cell imaging, we found that prior to rupture, the innermembrane is extruded into the cytoplasm via expanding BAX/BAK pores (Fig. 2). Our data demonstrate that – contrary to prevailing dogma – the mitochondrial inner membrane permeabilises during apoptosis.

Publications listed on page 94

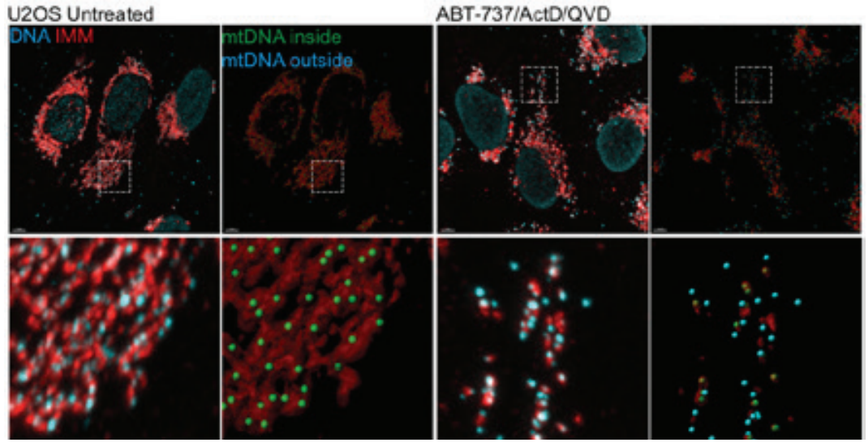

Figure 1
Mitochondria release DNA during cell death
U2OS cells were treated with the BH3-mimetic ABT-737 in the presence of caspase inhibitor qVD-OPH to engage cell death. Cells were stained for the mitochondrial innermembrane (red – anti-AIF) and mitochondrial DNA, mtDNA (blue – anti-DNA). Images were analysed for the presence of mtDNA within the mitochondria, denoted green or outside the mitochondria, denoted blue. Specifically during cell death (right lower) mtDNA is found to exit the mitochondria.

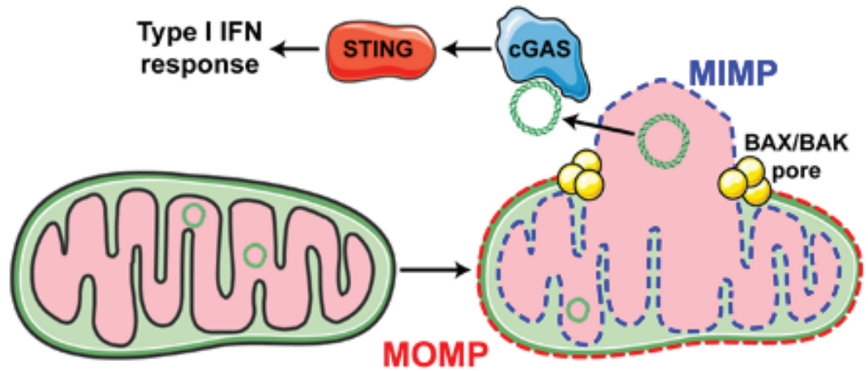

Figure 2
Innermembrane permeabilisation allows mtDNA dependent activation of cGAS-STING signaling
During mitochondrial apoptosis, following mitochondrial outer membrane permeabilisation (MOMP), pores in the outer membrane progressively widen. This allows extrusion of the mitochondrial inner membrane leading to mitochondrial inner membrane permeabilisation, mtDNA release and cGAS-STING activation. This underpins the immunogenicity of caspase independent cell death.

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 liver. At the same time, they are obligate substrates for anabolism of tumours originating in these organs, such as glioma and hepatocellular carcinoma. In particular, we are investigating the role of glutamine synthetase (GS) in the biology of glioblastoma and hepatocellular carcinoma. 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.

While in normal liver the expression of GS is strictly confined to pericentral hepatocytes (Fig. 1), 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. Based on this clinical observation, we are developing a β-catenin– and c-Myc–driven mouse model of HCC, in which the gene encoding for glutamine synthetase is conditionally deleted. Of note, the amplification of *c-Myc* is an aberrant genetic event frequently occurring in HCC, and both β-catenin and c-MYC control glutamine metabolism. While an active β-catenin promotes the expression of GS in HCC, in various types of cancer c-MYC drives the expression of glutaminase (GLS), which catalyses glutaminolysis.

By means of HPLC–mass-spectrometry–based metabolomics and cell biology approaches, we are studying the rewiring of carbon and nitrogen metabolism imposed by high GS expression, in murine and human models of liver cancer.

This study will shed light on the metabolic features imposed by specific oncogenic mutations in liver cancer, and it will pave the way for the identification of metabolic targets with therapeutic potential in a genetically defined subset of patients with liver tumours.

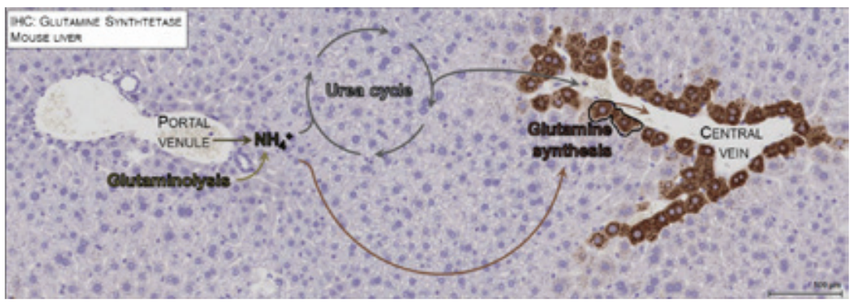

Figure 1
Liver zonation of glutamine metabolism
The liver is an ammonia-detoxifying organ and maintains homeostatic levels of circulating ammonia and glutamine. The functional unit of the liver constitutes an elegant example of metabolic zonation. In fact, the periportal region expresses a liver-type glutaminase which converts excess circulating glutamine into glutamate and ammonia. The latter flows downstream through hepatocytes competent in the urea cycle. These cells, despite having a low affinity for ammonia, constitute the bulk of the liver, and convert large amounts 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.

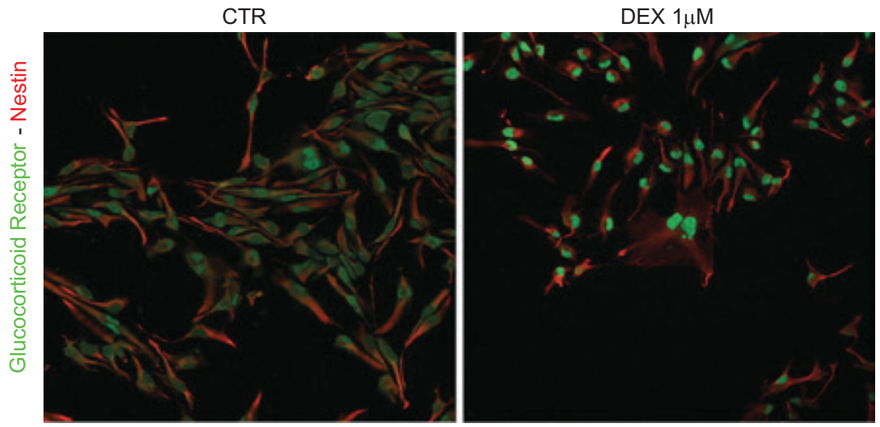

Figure 2
Glucocorticoid Receptor in glioblastoma cells
P3 human glioblastoma cells at early passages have been cultured as adherent monolayers in serum-free Plasmax medium containing growth factors and vehicle (ethanol, CTR) or 1 μM dexamethasone (DEX) for 72h. Cells were fixed, permeabilised and incubated with anti-glucocorticoid receptor and anti-Nestin antibodies. DEX induces morphological changes and the translocation of glucocorticoid receptor from the cytoplasm to the nucleus.

Identification of the metabolic advantages and 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 enhance the fitness of glioma cells (Fig. 2). While the anti-inflammatory action of glucocorticoids is indispensable for the clinical management of glioma patients, unwanted collateral effects of these drugs, including the rewiring of cell metabolism, may ultimately worsen the prognosis of brain tumour patients. On these bases, glucocorticoids constitute excellent candidates to design novel metabolic and combination therapies for the treatment of glioma.

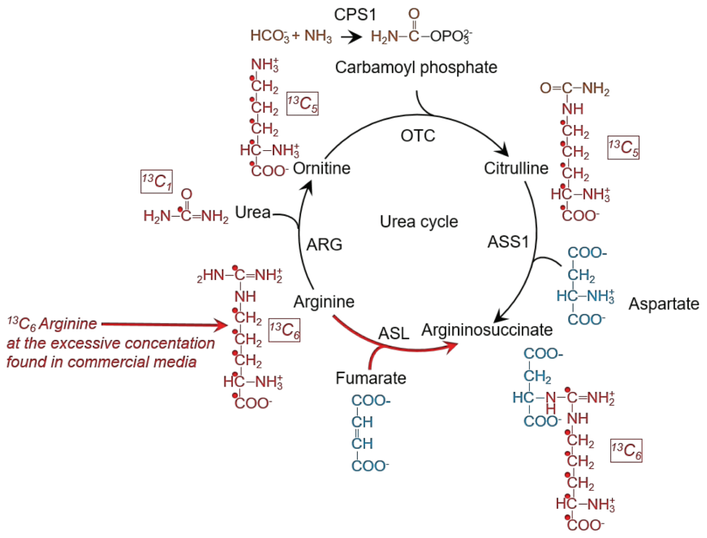

Figure 3
Schematic representation of the urea cycle and expected labelling of argininosuccinate from $^{13}C_6$ arginine upon supplementation with supra-physiological concentration of arginine such as those found in DMEM and RPMI
Under these commonly employed culture conditions the activity of argininosuccinate lyase (ASL) is reversed and produces argininosuccinate from fumarate and arginine. Therefore, one of the paradoxical consequences of employing media with excessive levels of arginine is the presence of argininosuccinate in ASS1-deficient cells. ARG: Arginase, OTC: Ornithine transcarbamylase, CPS1: Carbamoyl phosphate synthetase 1, ASS1: Argininosuccinate synthase 1.

Rethinking cell culture media to achieve more physiologically relevant *in vitro* results

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 focused in perfecting the formulation of historic media in recent years.

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). Consequently, a standard culture medium known as DMEM is far apart from the nutrient levels found in normal human blood; for example, glucose in DMEM is at fivefold the normal glycaemia. A similar ratio applies to glutamine, the most abundant amino acid in circulation. Conversely, five non-essential amino acids normally circulating in blood are missing from DMEM.

On this basis, we produced 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. It has been successfully tested in a variety of cell culture systems, including murine normal, stem and cancer cells, as well as in established and primary human cancer cells derived from several tumour types.

The availability of a physiologically relevant cell culture medium will reduce the inconsistency between *in vitro* and *in vivo* results (Fig. 3), thus favouring more translational biomedical research.

Publications listed on page 94

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MATHEMATICAL MODELS OF METABOLISM

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[1]Left during 2018
[2]European Union Horizon 2020

Metabolism is essential for life, and its alteration is implicated in multiple human diseases. The transformation from a normal cell to a cancerous cell requires metabolic changes to fuel the high metabolic demands 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 microenvironmental 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. First, we aim to uncover the metabolic objectives and metabolic constraints upon which natural selection is acting. Second, we aim to determine which known (and yet to be discovered) molecular alterations are driving the deterministic or stochastic occurrence of the optimal metabolic modes.

Formate metabolism in cancer

In his 1956 landmark paper, Otto Warburg hypothesised that cancer is caused by mitochondrial defects that result in increased rates of glycolysis with lactate overflow. Today, increased glycolysis is an established hallmark of cancer metabolism and forms the scientific basis for Positron Emission Tomography (PET) scans. In contrast, the Warburg hypothesis that cancers harbour defective mitochondria has remained controversial. Recent evidence indicates that some tumours have rates of glucose oxidation comparable to those observed in normal tissues, challenging the assumption that cancer cells are characterised by defective mitochondrial metabolism. A pathway that relies on functional mitochondria is the oxidation of the third carbon of serine to formate. Formate produced in the mitochondria is released into the cytosol, where it supplies the one-carbon demand for nucleotide synthesis. Work from our laboratory has shown that cancer cells produce formate at rates that exceed the biosynthetic demand of one-carbon units, resulting in formate overflow from cells (Meiser *et al.*, Sci Adv 2016; 2: e1601273 and Meiser *et al.* Nat Commun 2018; 9: 1368). However, the selective advantage of this phenotype remains to be elucidated.

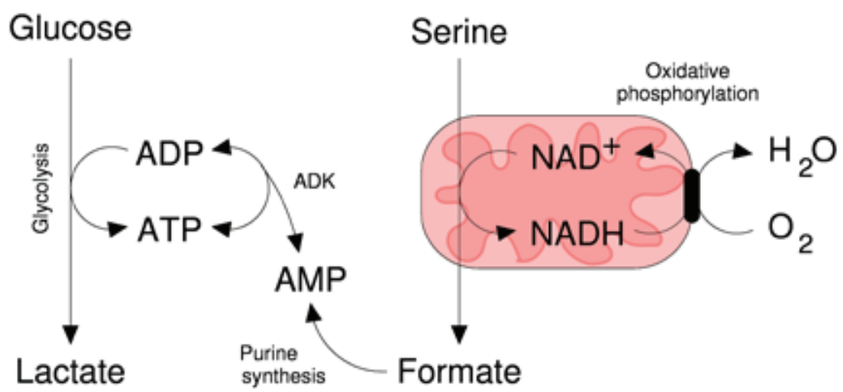

Figure 1
Proposed biochemical mechanism where formate links glycolysis to oxidative phosphorylation

In 2018 we had a major breakthrough. We discovered that high rates of mitochondrial formate production, together with energy homeostasis mechanisms, induce glycolysis. Specifically, mitochondrial one-carbon metabolism oxidises the third carbon of serine to formate. Excess formate production induces an increase in the rate of purine synthesis resulting in higher AMP levels. Due to energy homeostasis mechanisms, the increase in AMP induces an increase in the levels of ADP and to a lesser extent of ATP, overall resulting in an increase of ADP. The increase in ADP thermodynamically pushes glycolysis, which in turn induces an increase in proliferation and cell migration. In the absence of mitochondrial formate production, all these effects can be recapitulated by formate supplementation.

The proposed biochemical mechanism has a number of implications for and beyond cancer metabolism. Previous work at the Institute, from the laboratory of Karen Vousden, has shown that some cancer cells are dependent on mitochondrial metabolism of serine to formate for their growth and survival. The homozygous deletion of enzymes in mitochondrial serine-to-formate metabolism is embryonic lethal in mice and the phenotype can be rescued by formate supplementation. Mitochondrial serine metabolism is also essential for T-cell activation. It remains to be elucidated whether mitochondrial formate production is inducing glycolysis in those contexts and to what extent the requirement of mitochondrial serine metabolism is determined by this mechanism. Finally, since excess formate production results in formate overflow from cells, the link between formate and glycolysis could act in a cell non-autonomous manner as well.

Publications listed on page 94

CANCER METASTASIS AND RECURRENCE

www.beatson.gla.ac.uk/tom_bird

LIVER DISEASE AND REGENERATION

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Paterson Endowment Fund
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Hepatocytes are the key target for regenerative therapy for patients with liver disease and are the source of liver cancers (hepatocellular carcinoma, HCC). These cells show immense regenerative capacity, but despite our current understanding of the mechanisms that control liver regeneration, no therapeutic breakthroughs have been achieved to date. It is the aim of my group to understand what makes some hepatocytes regenerate whilst others do not, and to unpick the molecular pathways that underpin the transformation of regenerating hepatocytes into malignant hepatocytes.

Mechanisms controlling hepatocyte proliferation

The Wnt/β-catenin signalling pathway is crucial for establishing and maintaining the zones of the liver in which we believe that the regenerative cells reside. Activation of the Wnt pathway is sufficient to cause hepatocytes to divide and the liver to grow. It also infers hepatocytes with a greater propensity to regenerate than those without its activation. However, when this occurs, anti-proliferative pathways are also activated, preventing ongoing liver growth. We are investigating the nature of these pathways, 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 renders it incapable of participating in regeneration. In severe liver injury we have shown that senescence may occur in response to injury (Fig. 1). We are investigating the pathways by which this process is activated and are currently performing preclinical trials in models of acute severe liver injury to prevent senescence formation and improve regeneration. Our recent work suggests senescent hepatocytes may affect their surrounding environment in many ways, including immune activation, matrix deposition and the induction of senescence in other cells. We have started to characterise the phenotype at single-cell level within this environment. We are now studying ways to interfere with such

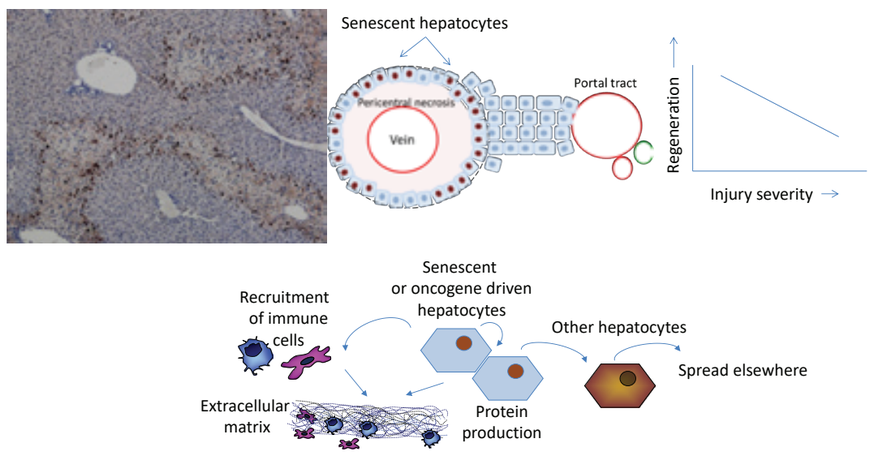

Figure 1
Damage-induced senescence in the liver
Following acute paracetamol toxicity, a rim of senescence develops around the area damaged by the toxic insult (pericentral). Instead of local regeneration, hepatocellular proliferation is activated elsewhere. However, when injury becomes increasingly severe, liver regeneration reduces progressively. The senescent cells are able to affect their environment and influence tissue resident populations, in addition to stimulating cell recruitment and further cell senescence. (Adapted from Bird *et al.* Sci Transl Med 2018; 10: eaan1230).

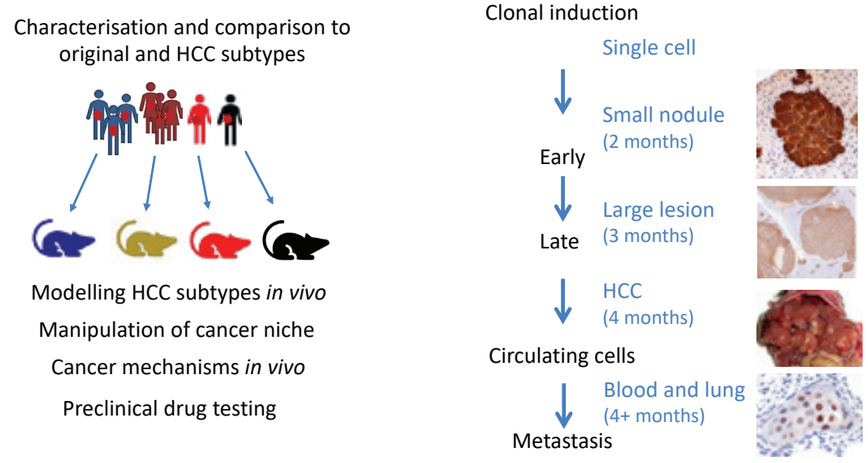

Figure 2
Human HCCs can be grouped into different functional and genetic subclasses
We are mimicking the genetic alterations in human HCC subclasses using *in vivo* models. Our strategy is to induce clonal hepatocytes with these targeted genetic alterations and then follow the clones' development into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

spreading senescence as a means to develop treatment for states of regenerative failure e.g. fulminant liver failure, alcoholic hepatitis and small-for-size syndrome.

Transformation of regenerative hepatocytes into malignancy

Whilst the Wnt/β-catenin pathway plays a role in regeneration, it is also the most frequent site of mutations in liver cancer. The actions of active β-catenin in hepatocytes are different to those in other organs, and typically the mutations which occur in HCC prevent current therapies targeting the pathway from working in this condition. We are investigating how the blockade of proliferation imposed by β-catenin on hepatocytes may be broken during cancer formation and if new therapies targeting β-catenin downstream of its destruction complex might be effective in this condition. We are also working on studying the role β-catenin plays in promoting evasion of tumour detection by the immune system.

We have developed a number of models of HCC utilising a combination of targeted genetic manipulations 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 to create models for each subtype of human cancer (Fig. 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 response to therapies. Using the advanced facilities within the 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. Our aim, with the input of other groups within the Institute, is to map the evolution of the tumours and test therapies aimed at preventing tumour initiation, expansion and metastasis.

Early detection of hepatocellular carcinoma

In the UK, 10–20% of the population are potentially at risk of liver disease, and 50,000 adults in the UK estimated to have cirrhosis as a result of a trebling in incidence in the last 30 years. Hand in hand with chronic liver disease, primary liver cancers are becoming more common, having trebled in the last 15 years. Liver disease is reversible, as is the risk of HCC. HCC is potentially curable, providing it is detected at an early stage. However, 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 who may be at risk of liver cancer. We hope to provide a rationale for inclusion of these biomarkers in routine clinical practise to facilitate the early treatment and cure of HCC in those at risk. We are collaborating with experts in public health and statistics to gather and analyse additional data collected from across Scotland and have already shown that by the application of novel statistical analysis of dynamic changes in serum biomarkers in individual patients we are able to detect HCC in its early forms. With the integration of additional clinical variable and other biomarkers, we aim to improve the accuracy of this approach and move towards clinical trials.

Publications listed on page 82

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MOLECULAR CONTROL OF EPITHELIAL POLARITY

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Our group studies the gain and loss of collective cell polarity and invasion in prostate 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 parallelism. 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.

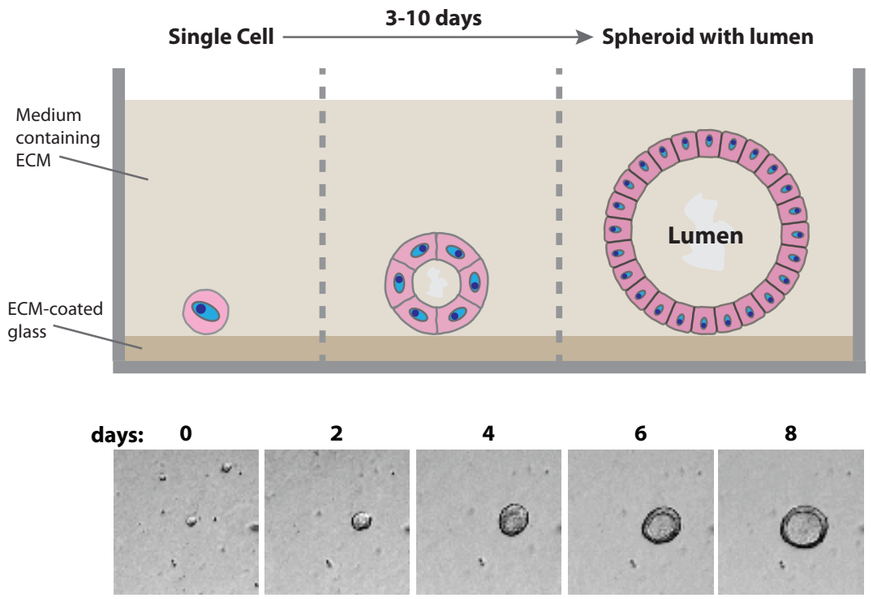

Figure 1
By culturing cells on glass-bottomed chambers coated with extracellular matrix (ECM), we direct the self-assembly of single cells into multicellular spheroid structures with a single, central lumen. This process occurs over 10 days, allowing us to study the dynamics of tissue formation.

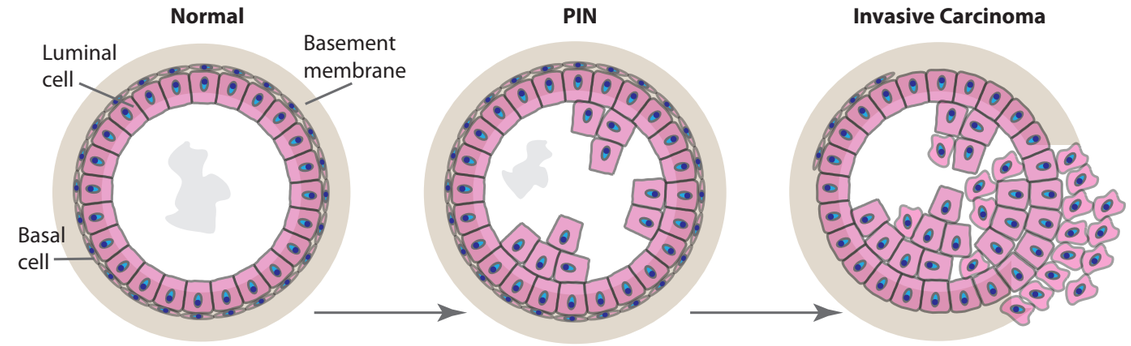

Figure 2
3D cultures of cells to form cysts (also called spheroids or organoids) also allows us to model the loss of normal tissue architecture that occurs in cancer. For example, the progressive disrupted organisation of Normal, to Prostatic Intraepithelial Neoplasia (PIN), to Invasive Carcinoma typifies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

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 family's influence on prostate cancer cell invasion.

In collaboration with the Ismail and Shanks groups, 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 collaborated with the Shanks lab / HICAR facility to develop a functional genomic approach to systematically evaluate each member of the Podxl interactome for its role in invasion from spheroids. Our current aim is for a rigorous dissection of the exact cooperating protein modules that promote Podocalyxin-driven invasion. Our future aim is to understand which of these *in vitro* modulators of invasion are consistently altered in prostate 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.

In collaboration with Samantha Stehbens (University of Queensland, Australia), we are investigating the effect of FGFR mutations in collective cell invasion in 3D cultures. This work is revealing that collective polarity defects underpin patient mutations in FGFR2.

Publications listed on page 83

LEUKOCYTE DYNAMICS

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[1]National Heart & Lung Institute Foundation
[2]Imperial College London President's PhD Scholarship
[3]Now research scientist in Owen Sansom's group

The immune system can exert both anti- and pro-tumour activity, depending on many factors. 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. The role of immunity in cancer is complicated as immune cells can kill cancer cells and stabilise the primary tumour to help prevent spread but 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.

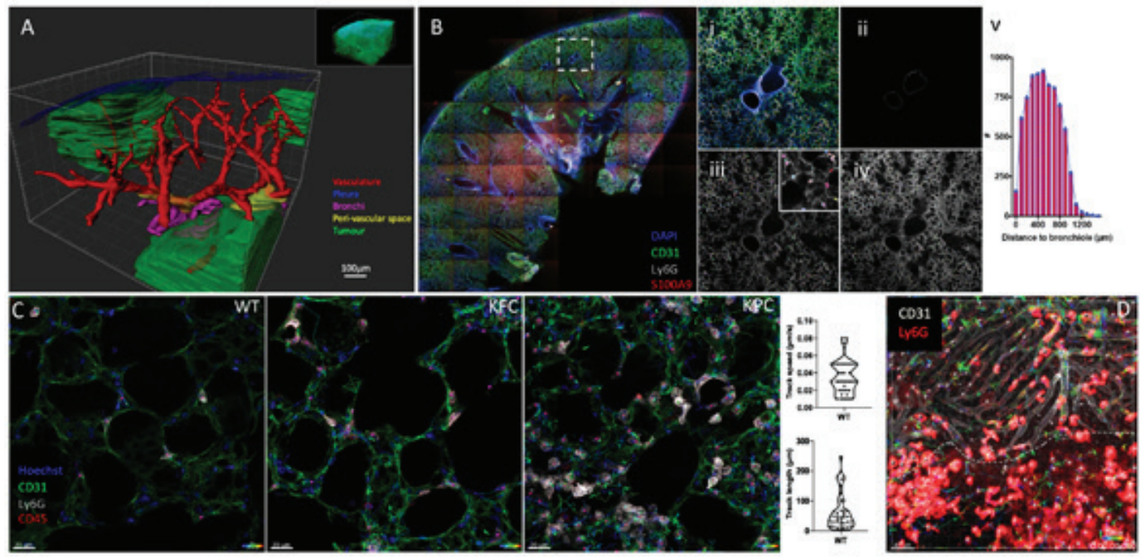

Figure 1
Investigating leukocyte dynamics in cancer using multiscale advanced light microscopy.
A) 3D segmentation and reconstruction of tissue features from an optically cleared model lung adenocarcinoma imaged by multiphoton microscopy.
B) Localisation and quantification of neutrophil localisation in the lung of pancreatic cancer model. i-v) Cells and anatomical features are segmented and their relationships quantified in whole lung slices.
C) Neutrophil motility quantified in live *ex vivo* lung slices from control and pancreatic cancer models.
D) Still from an intravital timelapse of neutrophil dynamics around a colorectal liver metastasis. All Images acquired with the help of the Beatson Advanced Imaging Resource.

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; and 2) uncover cancers with the strongest or most manipulable interaction with particular immune cells. We continue to collaborate with several groups here at the Institute to investigate this in state-of-the-art models. For example, we are currently investigating the mechanisms that regulate leukocyte dynamics in lung cancer by imaging large areas of optically cleared tissue in a lung adenocarcinoma model (Fig 1A; F. Fercoq and X. Raffo in collaboration with Daniel Murphy's lab); metastasis to the lung from pancreatic cancer using a combination of high-content imaging and analysis in fixed lung tissue and live *ex vivo* lung tissue imaging in precision-cut lung slices (Fig 1B,C; A McFarlane in collaboration with Jim Norman's and Jennifer Morton's labs) and metastasis to the liver from colorectal cancer directly *in situ* by intravital microscopy (Fig 1D; J Mackey and R Jackstadt in collaboration with Owen Sansom's lab).

Publications listed on page 83

IMMUNE CELLS AND METASTASIS

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Our lab focuses on the regulation and function of γδ T cells in tumour progression, using genetically engineered mouse models of cancer. γδ T cells are a unique cell population – they are small in number but have major influences on neighboring cells during immunity. We aim to understand how γδ T cells behave in tumour-bearing mice, specifically in pre-metastatic organs, and to uncover regulatory pathways governing these cells that may be manipulated to counteract metastasis formation. Our research is centered on three main cancer types: breast, colon and pancreas.

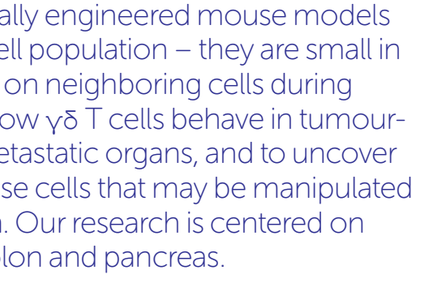
γδ T cells in breast cancer

A subset of γδ T cells promotes mammary tumour metastasis through expression of the cytokine IL-17 (Coffelt *et al.*, Nature 2015; 522: 345–8). Last year, we found that this subset of IL-17-producing γδ T cells expresses the receptor NKG2D – a molecule more commonly associated with natural killer (NK) cells and cancer cell killing. Our work to understand the function of this killing receptor on pro-metastatic γδ T cells continued into 2018. We found that NKG2D-expressing γδ T cells are enriched in lung tissue, when compared to lymph nodes, spleen, liver or thymus. These data suggest that they may have an important role in metastasis, since the lung is the primary site of cancer cell dissemination in mice. Interestingly, NKG2D-expressing γδ T cells are not present in lungs after birth, but arise sometime after weaning. We are currently exploring the idea that NKG2D is involved in regulation of IL-17 expression in γδ T cells. We have profiled various cell populations in the lung to determine where the ligands for NKG2D are expressed and how mammary tumours affect ligand expression. This analysis uncovered monocytes as the main producers of NKG2D ligands, so we will investigate the relationship between monocytes and γδ T cells over the coming year. We have secured funding from Breast Cancer Now for this project. All our work in breast cancer is done in collaboration with Karen Blyth and her team.

To gain a better understanding of γδ T cell heterogeneity in the lung, we have performed single-cell sequencing on these cells in collaboration with Kristina Kirschner (University of Glasgow) and Nizar Batada (University of Edinburgh). An unbiased transcriptional analysis of 1000s of individual cells revealed two major clusters of γδ T cells in accordance with published literature that largely segregate on cytotoxic molecules (Fig. 1a). Within these two major clusters, we identified populations expressing T cell receptor signalling molecules, co-stimulatory molecules, T cell checkpoint inhibitors, lymph node trafficking molecules, NK cell markers, cytokines (such as IL-17) and cytokine receptors, as well as genes involved in NOTCH signaling. This in-depth analysis of γδ T cells at the single-cell level provides further insight into γδ T cell biology in homeostasis and cancer.

γδ T cells in colorectal cancer

In collaboration with Owen Sansom and his lab, we are investigating the role of γδ T cells in various mouse models that recapitulate distinct molecular subtypes of colon cancer. We have found that the absence of γδ T cells fails to influence tumour growth and survival in models driven by loss of the tumour suppressor Apc. At the same time, we profiled the phenotype of γδ T cells in two other models of colorectal carcinoma, which indicate that γδ T cells may be important. We found that IL-17-producing γδ T cells are increased in the metastatic *Villin-Cre;Kras*^{G12D}*;Trp53*$^{F/F}$*;Nicd1*$^{F/+}$ model and, in a similar fashion to our observations in metastatic mammary tumour models, we find that the increase in IL-17-producing γδ T cells occurs in visceral organs in addition to the primary tumour. Over the next year, we will determine whether IL-17-producing γδ T cells play a role in primary tumour growth or metastasis formation in this model. We have also been using a model of microsatellite instability, which has provided some surprising results. Unlike any other cancer model we have profiled, γδ T cells make up a large proportion of infiltrating T cells in microsatellite unstable tumours (Fig. 1b). We have shown that these γδ T cells produce IL-17 and that their infiltration correlates with neutrophil influx. In the future, we aim to determine whether they are required for tumour growth and the mechanisms by which they are recruited to tumours.



Seth Coffelt giving a tour of the labs to Rebecca Scott, who underwent treatment for ovarian cancer in 2017. Photo credit: Steve Welsh

γδ T cells in pancreatic cancer

Using the *Kras*^{G12D}*;Trp53*^{R172H}*;Pdx1-Cre* (KPC) model, we are exploring the ability of γδ T cells to influence pancreatic ductal adenocarcinoma (PDAC) and liver metastasis. This work is done in collaboration with Jen Morton. We crossed the KPC model with γδ T cell–deficient (*Tcrd*$^{-/-}$) mice and measured neutrophils in the circulation of these mice. In contrast to our previous observations in mammary tumour–bearing mice, we found that neutrophils were unaffected by the absence of γδ T cells (Fig. 1c), indicating that γδ T cells are not required for neutrophil expansion in the presence of PDAC. When we profiled conventional T cells and NK cells in livers of tumour-bearing mice, however, we noticed that NK cell activity was increased in γδ T cell–deficient mice, while T cells remain unchanged. These data suggest that γδ T cells may communicate with NK cells to suppress their function in liver tissue. Over the next year, we will examine the crosstalk between NK cells and γδ T cells and measure any impact on pancreatic cancer metastasis.

Publications listed on page 84

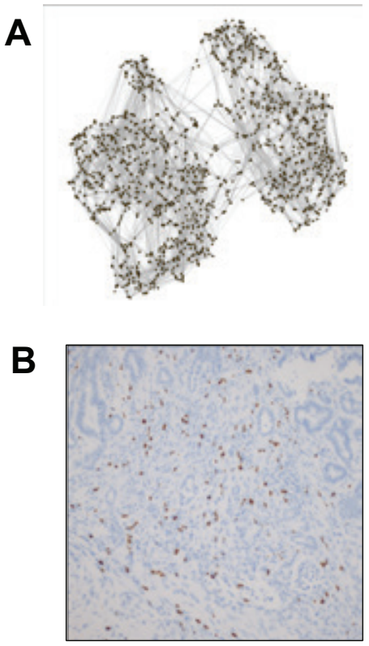


Figure 1
γδ T cells in mammary, pancreatic and colon cancers
(a) γδ T cells sorted from lungs of wild-type mice and analysed by single-cell sequencing. Analysis shows that two main populations of γδ T cells reside in the lungs under homeostatic conditions.
(b) γδ T cell abundance in microsatellite unstable tumours of the gut as determined by RNAscope for the *Trdc* transcript.
(c) Neutrophil counts in blood of wild-type (WT) mice, tumour-bearing KPC mice, γδ T cell–deficient (*Tcrd*$^{-/-}$) mice and tumour-bearing, γδ T cell–deficient KPC mice.
Each dot represents one mouse. P value was determined by one-way ANOVA.

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CELL MIGRATION AND CHEMOTAXIS

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[1]CRUK Multidisciplinary Award

One of the most damaging aspects of cancer is metastasis, in which cells spread beyond the tumour in which they arose and colonise other organs. In normal organs, and most early solid tumours, cells do not migrate. However, when tumours become metastatic, suppression of cancer cell migration may be lost – cells spread into the blood and lymph systems to form secondary tumours. It is believed that cells cannot spread or move efficiently unless they are steered by something. However, it is not understood what provides the directional steer, or how cells manage to read and respond to it. Our group brings together multiple tools, from mathematical modelling to cell biology and biochemistry, to improve understanding of how cell migration is controlled.

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, 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 metastatic cancer.

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.

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, homogenous 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 86

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 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 show *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 cargos. 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 show 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 show 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 propose in this study that the ciliary machinery has been repurposed by T cells to generate and maintain polarized segregation of signals at the immune synapse.

Publications listed on page 86

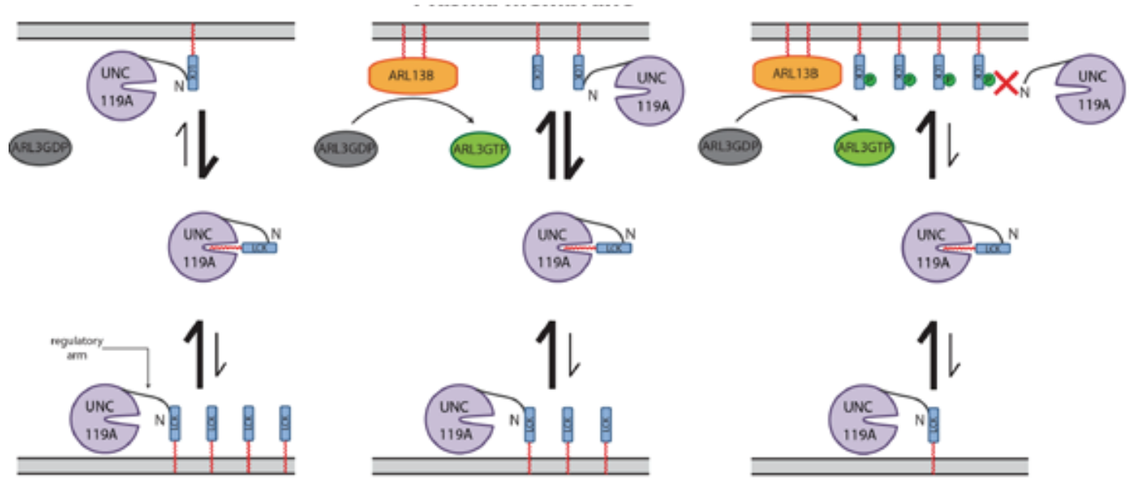

Figure 1
A working model of the interplay between ciliary ARL3 and LCK trafficking and phosphorylation
Myristoylated LCK at the endomembranes is extracted by UNC119A through a regulatory arm–kinase domain interaction as well as hydrophobic pocket-myristoyl group binding. Solubilised LCK can then be transported in the cytosol, but, in the absence of active ARL3GTP at the plasma membrane (left), very little release of LCK occurs at the cell surface. Localisation of guanine exchange factors (GEFs) such as ARL13B at the plasma membrane drives activation of ARL3. LCK released by ARL3GTP can then anchor to the plasma membrane, where it can be extracted again by UNC119A (middle). Phosphorylation of LCK due to its activation blocks the interaction between the kinase domain and UNC119A (right), thereby trapping LCK at the plasma membrane.

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MIGRATION, INVASION AND METASTASIS

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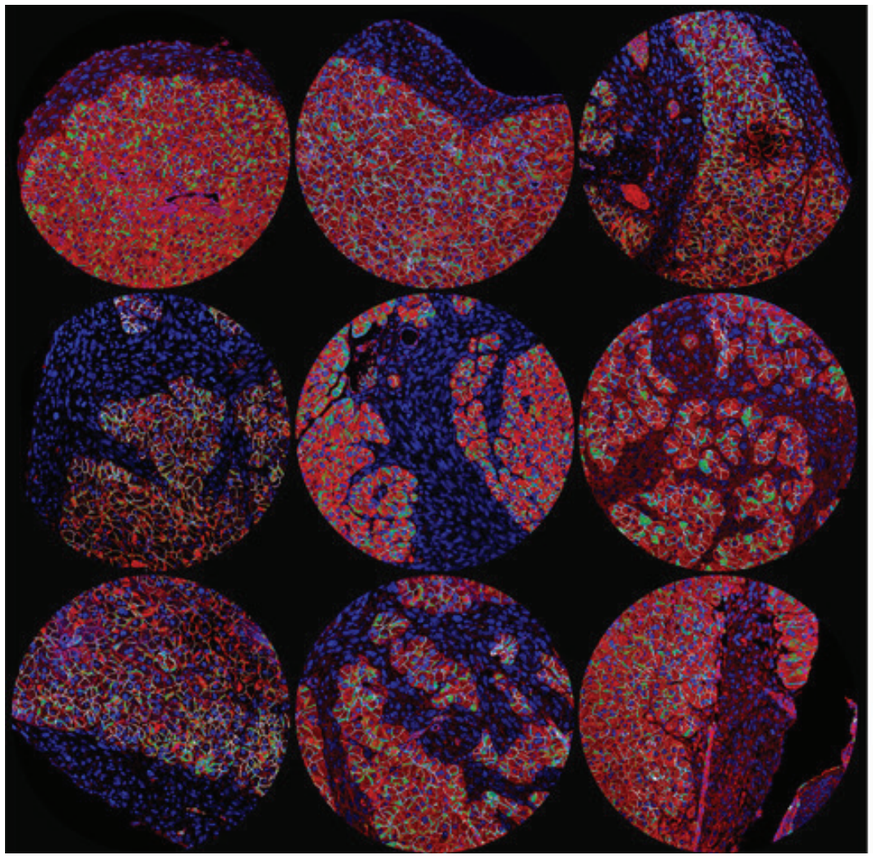
Cancer metastasis and recurrence after treatment still account for the vast majority of cancer deaths and new strategies are urgently needed in these areas. Reasons why metastasis is difficult to target include difficulty of detecting or eradicating disseminated tumour cells, lack of understanding of the mechanisms of spread and heterogeneity of tumours. Our group aims to improve understanding of the mechanisms of cancer metastasis with the goal of identifying new strategies to improve outcomes for patients with metastatic cancer. We study mechanisms of cell migration control, Rho-family GTPase signalling and mechanosensing by tumour cells. We are also interested in crosstalk between mechanosensing and metabolism. Our focus is mainly on pancreatic cancer and melanoma, but our basic science is relevant to multiple cancer types and to normal developmental migration.

The actin-nucleating protein N-WASP is implicated in cancer cell invasion and metastasis due to its ability to trigger actin assembly and link with signalling pathways via Rho GTPases that control both actin-based motility and vesicle trafficking. This year, clinical research fellow Dr Hayley Morris discovered that N-WASP is also important for maintenance of normal tissue homeostasis in the intestine and colon and can act as a suppressor of tumourigenesis in an APC-driven model of colorectal cancer. Additionally, postdoc Dr Amelie Juin implicated N-WASP in a chemotactic signalling loop regulating invasion and metastasis of pancreatic ductal adenocarcinoma (manuscript under review). Loss of N-WASP in a KRas- and mutant p53– driven model of pancreatic ductal adenocarcinoma (PDAC) led to reduced metastatic spread to distant sites such as the liver and peritoneal cavity. N-WASP is thus a promising but complex target, due to its strong role in invasion and metastasis, combined with its contrasting requirement for maintaining normal tissue homeostasis in the early stages of cancer progression. Amelie and scientific officer Heather Spence are also investigating the role of the sorting and trafficking protein strumpellin in cancer metastasis in both prostate cancer (with Rachana Patel and Hing Leung) and PDAC.

Rac1 is well known to be important in Ras-mediated transformation of cancer cells and is one of the more commonly mutated genes in sun-exposed melanomas. Rac1 regulates actin dynamics via a direct interaction with the Scar/WAVE complex, which generates new actin filaments at the leading edges of cells. In a collaborative effort with Robert Insall's group, we discovered a new regulator of actin dynamics, CYRI (in humans, CYRI-A and CYRI-B), encoded by the *FAM49* gene. CYRI is a Rac1-interacting protein that negatively regulates Rac1 signalling to activate the Scar/WAVE Complex. CYRI is well conserved in evolution and represents an example of a 'local inhibitor' of actin-based protrusions. Postdoc Jamie Whitelaw and PhD student Anh Le are further investigating the role of the Scar/WAVE complex and CYRI-A/B in the control of actin dynamics. PhD student Savvas Nikolaou has found CYRI-B to be particularly highly expressed in PDAC and is investigating a possible role in regulation of Rac1 activity during PDAC progression. Postdoc Karthic Swaminathan is investigating how the Scar/WAVE complex controls melanocyte migration and melanoma progression. Another downstream target of Rac1 is the actin bundling protein fascin-1, which we continue to collaborate on with the Institute's Drug Discovery Unit and also with David France and Sarah Memarzedah in the Department of Chemistry at the University of Glasgow to make a fascin-targeting degradation reagent using the PROTAC ubiquitin targeting method.

Figure 1
Tissue sections from pancreatic cancer metastatic nodules showing DNA/nucleus in blue and E-cadherin cell junctions in green.

Photo Credit: Dr Amelie Juin



Pancreatic tumours are particularly stiff due to accumulation of collagen-containing stroma. They are also nutrient- and oxygen-depleted, potentially leading to high selective pressure for metabolic plasticity to overcome the hostile environmental challenges. Actin dynamics for cell migration require ATP turnover, and tumour cells have particularly high metabolic demands due to uncontrolled proliferation and poor nutrient access. We are investigating how the stiff environment of the tumour changes pancreatic cancer cell metabolism and how metabolic pathways are coupled with cell migration and the actin cytoskeleton. Postdoc Nikki Paul is on an MRC-funded project to screen for metabolic regulators that regulate the cytoskeleton and motility properties of PDAC cells. Student Vassilis Papalazarou is funded by the Cancer Research UK Glasgow Centre as a collaboration with the Bioengineering group of Manuel Salmeron-Sanchez (University of Glasgow) to study how mechanosensing couples with metabolism in PDAC cells. We also collaborate with Oliver Maddocks (University of Glasgow) to perform metabolomics on PDAC cells. Our studies are revealing how cancer cells can still remain exquisitely mechanosensitive and enter into states of relative dormancy or active growth depending on mechanical cues. Cancer dormancy accounts for many types of relapse of disease after treatment and we urgently need more information about the dormant state of cancer in order to develop new treatments against recurrence.

Publications listed on page 88

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PRECLINICAL PRECISION PANCREAS

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Pancreatic cancer is predicted to become the second most common cause of cancer death in the western world by ~2025. The focus of our research is to better understand the disease and identify more effective and targeted therapies. In particular, we use genetically engineered models to study different genetic and transcriptomic subtypes of the disease, and determine how we can tailor treatments to these tumours. Our lab is part of PRECISION-Panc, a multi-disciplinary network that aims to learn more about the disease and pave the way for clinical trials of personalised therapies by aligning preclinical discovery and clinical development.

Pancreatic Cancer Modelling

Pancreatic cancer is a genetically complex disease, with many genes mutated at low frequency. Whilst mutations in KRAS, TP53, SMAD4 and CDKN2A are common, these are difficult to target therapeutically. Conversely, many of the genes mutated or pathways deregulated less frequently may be clinically relevant targets in subsets of patients. We use genetically engineered mice to model these subsets of patients who may ultimately benefit from more personalised approaches to treatment based on their mutations. KPC mice, which express endogenous mutant KrasG12D and p53R172H targeted to the pancreas (using Cre-Lox technology), develop pancreatic tumours reminiscent of the human disease, whilst novel models also allow us to manipulate genes in different cellular compartments within the tumour, so as to better understand the complex signalling network that exists within the tumour microenvironment. By layering further genetic aberrations onto this model we can study the importance of various signalling pathways in tumourigenesis, and use these mice to test new therapies and combinations. For example, mutations in PTEN/AKT/mTOR pathway components are found in up to 10% of patients. We previously showed that these tumours might be exquisitely sensitive to mTORC1 inhibition, while tumours lacking these aberrations were sensitive to mTORC2 inhibition (Driscoll *et al.*, Cancer Res. 2016; 76: 6911–23). Thus, we are currently investigating which therapeutic combinations are most effective in targeting signalling pathways downstream of Kras, and in which genetic setting.

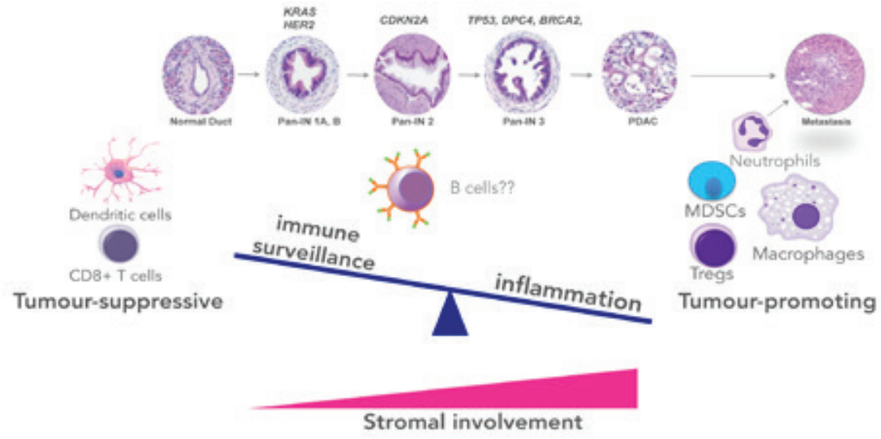

Figure 1
The role of the immune system in pancreatic cancer progression
Crosstalk between tumour cells and the microenvironment can affect cell proliferation, survival, metabolism, immune response and response to different therapeutic agents, while therapeutic agents in turn alter these signals and change the tumour subtype.

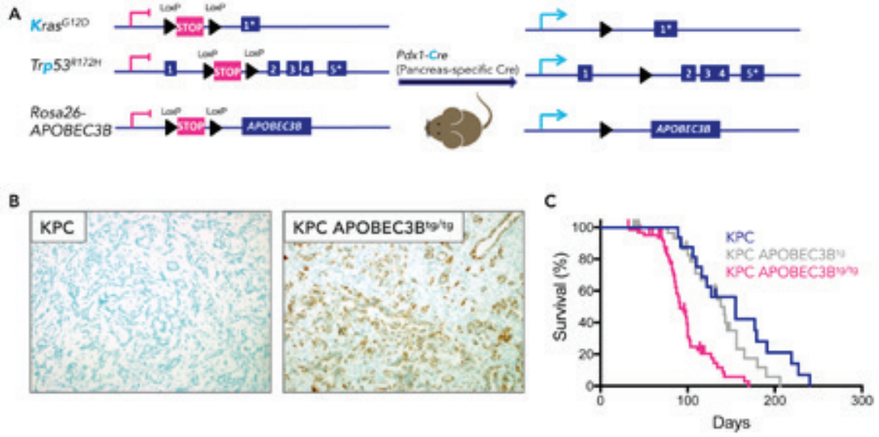

Figure 2
The KPC APOBEC mouse model
(a) Generation of an APOBEC3B-expressing KPC (*Pdx1-Cre; Kras*$^{G12D/+}$*; p53*$^{R172H/+}$)-based mouse model of pancreatic cancer.
(b) RNAscope shows expression of APOBEC3B in APOBEC3B-expressing KPC tumours. Scale bar = 100μM.
(c) Kaplan–Meier survival analysis shows significant acceleration of pancreatic cancer in APOBEC3B-overexpressing KPC mice.

The pancreatic cancer immune microenvironment

A prominent feature of pancreatic cancer is the dense desmoplastic stroma, which can account for up to 90% of the tumour volume. This microenvironment includes fibroblasts, stellate cells, immune cells, blood vessels and extracellular matrix proteins, and is characterised by significant infiltration of immune cells, but a distinct lack of $CD4^+$ and $CD8^+$ T cells (Fig. 1). Thus, immunotherapies designed to activate effector T cells by blocking ligation by immune checkpoint ligands such as PD-L1 and CTLA-4 have had little efficacy in PC despite promise in other cancers. We previously found that ablating neutrophils homing to pancreatic tumours could enhance T cell infiltration, providing a therapeutic opportunity for PD1-blocking immunotherapy (Steel *et al.*, Cancer Cell; 29: 832–45). We have also found that inhibiting macrophages, via CSF-1R inhibition, stimulated active effector T cells to infiltrate pancreatic tumours, which was sufficient to block tumour growth and extend survival in KPC mice (Candido *et al.*, Cell Reports; 23: 1448–60). We are currently trying to interrogate how tumour-associated macrophages can drive tumourigenesis.

Recent studies have also suggested that depletion of B cells, or inhibition of B cell signalling, can reduce tumour growth in xenograft models of pancreatic cancer. However, clinical trials targeting B cells in pancreatic cancer have been conducted recently and the results have been very disappointing. We have now found in our lab, using a more clinically relevant autochthonous model, that B cells are more likely tumour suppressive. We are now investigating the mechanisms behind this, and the microenvironmental differences that are observed in spontaneous versus allograft models.

Modelling Genomic Instability

Recent data have suggested that genomic instability and increased mutation burden may render tumours sensitive to DNA-damaging therapeutic agents, but also predict the efficacy of immune checkpoint inhibitors. To investigate this, and to create models in which to test response to therapies, we have modelled the effects of deletion of DNA damage repair genes that are mutated in pancreatic cancer, e.g. *ATM*, and *BRCA¬-1& -2*. We find that tumourigenesis is accelerated in, for example, ATM-deficient mice, suggesting at least some degree of genomic instability. Pharmacological inhibition of the DNA damage repair (DDR) pathway using ATR and PARP inhibitors showed some efficacy in these models; however, there was still a lack of infiltrating T cells. Thus we are examining mutational burden, and investigating whether antigen presentation can be enhanced to render tumours sensitive to immunotherapy in combination with DDR-inhibiting agents.

We have also generated a model of *APOBEC* overexpression in pancreatic cancer. The APOBEC family of cytidine deaminase enzymes, whose normal function is to protect from viral infections, have been revealed as drivers of mutation in a variety of human tumours, including pancreatic cancer. We have engineered mice to express Cre-inducible *APOBEC3B* and crossed these with the KPC genetically engineered mouse model of pancreatic cancer (Fig. 2). We find that overexpressing *APOBEC3B* results in a poorer prognosis in these animals, and some changes in the immune microenvironment; however, there is still a lack of T cell infiltration into these tumours. We are currently performing genomic and transcriptomic analyses to determine mutational burden and tumour subtype in an effort to identify therapeutic opportunities in patients bearing this signature.

Publications listed on page 89

INTEGRIN CELL BIOLOGY

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One of the main challenges that we face in treating cancer is the likelihood that, at the time of diagnosis, malignant cells have already left the primary tumour and spread to other organs. Thus, even following complete removal of the primary tumour, these disseminated cells can reside within 'primed metastatic niches' only to reappear later as metastases. We anticipate that a deeper understanding of the mechanisms underlying metastatic niche priming will enable us to predict patterns of metastasis and the likelihood of disease recurrence and will reveal therapeutic vulnerabilities.

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

1. To establish how metabolites released by primary tumours influence invasiveness and the priming of metastatic niches; and

2. To determine the mechanisms through which mutant p53s generate metastatic niches

By addressing these aims, we will identify circulating biomarkers that can predict recurrence, and discover how tumour metabolism influences cellular processes (such as cell migration and endosomal trafficking) which drive invasion, metastasis, and awakening of dormant disseminated tumour cells.

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

Using a comprehensive metabolomic screen, we have found that the landscape of serum metabolites is markedly different in women with metastatic breast cancer by comparison with healthy volunteers (Fig. 1a). We have found that we are able to recapitulate these changes in the serum metabolome using genetically engineered mouse models of mammary carcinoma (Fig. 1b). Importantly, levels of glutamate in the serum reflect tumour burden and acquisition of primary tumour invasiveness (Fig. 1b), and plasma aspartate and γ-amino butyric acid (GABA) concentrations increase as frank metastases form in the lung. Preliminary evidence indicates that glutamate and aspartate release from tumour cells is mediated by alterations in the expression of metabolite transporters of the SLC family, and that these alterations are triggered by the response of tumour cells to metabolic stresses. We are investigating how extracellular levels of glutamate, GABA and aspartate drive invasiveness and metastatic niche priming by activating plasma membrane receptors for these metabolites (i.e. the mGluR, NMDA and GABAR families) on fibroblastic and other cell types to influence extracellular matrix (ECM) deposition in metastatic target organs. Furthermore, we are interested in how these metabolites modulate the immune landscape of tumours and metastatic target organs.

What are the mechanisms through which the expression of mutant p53s and other pro-metastatic oncogenes generate metastatic niches?

We have found that key membrane trafficking events evoked by gain-of-function p53 mutations in primary tumours may be transferred via exosome-mediated mechanisms to cells in other organs. Indeed, exosomes from mutant p53–expressing pancreatic adenocarcinoma (PDAC) (but not in PDAC with p53 loss) can influence integrin trafficking in lung fibroblasts to alter the deposition of ECM proteins such as collagen VI in the lung (Fig. 2). This altered microenvironment provides migratory cues which lead to priming of the lung as a metastatic niche, thus providing a mechanistic rational for why mutant p53¬–expressing PDAC are more metastatic than their p53 null counterparts. We are investigating how pancreatic and colon adenocarcinoma can foster certain ECM microenvironments in metastatic target organs, and how this can promote recruitment of circulating tumour cells and components of the innate and acquired immune systems to break dormancy and drive metastasis. We are using a range of non-invasive ECM imaging available at the Institute to assess the priming of metastatic organs, and to explore pharmacological approaches to reducing metastatic niche formation to oppose cancer recurrence following surgery.

Publications listed on page 90

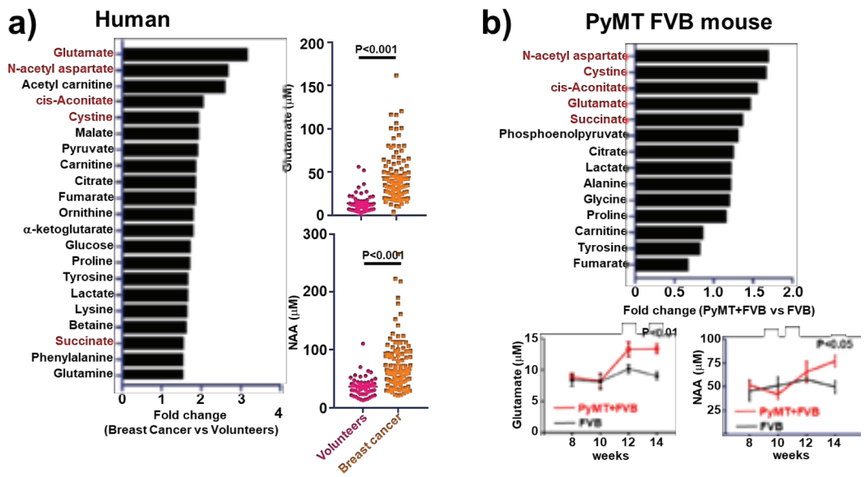

Figure 1
Circulating metabolites in mammary carcinoma in human and mouse.
Circulating metabolites which are significantly different between breast cancer patients and healthy volunteers (a) and MMTV-PyMT and non-tumour bearing FVB control mice (b). Metabolites which are common to human and mouse are highlighted in red. The graphs in (b) indicate that glutamate and N-acetyl aspartate (NAA) levels are significantly elevated in the serum of MMTV-PyMT mice as 12-14 weeks of age. Volunteers, n=35; breast cancer patients, n=120. Non-tumour bearing and MMTV-PyMT mice, n=30.

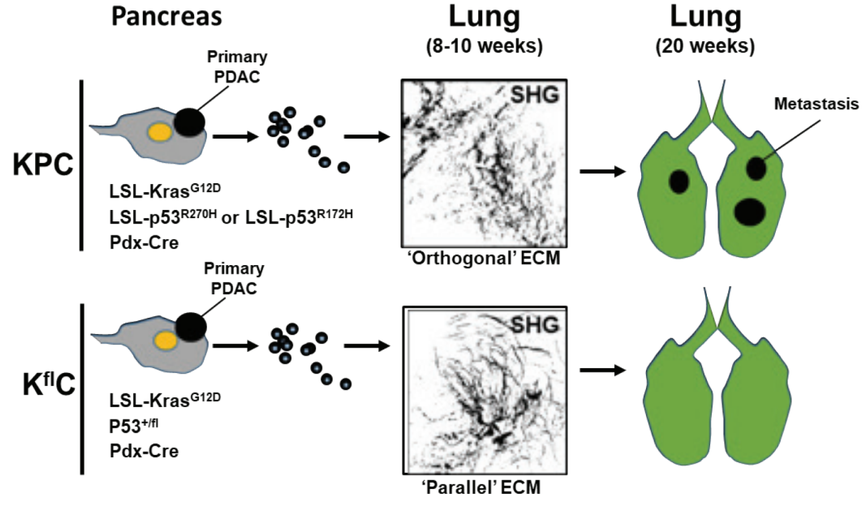

Figure 2
KPC and KflC models of PDAC to study metastatic niche priming.
The KPC model of pancreatic ductal adenocarcinoma (PDAC) is driven by autochthonous expression of mutant KRas and mutant p53 (either $p53^{R70H}$ or $p53^{R172H}$) in the pancreatic epithelium. The KflC model of PDAC is driven by mutant KRas in combination with a floxed allele of p53. Both models promote primary tumour growth with similar penetrance and speed. However, KPC, but not $K^{fl}C$, form metastases which are detectable at 20 weeks of age. Second harmonic generation (SHG) analysis indicates that KPC, but not KflC, mice display alterations to the extracellular matrix (ECM) of the lung at 8-10 weeks of age – prior to the establishment of detectable metastases. The lung ECM of KPC mice is different from that of $K^{fl}C$ mice in a number of quantifiable ways, such as a more orthogonal filament organisation.

COLORECTAL CANCER AND WNT SIGNALLING

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Colorectal cancer is the third most common cancer in the UK and the second most common cause of cancer mortality. The focus of our group is to understand the early changes associated with intestinal neoplasia in order to identify novel markers of the disease as well as new targets for therapy. The key intestinal tumour suppressor is the APC gene, which is mutated in approximately 80% of sporadic cancers but rarely in other cancers. This year our group was part of the SpecifiCancer Cancer Research UK Grand Challenge team funded to understand the basis of tissue specificity of the driver mutations in cancer. Central to our work is the use of novel inducible models of intestinal tumourigenesis that allow us to study the functions of specific tumour suppressor genes.

Elucidating the cell-of-origin for colorectal cancer

Loss of APC is the most common mechanism of deregulated Wnt signalling in colorectal cancer (80% of cancers carry this mutation). However, in liver cancers, *APC* is rarely mutated and instead the Wnt pathway is activated through mutation of β-catenin. While our laboratory has identified significant differences between *APC* and β-catenin mutations in terms of the level of Wnt signalling they potentiate, one question that still puzzled us was why activating mutations in β-catenin within an intestinal stem cell, which would be long lived and should allow ample time for accumulation of β-catenin, fail to transform the intestine. For many years, the rapid turnover of the intestine (4–6 days) had suggested that the intestinal stem cell was the most likely cell-of-origin, and our work had shown if we targeted *APC* loss to stem cells, mice would rapidly develop cancer. Non-stem cells could be transformed but with much less efficiency. To examine this further, we modelled the likelihood of cancer comparing a single activating mutation of β-catenin versus bi-allelic *APC* mutation. We took into account the likelihood of the mutation, the requirement for two *APC* mutations and the fact that it took much longer for a β-catenin mutation to produce a phenotype. Using these parameters, the model predicted that if the stem cell was the cell-of-origin, one would expect β-catenin mutations, but if you include in addition transit amplifying (TA) cells then bi-allelic *APC* mutations were much more likely. Interestingly, the human colon has many more TA cells than the mouse, which may explain why an *Apc* mutation leads to small intestinal tumours in the mouse and colonic tumours in man.

Inhibiting Wnt signalling *in vivo* in cancers that have lost APC: deleting BCL9/9L

Different thresholds of Wnt pathway activation are thought to be required for stem cell maintenance, regeneration, differentiation and cancer. However, the principle that oncogenic Wnt signalling could be specifically targeted remains controversial. Here, we examined the requirement of BCL9/9L, constituent members of the Wnt-enhanceosome, for intestinal transformation following loss of the tumour suppressor *APC*. While these genes were required for maintenance of Lgr5+ intestinal stem cells and intestinal regeneration, *Bcl9/9l* deletion had no impact upon normal intestinal homeostasis. Loss of *BCL9/9l* suppressed many features of acute *APC* loss and the subsequent deregulation of the Wnt pathway *in vivo*. This resulted in a level of Wnt pathway activation that favoured tumour initiation in the proximal small intestine and blocked tumour growth in the colon. Furthermore, *Bcl9/9l* deletion completely abrogated β-catenin-driven intestinal and hepatocellular transformation. We speculate these results support the *just right* hypothesis of Wnt–driven tumour formation. Importantly, loss of *BCL9/9l* is particularly effective at blocking colonic tumourigenesis and suppressing the impact of mutations that most resemble those found in human cancer (Gay *et al.*, Nat. Comms. 2019; 10: 723).

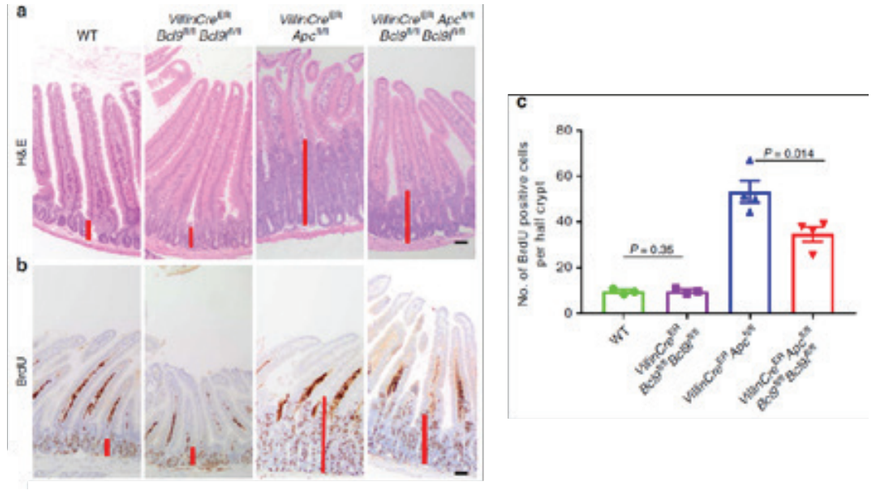

Figure 1: *Bcl9/9l* are required for intestinal transformation driven by *Apc* deficiency.
A) Representative images of haematoxylin/eosin stained wild-type (WT), *VillinCre^{ER} Bcl9/9l$^{fl/fl}$*, *VillinCre^{ER} Apc$^{fl/fl}$* and *VillinCre^{ER} Apc$^{fl/fl}$ Bcl9/9l$^{fl/fl}$* intestinal tissue sampled at 4 days post-Cre-induction.
B) Mice were injected with BrdU prior to sampling, with subsequent immunohistochemical staining of BrdU incorporation highlighting proliferative cells in these tissue specimens as described in (A). Red bars indicate the proliferative crypt. Scale bar: 50 μm.
C) Quantification of cellular proliferation indicated by BrdU incorporation in small intestinal tissues from tissue specimens as described in (A).

Inhibiting Wnt Signalling when the Wnt pathway is not mutated: RAL GTPases

Alongside loss of the tumour suppressor *APC*, colorectal cancers are commonly driven by activating mutations in the oncogene *KRAS*. The Ral GTPases are effectors of RAS signalling and can be seen by implication as potential therapeutic targets for KRAS mutant colorectal cancers and for RAS mutant cancers more broadly. As part of a collaborative effort with Julia Cordero's group (University of Glasgow), we identified the Ral GTPases as critical regulators of Wnt signalling and maintenance of the intestinal stem (ISC) and enteroblast populations of the *Drosophila* midgut and intestinal stem cell compartment in the mammalian intestine. In *Drosophila*, *Rala* was required within ISCs for efficient regeneration downstream of *Wingless (Wg)* signalling, while in the murine intestine, genetic deletion of either mammalian orthologue (Rala or Ralb) resulted in both reduced ISC function and Lgr5 positivity, hypersensitivity to Wnt inhibition and impaired tissue regeneration following damage. Ablation of both mammalian orthologues resulted in rapid crypt death. Mechanistically, we found that the Ral GTPases were required for efficient potentiation of the Wnt signalling pathway through participation in the internalisation of the Wnt receptor Frizzled-7. As a result, ligand-dependent Wnt signalling required Ral GTPase function for efficient activation, implicating Ral GTPase function in definition and maintenance of the intestinal stem cell pool. Intriguingly, as a result of their association with Wnt receptor internalisation, where activation of the Wnt signalling pathway is uncoupled from a dependency upon secreted Wnt ligands, as in the context of APC deficiency, Ral GTPase are no longer required for efficient pathway activation (Johansson *et al.*, *Cell Stem Cell*, 2019; 24: 592–607.e7). As a number of the Ral GEF (guanine nucleotide exchange factor) molecules which control Ral GTPase activity are in turn regulated by the RAS GTPase molecules, our ongoing research in this area focuses upon potential cross-talk between the RAS and Wnt signalling pathways.

Publications listed on page 91

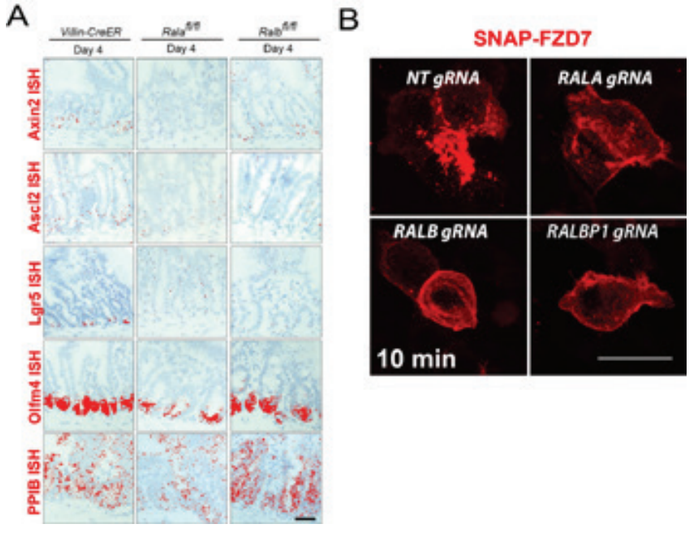

Figure 2: Ral GTPases are required for efficient activation of Wnt signalling.
A) Representative images of *VillinCre^{ER}*, *VillinCre^{ER} Rala$^{fl/fl}$* and *VillinCre^{ER} Ralb$^{fl/fl}$* intestinal tissue sampled at 4 days post-Cre-induction, stained by RNA in situ hybridisation (RNAscope) for the intestinal stem cell markers *Olfm4* or *Ascl2*, Wnt target genes *Lgr5* or *Axin2*, or the positive control *Ppib*, with red dots representing individual mRNA molecules. Scale bar: 50 μm.
B) Subcellular localisation of the Wnt-receptor Frizzled-7 visualised through confocal imaging of SNAP-tag labelled receptors following stimulation of HEK293T cells with serum for 10 minutes. Internalisation is assessed in populations of cells where *Rala*, *Ralb* or their common effector gene *Ralbp1* have been disrupted using CRISPR-Cas9 gene editing technologies and compared to control (NT gRNA) cells.

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 soluble factors, extracellular matrix (ECM) components and modifiers, 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 (Fig. 1) in breast and high-grade serous ovarian (HGSO) cancers because these tumours contain a sizeable proportion of stroma, which is densely populated by CAFs. Furthermore, CAFs have been shown to play key roles in the progression of both diseases. Importantly, ovarian cancer cells have few recurrent mutations and this limits the availability of targeted therapies against the cancer cells. Therefore, 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 promote invasive behaviour of the cancer cells and support their uncontrolled growth, and how CAFs facilitate tumour growth and the spread of metastases by altering the tumour vasculature. 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 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, the normal fibroblasts become activated. This activation induces extensive reprogramming of gene expression and protein levels, such that CAFs become highly contractile and secrete plethora of soluble factors and ECM components that promote the progression of cancer (Santi *et al.* Proteomics 2018; 18:e1700167). It is therefore crucial to better understand 1) the mechanisms that lead to 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; 123: e22–e36; Patella *et al.*, Mol Cell Proteomics 2015; 14: 621–34; Diaz *et al.* J Cell Sci 2017; 130: 697–711, Hernandez-Fernaud, Ruengeler *et al.*, Nat Commun 2017; 8: 14206), and we integrate this innovative technology in our research to tackle the above questions and provide new levels of understanding of CAF biology.

CAFs & invasion

We have previously established a method for global quantitative MS–proteomic analysis of secreted proteins in cell culture (van den Biggelaar *et al.*, Blood 2014; 123: e22–e36; Hernandez-Fernaud, Ruengeler *et al.*, Nat Commun 2017; 8: 14206) and discovered that the chloride intracellular channel protein 3 (CLIC3) is one of the most upregulated and heavily secreted proteins in breast and ovarian cancer CAFs. Using an innovative technique to measure cysteine oxidation that we have recently developed (van der Reest *et al.* Nat Commun 2018; 9:1581), we could also establish an unprecedented role for this protein in the extracellular environment: CLIC3 is a pro-invasive oxidoreductase able to promote tumour invasion and blood vessel growth by increasing ECM stiffness though the reduction (activation) of the extracellular transglutaminase TGM2 (Hernandez-Fernaud, Ruengeler *et al.*, Nat Commun 2017; 8: 14206). We are currently investigating the role of stromal CLIC3 in HGSO cancers to assess its potential as an effective target for this type of cancer, particularly for blocking metastasis, which is the major cause of patient lethality.

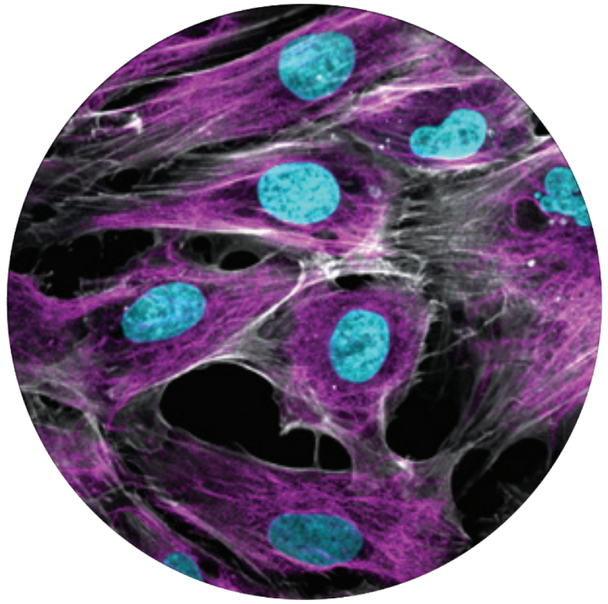
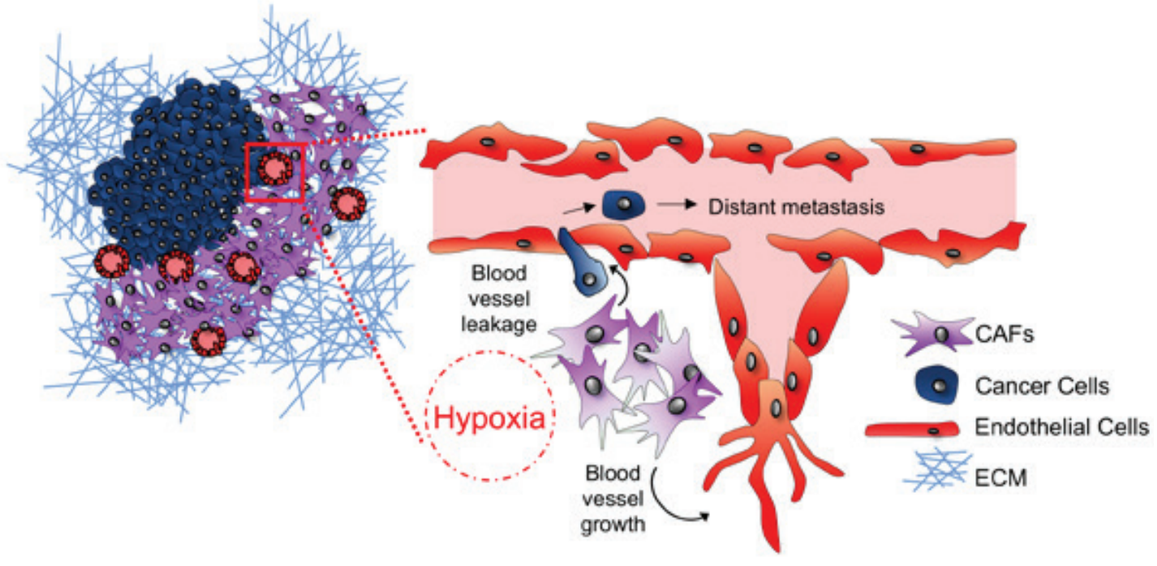


Figure 1
Immunofluorescence staining of breast cancer CAFs
Cyan = cell nuclei; magenta = vimentin filaments; grey = actin cytoskeleton

CAFs & hypoxia

Intratumoral hypoxia is typical in aggressive cancers and causes the formation of leaky blood vessels, which contribute to tumour metastasis and reduce the efficacy of therapeutic treatments. Using 3D co-cultures of breast cancer CAFs and ECs, we have found that hypoxia exacerbates the ability of CAFs to promote vascular endothelial growth factor (VEGF)-dependent EC sprouting and invasion, which are the key steps for the formation of new blood vessels and to create leaky vessels in tumours (Fig. 2). Extensive proteomic analysis of hypoxic CAFs showed that hypoxia induces a strong upregulation of the uncharacterised protein NCBP2-AS2. We have discovered that NCBP2-AS2 in CAFs is important to support the release of VEGFA induced by hypoxia, which is the most potent inducer of tumour blood vessel growth and leakage. Importantly, genetically targeting NCBP2-AS2 in hypoxic CAFs blocks their ability to promote VEGF-dependent EC sprouting/invasion and blood vessel growth (Kugeratski *et al.* Science Signaling 2019; 12: pii: eaan8247). Our discovery may have an important impact in cancer because targeting NCBP2-AS2 holds promise to block the formation of a dysfunctional vasculature in hypoxic cancers, thus reducing metastases and improving efficacy of therapeutic treatments.

Figure 2
When there is intratumoral hypoxia, CAFs release factors that promote blood vessel growth. (Cartoon by Alice Santi)



CAFs & 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 the cancer cells, the knowledge of the metabolism of stromal cells is lagging behind. Currently, we are investigating the metabolic pathways in breast cancer CAFs that are key to support their pro-tumourigenic and pro-invasive functions and how we can effectively target them.

Publications listed on page 95

DRUG DISCOVERY

DRUG DISCOVERY UNIT

Co-Heads

Justin Bower

Heather McKinnon

Chemistry

Justin Bower
Kenneth Davies
Stuart Francis
Claire Gardner
Callum MacGregor
Duncan McArthur
Charles Parry
Angelo Pugliese
Mairi Sime
John Taylor
Christopher West

Biology

Amy Bryson
Jonathan Clark
Daniel Croft
Sebastian Greenhough
Susan Macdonald
Craig Mackay
Laura McDonald
Heather McKinnon
Mokdad Mezna
Francesca Pellicano
Dominika Rudzka

Structural Sciences

Kenneth Cameron
Nichole Cerutti
Andrea Gohlke
Christopher Gray
Marta Klejnot
Jennifer Konczal
Camila Pasquali
Katie Pollock
Alexander Schuettelkopf

Lab Support

Michael Kilday

Infomatics Manager

Daniel James

The Drug Discovery Unit operates at the interface between discovery biology and clinical development. We provide a mechanism by which fundamental biology being undertaken within the Beatson Institute can be translated into new medicines for patients where there is a clear unmet medical need. We work collaboratively with the Institute's research scientists to identify areas of biology fundamental to the progression of cancer and target these to develop novel medicines that will positively impact the lives of patients.

Drug discovery is a multi-faceted scientific endeavour, involving expert scientists in cancer biology, chemistry, biophysics, biochemistry, structural sciences and clinical research. High quality, reproducible science is central to drug discovery and we have developed a collaborative team with a robust approach to rapidly progressing novel therapeutic opportunities. Working closely with Beatson scientists and clinicians, we have identified a number of exciting new targets that are being progressed in our laboratory.

Identifying new cancer target is the key initial step in the drug discovery process. Once this happens, a number of parallel drug discovery activities ensure a rationalised progression through discovery research to generate a new drug, ready to test in patients. We have built a focused capability that enables us to execute even the most challenging projects and successfully progress them through the drug discovery process. In order to understand how to modulate the function of a target (typically a protein) that is important in the progression of cancer, we need to understand how it works and how best to stop it working. By identifying molecules that interact with our target of interest we can evaluate their potential to modulate target function and gain valuable insight into their importance in cancer.

Drawing on the world-class research at the Beatson Institute is a key remit of the Drug Discovery Unit. As such, we interact closely with research groups within the Institute to identify novel and exciting targets for translation into the drug discovery paradigm. In collaboration with

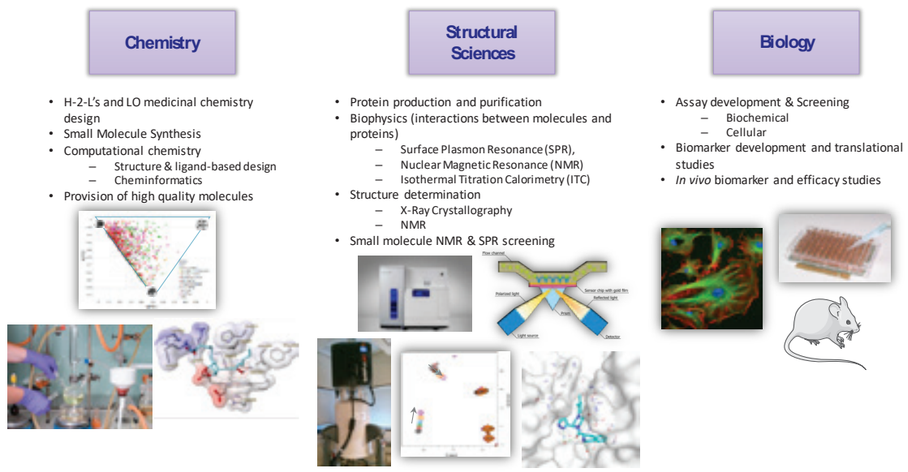
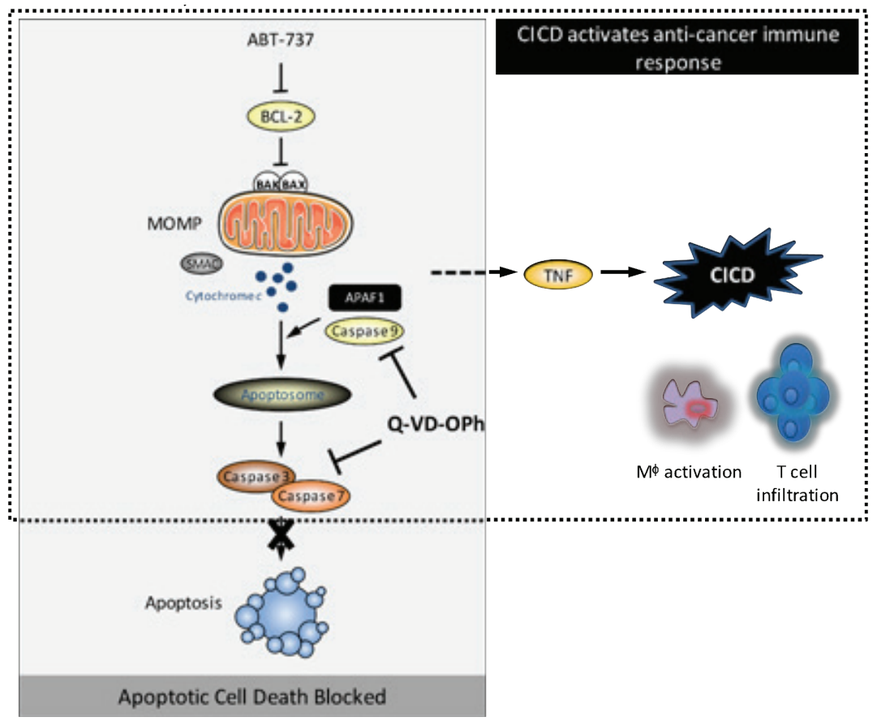


Figure 1 Drug Discovery Unit Capability & Expertise

Figure 2
Caspase inhibition with the peptide pan-caspase inhibitor Q-VD-OPh blocks apoptotic cell death induced by ABT-737 and re-routes cancer cells to die via CICD.
CICD is immunogenic and activates an anti-cancer immune response to destroy the remaining tumour cells.



Professor Stephen Tait, we are aiming to identify small molecule inhibitors of the caspase proteases that can be combined with standard-of-care chemotherapeutics to promote a caspase-independent cell death in tumours.

Targeting cancer cells to die by Caspase-Independent Cell Death (CICD)

Apoptosis represents the primary mechanism by which cancer cells die when exposed to anti-cancer therapies. The first committed stage in cell death is permeabilisation of the mitochondrial outer membrane (a process called MOMP). BH3 mimetics such as ABT-737 induce MOMP, which leads to the subsequent activation of the caspase cascade. By its nature, apoptosis is limited to the specific cells affected by treatment and furthermore, can promote oncogenesis in remaining cells, leading to recurrence. Once cells undergo MOMP, this is a point of no return for cell viability and will result in cell death. However, seminal work in Stephen Tait's group has demonstrated that a block of the caspase cascade (notably caspase-9) causes cells to be re-routed from a rapid apoptotic death to a delayed caspase-independent cell death (CICD) that is pro-inflammatory. Blockade of caspase 9 *in vitro* and *in vivo* leads to activation of pro-inflammatory M1 macrophages and immune clearance of tumours, which is not seen in immune-compromised mice.

Working closely with Professor Stephen Tait and his group, we have made excellent progress in developing a robust screening cascade that utilises our in-house biophysics, crystallography, biochemistry and cell biology expertise. By performing druggability and structural assessments of the caspase family, we have identified potentially druggable binding sites. With that in mind, we have developed a strategy to screen for small molecules that could perturb the function of these caspases.

To facilitate our drug discovery efforts, we have developed and validated biochemical assays for measuring activities of a number of caspases. All assays use a tetra-peptide substrate (e.g. DEVD for caspases 3 and 7) labelled with the fluorescent molecule amino-4-trifluoromethylcoumarin (AFC). Cleavage of this fluorophore leads to a concomitant increase in the fluorescence signal, allowing for detection of caspase activity modulation. We have utilised a small panel of commercially available caspase inhibitors to validate these assays and show their suitability for compound screening (Fig. 3). By screening an in-house library of ~750 irreversible compounds, we have identified a number of chemical hits for caspase 3/7 inhibition and have shown the assay is suitable for a larger high-throughput screening campaign.

We have characterised appropriate cellular assay systems to measure caspase inhibition and changes in cell viability, and therefore CICD. When used alone the BH3 mimetic ABT-737 induces MOMP, caspase activation and a rapid apoptotic form of cell death (Fig. 4). Treatment

with a pan-caspase inhibitor (such as Emricasan, Q-VD-OPh or Z-VD-fmk) in combination with ABT-737 prevents cells from dying via apoptosis but instead promotes a delayed, non-apoptotic cell death that is independent of caspase 3, 7 and 9 activity (Fig. 4). Moreover, these HCT116 cells undergoing CICD upregulate the expression of a number of cytokines and chemokines, such as *CSF2*, *CXCL1* and *TNFα*, involved in pro-inflammatory signalling and immune cell activation (Fig. 5).

Taking a multi-disciplinary approach, which is the foundation of all drug discovery projects, has enabled rapid target validation and assay development in the caspase project. Working with Stephen Tait provides the depth of expertise in this complex area of cell death in cancer, helping to guide the key biological questions that will enable us to target this cellular process in the most effective manner. This model of close collaboration is the basis of all drug discovery projects at the Institute and ensures even the most challenging targets are approached with a strong and far-reaching capability that allows for efficient and successful progression of promising new treatments in cancer.

Publications listed on page 84

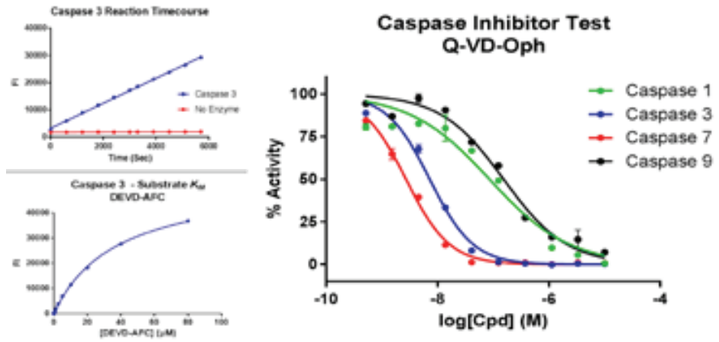


Figure 3
Biochemical Assay Development: Reaction time course and substrate K_M for Caspase 3.
Caspase panel response to inhibition with Q-VD-OPh.

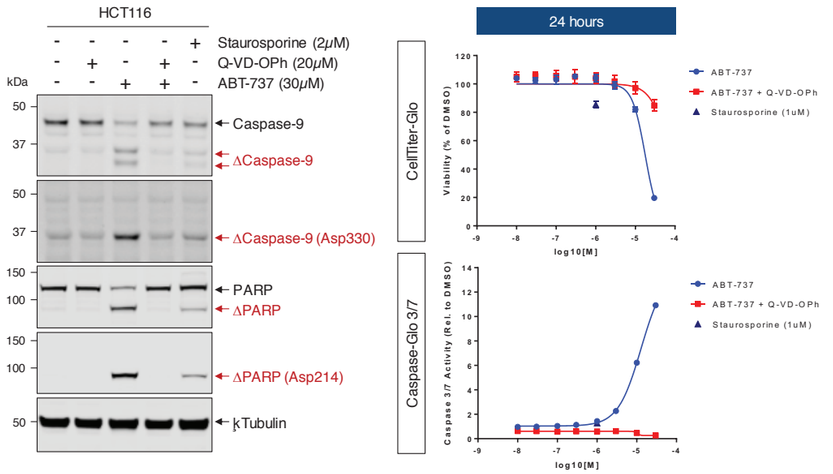


Figure 4
Caspase inhibition with Q-VD-OPh in combination with ABT-737 leads to a non-apoptotic cell death in HCT116 cells that is independent of caspase 3, 7, and 9 activity.

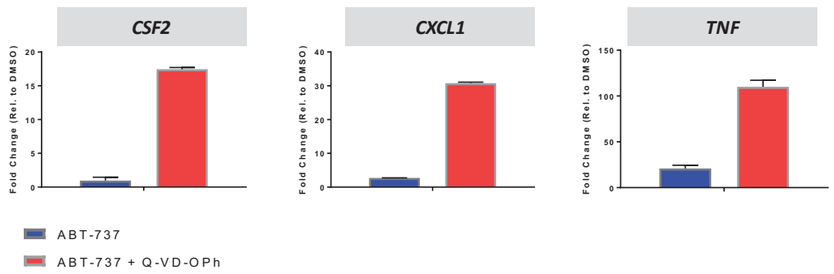


Figure 5
CICD upregulates the expression of pro-inflammatory cytokines and chemokines involved in pro-inflammatory signalling and immune cell activation.

BEATSON ADVANCED IMAGING RESOURCE (BAIR)

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 key cells of 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.

Beatson Advanced Imaging Resource (BAIR) scientists work 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 plot or uses sorted cells. We try 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 keen to help researchers identify how our methods can be used to test key hypotheses and help them to design experiments that make the most of the resources we have. We also help to identify new technology and methodology that allow our researchers to take the most elegant approaches.

I can't believe it is already the third time we have had a BAIR imaging competition. Once again, an excellent field ranging from high-content to super-resolution. A difficult choice for all of our voters. This year Zeiss provided high-end binoculars for first prize, won by Nikki Paul! Some of the stunning pictures that exhibit the excellent imaging performed in the BAIR are showcased throughout the report.

Imaging across different spatial and biological complexity scales

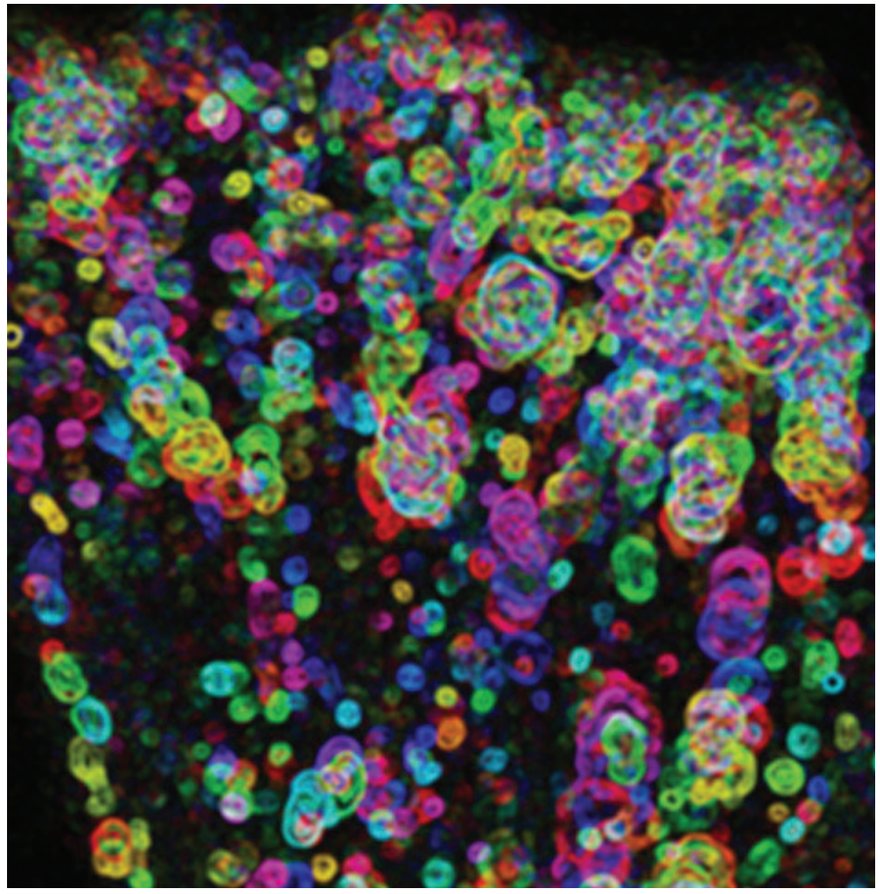
We now have the expertise and instruments to:

- 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 and plate handling for very high-throughput imaging experiments to analyse cell behaviour over thousands of experimental conditions via high-content imaging
- Image and spatially separate and quantitate up to seven markers in thick tissue 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 superresolution imaging
- Address cell function in 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.

Publications listed on page 83

Lysosome trafficking by Nikki Paul, winner of the 2018 BAIR imaging competition. PDAC cell expressing GFP-ATP6V0D1 in lysosomes. Imaged every 1 minute for 30mins using Zeiss 880 LSM with Airyscan. Each frame coloured differently over time.



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 analysis has continued to grow, and this has been accompanied by an increasing interest in using computational and machine learning approaches to interpret imaging and proteomics data. In order to meet these demands, another bioinformatician (Robin Shaw) joined our team in July 2018. The last year has also seen a growth in the use of machine learning approaches to help characterise our data, and we have seen an increasing demand to apply quantitative approaches to the analysis of microscopy and imaging data generated by the Beatson Advanced Imaging Resource (BAIR; pp 66).

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.

PROTEOMICS

Head
Sara Zanivan
(see page 52)

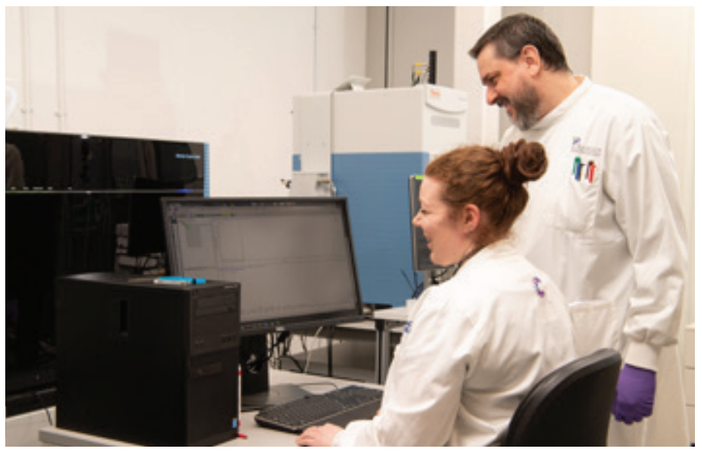
Scientific Officers
Kelly Hodge
Grigorios Koulouras
Sergio Lilla

Proteins constitute 50% of the cell (dry) mass and mass spectrometry (MS)-based proteomics is key to unravelling the identity and function of each protein. The proteomics facility is working with cutting-edge mass spectrometry 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 at the Institute and outside, and we actively develop MS-based proteomic platforms to address a variety of questions to help scientists better understand the mechanisms that regulate various aspects of cancer.

To achieve this, we are well equipped with three nano-liquid chromatography (nLC)–MS systems. This year we have installed the newest-generation Orbitrap instrument, Fusion-Lumos; we also have a Q-Exactive HF and an Orbitrap Elite. 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 pieces of software, of which MaxQuant is the most used, for highly accurate label-free or label-based quantitative analysis. Moreover, we use Skyline for the analysis of RM data. Finally, we use Perseus and Scaffold for data analysis and dissemination.



This year we have recruited Kelly Hodge as a new senior scientific officer, who is expert in sample preparation for proteomic analysis and MS data analysis. We have also recruited our first bioinformatician, Greg Koulouras, who is expert in web and Android programming and in working with big data and sensitive personal information. With Greg, we are currently working at an ambitious project of developing a cancer-centric database for the navigation through and analysis of MS–proteomic data.

During 2018, we have worked with many of the groups at the Institute and significantly contributed to the success of their research. Following the development of our novel stable isotope-based method, SICyLIA, for the global measurement of cysteine oxidation we are now further developing the approach to enable dynamic measurement of cysteine oxidation and to achieve higher depth of our analyses in a shorter time.

We are continuously striving to develop methods to answer more complex biological questions using proteomics and improve the methods currently in place to enrich the quality of the data that the facility can provide. We have now set up tandem mass tag (TMT)–based labelling approaches to multiplex up to ten samples in one. Moreover, we have been working to improve the depth of the proteomes and sub-proteomes achieved starting with small amounts of sample, e.g. FACS-sorted immune cells, using high-pH reverse-phase LC fractionation. This enables us to perform improved global proteomic, sub-proteomic and post-translational modification analyses of primary cells, circulating blood cells and 3D organotypic cell cultures.

Publications listed on page 95

METABOLOMICS

Head
Gillian Mackay

Scientific Officers
David Sumpton
Giovanny Rodriguez-Blanco

Graduate Student
Rachel Harris

The Metabolomics facility employs state-of-the-art mass spectrometry techniques to measure small molecules (metabolites) and explore changes in metabolic pathways in cancer cells. The facility supports many cancer metabolism research projects within the Institute. We are also responsible for the analysis of lipids and are currently validating simplified extraction methods for lipidomics analysis. For a third year, we were involved in organising and delivering a practical metabolomics course at Cold Spring Harbor Laboratory in the USA. The team has expanded and a new PhD student studying metabolomics of BRAF mutant melanoma has joined the group.

Our metabolomics platform is currently focused on the use of our two Thermo Scientific LC-MS systems (Q Exactive Plus and Q Exactive) with their high-resolution, accurate mass, Orbitrap technology, used for both targeted analysis and more in-depth metabolite profiling (untargeted). We have well-established LC–MS methods using HILIC chromatography. Our Thermo Scientific™ TSQ Altis™ triple quad mass spectrometer is used for specific targeted LC–MS/MS analysis, offering increased sensitivity and specificity for known metabolites, such as in the measurement of lysophosphatidic acids (LPAs). 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 acetate.

We work closely with the groups of Saverio Tardito, Alexei Vazquez and Jim Norman and also support several other research groups within the Institute who have specific interests in cancer metabolism. We provide advice for sample preparation for a range of sample types including cell extracts, plasma, urine and tumour tissue, and support for data analysis, based on Thermo Scientific™ TraceFinder™ and Compound Discoverer™ software.

With our targeted approach to metabolomics, we have increased the number of metabolites we can identify on our LC–MS platform to 400, which includes a broad range of metabolites of different classes. Potentially all 400 metabolites can be detected in a single analysis. Experiments using stable isotope tracers (such as ^{13}C glucose in the medium) enable us to examine the intracellular kinetics and the proportional distribution of metabolites produced from the tracer. We can also quantify metabolite abundance and calculate metabolite exchange rates between cells and the medium in which they are grown.

Using an untargeted approach, we look for novel metabolic changes, by identifying compounds showing altered abundances in our cancer models. We use Thermo Scientific's™ Compound Discoverer™ software, where we can link to other Thermo Scientific™ tools, including their mzCloud™ database of fragmentation spectra. Changes in metabolites can be shown using various statistical approaches, such as PCA and OPLS-DA, and metabolites are identified using a range of factors, such as accurate mass, adducts, isotopes and fragmentation spectra, comparing with the Human Metabolome Database (HMDB) and other databases. We are using this technique for a few specific *in vivo* and clinical projects.

After Jurre Kamphorst, the lipidomics research group leader, and their group's mass spectrometrist, left during 2018, we have become responsible for all methods for lipid analysis in the institute.

We are investigating various approaches to lipidomics methodology and data analysis. We have developed a simplified one-phase extraction method and are currently validating this method for cell culture experiments. This methodology shows a good coverage of different lipid species, and allows the use of protein content from the same sample for normalisation. We use high-resolution Orbitrap mass spectrometry (Q-Exactive) in single polarity mode and data-dependent fragmentation acquisition (ddMS2), to identify the lipids using both accurate mass and fragmentation patterns. We apply two complementary approaches for lipidomics data processing. LipidSearch (Thermo Scientific) mainly identifies lipid classes in the sample. For lipid quantitation, our workflow involves a recently developed open source software called LipiDex, which integrates with Compound Discoverer™ (Thermo Scientific™) software. We use databases such as LipidBlast that contain *in-silico* fragmentation information for more than 30 lipid classes. The data obtained can be processed using both univariate and multivariate statistics to identify changes in lipid composition in particular biological conditions.

In collaboration with the oncometabolism laboratory, led by Saverio Tardito, our new PhD student is aiming to identify metabolic targets in BRAF mutant melanoma through comprehensive metabolomics. In particular, we are interested in the metabolic reactions which are critical for the development of resistance to BRAF inhibitor drugs. Melanoma patients who acquire resistance to BRAF inhibitors eventually relapse, and novel therapeutic options are needed to impair the progression of these tumours.

Publications listed on page 88

TRANSGENIC MODELS OF CANCER

Head
Karen Blyth

Research Scientist
Nicholas Rooney

Scientific Officers
Dimitris Athineos
Sandeep Dhayade
Susan Mason

Graduate Students
Adiba Khan
Narisa Phinichkusolchit
Alessandra Riggio
Kerri Sweeney[1]

[1]Breast Cancer Now

Our lab uses *in vivo* models to recapitulate human cancer and interrogate all 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 RUNX1/CBFβ transcriptional complex and pro-survival factor MCL1 contribute to tumour progression and metastasis.

Modelling cancer *in vivo*

The ability of cancers to spontaneously grow at the site of origin, to invade surrounding tissue, and colonise distant organs is a complex process. So interrogating aspects of this multifaceted behaviour in a monolayer setting within a dish can have limitations. It is key therefore to use physiologically relevant models in which tumours arise and mature in their natural environment *in situ*. In this way, tumour cells directly and spatially co-evolve with stromal fibroblasts, immune cells and the endothelium, recapitulating a more accurate tumour microenvironment, and have to negotiate biological barriers in order to metastasise. In addition, many anti-cancer drugs fail in the clinic because although they are effective in simplified tissue culture models, the nuances of taking these drugs into the whole animal setting cannot be ignored. Our group uses preclinical models such as xenograft, allograft and genetically engineered mouse models to translate and vindicate the findings from *in vitro* analyses *in vivo* for the benefit of cancer patients. The lab collaborates with many of the groups at the Institute as well as with local, national and international collaborators. For example, we have ongoing projects with Seth Coffelt (Beatson Institute) and Patricia Roxburgh (University of Glasgow), testing novel therapeutic combinations to treat breast and ovarian cancer; with Oliver Maddocks (University of Glasgow), looking for metabolic vulnerabilities *in vivo*; with Sara Zanivan (Beatson Institute), interrogating the role of the tumour microenvironment in metastasis; and with Melchiorre Cervello (CNR, Italy), assessing novel players in steatosis and hepatocellular carcinoma (funded by Associazione Italiana per la Ricerca sul Cancro).

As one of the hallmarks of cancer is the adaptation to restrictive energetic sources, the cellular metabolism of tumours is often very different to the corresponding normal tissue from which that cancer emerges. Thus it is possible that such altered metabolism may provide biomarkers for disease progression and/or an Achilles heel for therapeutic intervention. Together with Jim Norman's lab and under the auspices of SEARCHBreast (https://searchbreast.org/), we have created a useful tissue resource from the MMTV-PyMT model of breast cancer to explore this in an *in vivo* setting. Indeed, certain metabolites (e.g. glutamate) are increased in the serum of our preclinical model during disease progression. Excitingly, these same metabolites are increased in the serum of breast cancer patients exemplifying the power of our preclinical models. Using this resource we have also collaborated with Alexei Vasquez to show that serine is increased in mammary (and intestinal) tumours. This results in increased serine catabolism to formate, with higher levels of formate in the tumour tissue and serum of disease-bearing animals, revealing a novel mechanism of how tumours can cope under the pressures of energetic restriction.

MCL1 as a prognostic indicator and drug target in breast cancer

Breast cancer remains the most common cancer in the UK and the second biggest cause of cancer death in women. Whilst great strides have been made in treating breast cancer, there is still an ongoing need to understand the disease better, and we have several projects in the lab to study this. In collaboration with Kirsteen Campbell and Stephen Tait (funded by Breast Cancer Now), we have been studying MCL1 as an exciting and novel target in breast cancer. MCL-1 is renowned for its role in leukaemia, but we showed that high levels of MCL-1 protein were also found in samples from breast cancer patients, and furthermore, high MCL-1 correlated with significantly reduced patient survival. Using *in vivo* models, we have exciting data to prove that targeting MCL-1 slows tumour growth and causes regression of pre-existing disease.

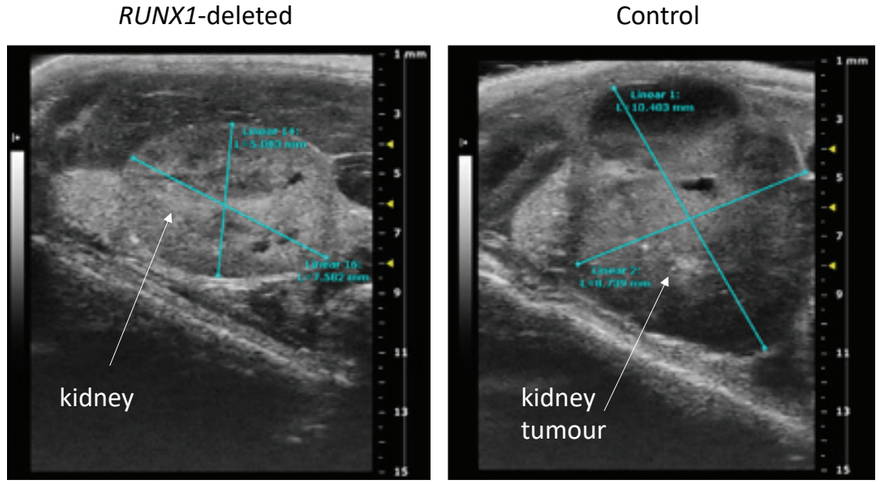


Figure 1
Ultrasound images of kidneys from an orthotopic xenograft model where renal adenocarcinoma cells (786-O) were CRISPR/CAS9 deleted for RUNX1 (left) or control (right). Increased size of the kidney effaced with tumour is indicated in the control kidney (shown by blue measurements).

The *RUNX/CBFβ* transcriptional complex in breast and other epithelial cancers

Two other understudied players in breast cancer are the RUNX1 transcription factor and its obligate DNA binding partner CBFβ. RUNX1 has an enigmatic role in breast cancer, and PhD students Alessandra Riggio and Kerri Sweeney have been using *in vitro* and *in vivo* models to probe its putative tumour suppressor and/or pro-oncogenic properties. In two independent genetic models of breast cancer, RUNX1 acts to restrain tumour onset, with data linking *Runx1* to mammary cell stemness. Nicholas Rooney in the lab has been investigating a new role for the RUNX proteins in kidney cancer, where RUNX1 (and related family member RUNX2) is expressed in human kidney cancer patients with prognostic indication. When *RUNX1* is deleted in renal cell carcinoma (RCC) cells, growth is compromised and the cells have a reduced capacity to form tumours in an orthotopic xenograft model (Fig. 1). Furthermore, deletion of RUNX1 delays disease onset and progression in a genetic model of kidney cancer. We have been utilising RNA sequencing to understand the global gene expression changes that occur upon RUNX1 deletion.

We were delighted that Alessandra Riggio successfully defended her PhD thesis, '*The role of RUNX1 in genetic models of breast cancer*', in February. Although sad to see Alessandra leave, we were delighted to welcome two new students this year; Narisa Phinichkusolchit and Adiba Khan, who have respective projects investigating the role of *Mdm2* in tumourigenesis and CBFβ in breast cancer.

Publications listed on page 82

TRANSGENIC TECHNOLOGY

Head
Douglas Strathdee

Research Scientist
Eve Anderson

Scientific Officers
Cecilia Langhorne
Farah Naz Ghaffar

The Transgenic Technology Laboratory makes use of molecular genetic approaches to understand the function of genes in the development of cancer. Using stem cells allows us to introduce precise alterations into the genome by techniques such as genome editing or gene targeting. Using this type of method allows us to introduce genetic alterations into cells which can accurately reproduce the mutations observed in human cancers. Additionally, it is also possible to introduce a number of different mutations into the same cells. This allows us to reproduce the combinations of genetic changes seen in cancer and understand how those changes interact during the development of the disease.

Using stem cells to generate disease models

Embryonic stem (ES) cells have properties which make them useful for generating disease models. First, they have high rates of homologous recombination, which allows the introduction of very precise genetic alterations. This ensures we can introduce identical changes to those detected in human cancers. Following the introduction of the desired genetic changes these cells can then be differentiated into a variety of different cell types. Consequently, this allows us to study gene function in the development of cancer, in cells types derived from the ES cells, directly relevant to the tissue of origin of human cancer that we are modelling.

This year, we have collaborated with a number of research groups using this type of technology to generate a variety to different types of genetically altered alleles, including conditional knockouts, point mutations and marker genes. These can be introduced by homologous recombination or by genome editing.

More complex alleles to suit different models

Increasingly we are making use of more complex allele designs to enable more accurate modelling of disease progression. Often the genetic changes seen in human cancers are point mutations, where only a single base is altered in the genetic code for a particular gene. Although small, these changes can have a major impact on the function of the altered protein, for example the mutations which activate oncogenes such as Ras. In disease these fine alterations are only found altered in the cancer and not throughout the rest of the organism.

In order to accurately model these sorts of changes we have created a number of conditional point mutation methods, which can give both temporal and spatial control over the expression of the mutant allele. This allows us to control when and where the altered protein is produced, and can ensure that this mutant protein is only made in a subset of cells, a situation which more closely resembles that seen in human disease. Recently, we have designed a number of these types of alleles for different projects. In each case the allele was designed in a different way and tailored specifically for the requirements of the individual project.

In the first example (Fig. 1A), a mixture of normal and mutated protein was required to be expressed in a particular cell type. Consequently, the targeted allele was designed in such a way that there is no expression prior to recombination. This is achieved by inserting a *lacZ* gene trap cassette into the gene at the same time as introducing a genetic change into the coding sequence of the last exon. Following recombination in heterozygous cells, the gene trap cassette is deleted and the cells now start to produce protein including the genetic alteration. These cells continue to express wild-type protein form the wild-type allele.

In the second example (Fig. 1B), only the mutant protein was required to be expressed in the relevant cells. Other cell types were required to express normal levels of the endogenous protein. As the desired alteration was again in the last exon, this was achieved in this case by duplication of the last two exons of the gene.

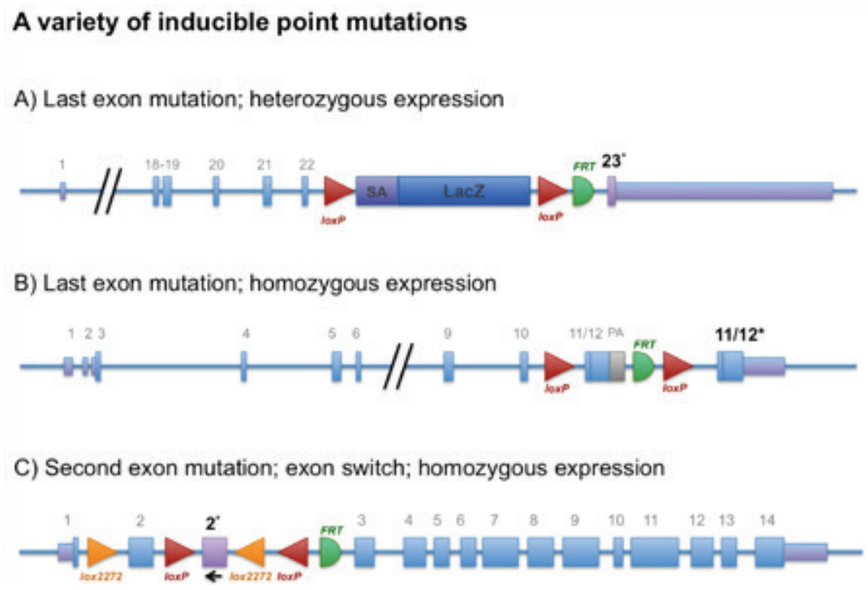


Figure 1
A variety of different inducible point mutant alleles designed to fit the criteria for different models
Each diagram represents a different allele. Blue numbered boxes represent exons. Red triangles are *loxP* sites. Orange triangles are modified *loxP* sites (lox2272). Green semi-circles are FRT sites. Exons with mutations are numbered in bold with asterisks(*).
(a) point mutation in the final exon; heterozygous expression required. There is a gene trap (*SA-lacZ*) cassette inserted in the allele, preventing expression. Following recombination at the *loxP* sites, the cassette is removed, allowing expression.
(b) point mutation in the final exon; homozygous expression required. Exons 11 and 12 are duplicated, grey box is an SV40 polyadenylation signal sequence. Following recombination, the wild-type exons 11 and 12 are deleted and replaced by those carrying the mutation.
(c) point mutation in the second exon; homozygous expression required. The second exon is duplicated but in the opposite orientation to the rest of the gene. Following recombination, at *loxP* and modified *lox* sites, the wild-type exon is deleted; the mutant exon is inverted and replaces the original wild-type exon.

The second copy of the exons was modified to incorporate the relevant mutation. Following recombinase expression, the recombination at the *loxP* sites removes the wild-type exons and replaces these with the exons carrying the mutation. Consequently, cells which are homozygous for this allele switch from producing the normal protein to the mutant protein following recombinase expression.

The last example (Fig. 1C) shows an allele where the desired mutation is in the second exon of the gene. In this case again, the cells should express the wild-type protein until recombination, and then the mutant protein after recombination. This was achieved in this case by placing an inverted copy of the second exon just downstream of the wild-type exon 2. As the altered allele is in the opposite orientation to the gene, it is not included in the RNA, and therefore not expressed prior to recombination. By using a combination of *loxP* and modified *loxP* sites, upon recombination the original exon is deleted and the modified exon is inverted to replace the original. Consequently, homozygous cells stop producing wild-type protein upon recombination and instead produce the genetically altered variant.

These types of approaches allow us to generate more sophisticated and accurate cancer models to uncover the functions of a variety of different genes and the roles that these can play in the progression of different cancer types.

Publications listed on page 93

TRANSLATIONAL MOLECULAR IMAGING (TMI)

Head
David Lewis

PET Chemist
Dmitry Soloviev

Staff Scientist
Gaurav Malviya

Medical Physicist
Caroline Findlay

Scientific Officers
Emma Johnson
Agata Mrowinska

The Translational Molecular Imaging facility collaborates and drives in vivo imaging research projects from the state-of-the-art preclinical models at the Beatson Institute through to clinical implantation at the West of Scotland PET Centre. Investment from the Beatson Cancer Charity of £240k/year has purchased critical radiochemistry infrastructure and funded a Medical Physicist to develop and translate quantitative PET imaging biomarkers.

In 2018, we have upgraded the radiochemistry laboratory in the Radiopharmaceutical Unit at the West of Scotland PET Centre to develop new carbon-11 and fluorine-11 labelled PET probes. Due to recent engineering works in the cyclotron and radiochemistry laboratory, we can now produce high-level carbon-11 labelled gaseous products. The safety and robustness of large-scale carbon-11 tracer productions has now been thoroughly demonstrated. Carbon-11 is a versatile radiolabel, tracing many endogenous metabolic pathways. These engineering works therefore enable flexible development of metabolic PET imaging probes for cancer imaging.
We have installed two identical universal automatic $^{11}C/^{18}F$ synthesisers (Synthra GmbH, Germany) in the R&D and GMP radiolabelling suites. Installation of the two synthesisers finished successfully in June 2018 and the first new tracers, [^{11}C]acetate, [^{18}F]fluoro-ethyl-tyrosine (FET), [^{11}C]methionine, [^{18}F]fluoro-thymidine (FLT) and [^{18}F] tetrafluoroborate (TFB), were made available for preclinical studies this year. Preparatory work to expand the list of available radiotracers has started with plans to produce (4S)-4-(3-[^{18}F]fluoropropyl)-L-glutamate (FSPG), [^{11}C]palmitate and [^{11}C]leucine for research use in 2019. Any radiotracer developed for preclinical research will be available for rapid translation to human studies at the PET Radiopharmaceutical Unit in Gartnavel Royal Hospital.

Collaboration with the group of Dr Andrew Sutherland, School of Chemistry at the University of Glasgow, has provided two novel tracers, a carbon-11 labelled S1P receptor and a fluorine-18–labelled TSPO tracer which, in collaboration with Hing Leung, will be piloted for imaging castrate-resistant prostate cancer.

Preclinical and Translational Imaging

Together with MRI, which provides functional and high-contrast soft tissue imaging, PET non-invasively assesses specific biological processes such as glycolysis, fatty acid synthesis, proliferation, redox, hypoxia, amino acid uptake, and protein and nucleotide synthesis.

In 2018, we started exploring the role of PET/MRI in phenotyping subtypes of colon cancer. Colon cancer has recently been stratified into four major subtypes, and Glasgow is leading a European-wide consortium, ACRCelerate: Colorectal Cancer Stratified Medicine Network, to enable better matching of colon cancer subtypes to therapeutic trials. In collaboration with Owen Sansom and using the collection of state-of the-art models at the Institute, we aim to develop non-invasive imaging for spatial and temporal stratification of colon cancer. Initial multiplexed PET imaging, probing glucose, nucleotide, amino acid and fatty acid metabolism (Fig. 2), has revealed subtype-specific differences in imaging phenotype. We aim to further validate this work to identify co-existing subtypes and to image subtype plasticity.

In collaboration with Karen Blyth and Jim Norman, we are exploring metabolic imaging in the PyMT breast cancer model to image tumour evolution from normal breast epithelium through invasive ductal carcinoma and metastasis. This project aims to find specific imaging biomarkers of aggressive and metastatic breast cancer which could guide patient management.

Publications listed on page 88

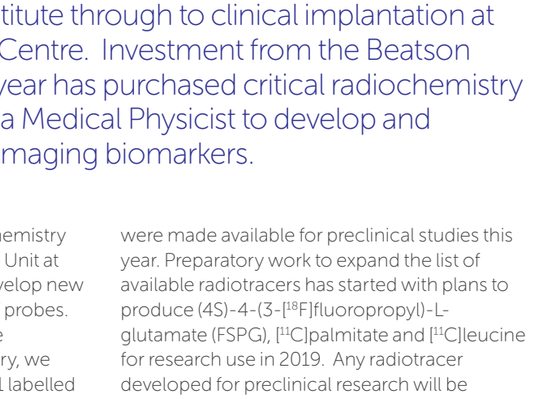


Figure 1
Radioactive hot-cells at the PET Radiopharmaceutical Production Unit at Gartnavel Royal Hospital containing one of the new state-of-the-art Synthra Wolpertinger modules (top left and right) and the refurbished GE Tracerlab (bottom left) with Dr Dmitry Soloviev, Senior PET Chemist. The Synthra Wolpertinger can make any ^{11}C- or ^{18}F-labelled metabolite, and the GE Tracerlab is used for research fluorinations. These different modules can be run in quick succession for near simultaneous production of ^{11}C and ^{18}F products for research use.

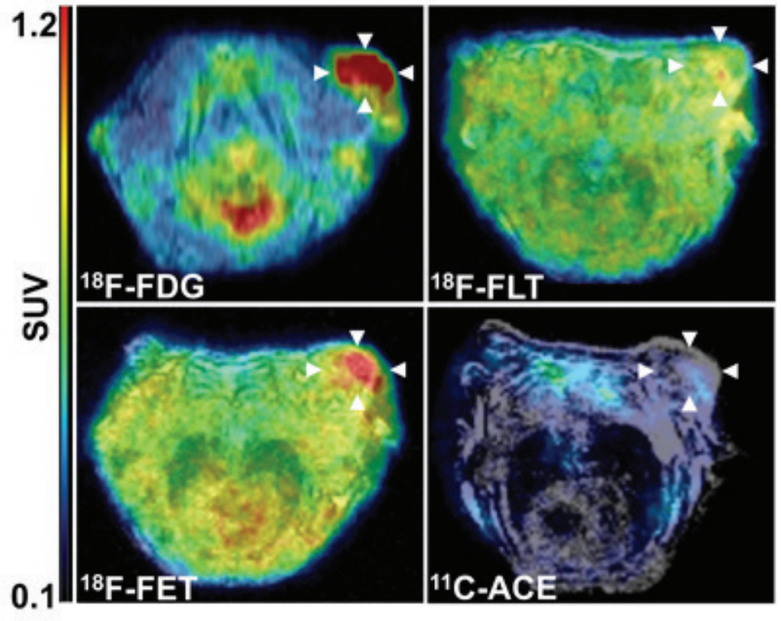


Figure 2
Sequential PET/MRI scans of metabolic radiotracer uptake in the same implanted colorectal cancer organoid.
FDG, [^{18}F]fluorodeoxyglucose; FET, [^{18}F]fluoroethyltyrosine; FLT, 3'-deoxy-3'[^{18}F]-fluorothymidine; ACE, [^{11}C]acetate.

RESEARCH FACILITIES PUBLICATIONS & OPERATIONS

RESEARCH FACILITIES

Head of Research Facilities
Sue Fowler

Research Facilities supports research groups at the Institute and the University of Glasgow groups based at the Beatson site. This year there has been investment in replacing ageing equipment. The Histology Facility replaced essential equipment including an autostainer, tissue processor and microtome. The Molecular Technology Service replaced their DNA preparation robot with a universal platform, and Central Services replaced a washer/dryer. In addition, Laboratory Management have replaced several essential pieces of core equipment, including cryofreezers and CO2 incubators. Building Facilities have been active with a number of projects to create more seating and meeting space and upgrade plant and laboratory areas.

Building Facilities

Alistair Wilson, Alex Kernahan, John Trivett

Building Facilities manage the outsourced services provision for catering, cleaning and janitorial services. We provide maintenance, support for the Institute's buildings, and manage alterations and refurbishments. We ensure that all statutory compliance issues with respect to buildings and systems are up to date. Use of the online helpdesk facility continues to be an effective means of logging reactive calls for maintenance and repair.

This year, there have been projects to replace existing plant and air handling units. Meeting room space was expanded by the installation of a meeting pod, and acoustic screens have created small private meeting spaces. A number of quiet desks have been added to meet the demand for additional write-up space. There have also been a number of laboratory alterations to facilitate installation of new equipment.

Central Services

Margaret Laing (Supervisor), Elizabeth Cheetham, Dilhani Kahawela, Kirstie McPherson, Jonny Sawers, Lauren Ure, Linda Scott, Tracy Shields, James Dyball, Beata Mosionek, Colin Graham

Central Services perform a wide range of duties that are essential for the support of the research groups across the site. These include cleaning and sterilisation of reusable laboratory glassware, sterilisation of consumables, and preparation of tissue culture solutions, bacterial culture media and *Drosophila* food. The team is also responsible for the cleaning and checking of equipment such as centrifuge rotors, X-ray processors, water baths and pH meters. The stocking of the tissue culture suites, and laboratory waste collections and autoclave processing to make waste safe are performed daily.

Histology Service

Colin Nixon, Barbara Cadden, Denise McPhee, Fiona McGregor, Gemma Thomson, Mark Hughes, Saira Ghafoor, Shauna Currie Kerr, Vivienne Morrison, Wendy Lambie

The Histology Service performs processing of tissue samples and cellular material from the wide range of cancer models developed within the Institute. This allows the 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 to allow sectioning and further investigation. All paraffin wax blocks sectioned are stained with haematoxylin and eosin in order to allow general analysis of cell morphology and structure. After initial analysis, more specialised histology stains can be performed if required to investigate specific tissue structures.

Where fixation is not required or disadvantageous to tissue structure and analysis, the facility offers a frozen section resource. Frozen tissue, embryos or cells can be sectioned and when required stained for examination using routine histological stains or immuno-histochemical or immunofluorescence staining methods.

Material for DNA/RNA investigation, PCR analysis and immunofluorescence staining can also be sectioned from both paraffin-embedded material and frozen tissue. 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 four 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 the 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 section, a cytospin preparation or frozen tissue section. 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.

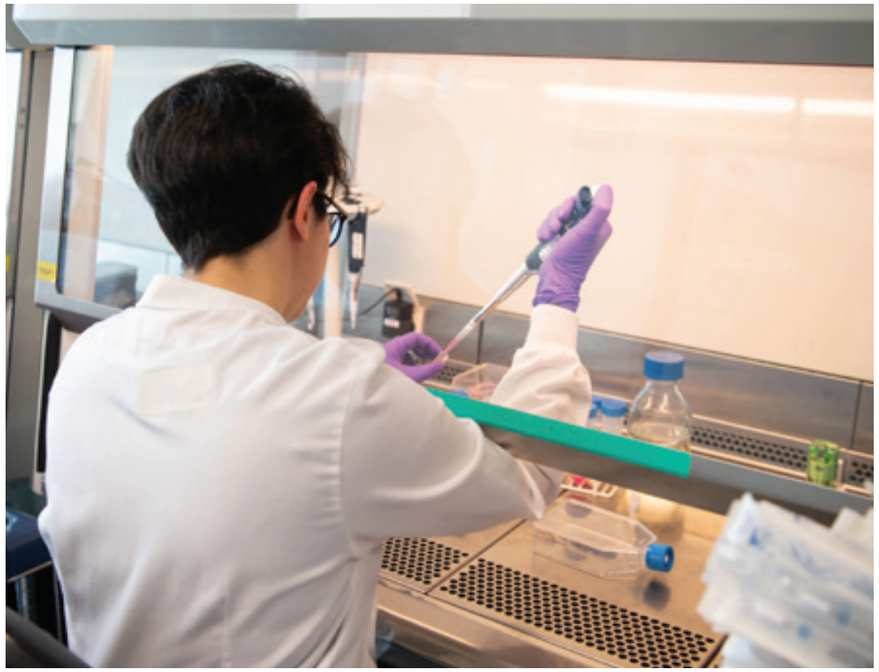
A recent advancement with the *in situ* hybridisation technique now means that when a probe is available or one can be specifically designed to the meet the researcher's needs, we can label and visualise much smaller targets, around 50 base pairs in size. This technique is now automated, allowing for 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 Indica HALO™ image analysis software, which 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 allows for 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.



Information Services

Peter McHardy, Iain White

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. All have central authentication, central file store and network printing. The servers have in excess of 1 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 Beatson 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 theatre.

Laboratory Management

Laura Bence, Richard Selkirk, Michael McTaggart, George Monteith, Michael Kilday, Karen Thomas

Laboratory Management are responsible for providing a number of vital support roles to the Institute. This includes 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 and genetic modification processes. Safety in regard to fire risk is also managed. As safety plays an important part of everyday life in the laboratory, and in running building services, it is essential that health and safety processes are reviewed and monitored regularly, that any training needs are rectified and that 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.

A major function of Laboratory Management is the overseeing of shared equipment servicing and 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 and use the latest state-of-the art technology. Several new pieces of equipment were then purchased. 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.

An essential role of Laboratory Management is the servicing and maintenance of core

equipment, and any systems that these require, such as carbon dioxide or nitrogen gas, is carefully managed and coordinated to ensure equipment breakdowns are kept to a minimum. Any equipment repairs are coordinated to ensure these are 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 pursued 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.

The Stores facility receives and processes all orders issued from the Institute and this year has processed an average of 1500 orders a month. A stock is held of a wide range of frequently used consumables with rapid re-stocking to ensure high-use materials are always available. Preferential pricing is obtained by maintaining good relationships with suppliers. As a result of good negotiations and better turnaround times from suppliers, we have been able to reduce the overall value of stock held without compromising supply lines to the laboratories. This year the Stores team have worked hard to instigate various supply agreements to ensure that costs are kept as low as possible and to ensure that Stores stock is readily available to researchers. This has resulted in considerable savings for the Institute, e.g. since September 2018 we have saved £5376 by purchasing one of the key Stores consumable items in bulk. 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. We continue to review the services provided by Stores to 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.

Molecular Technology and Reagent Services

Billy Clark, Jillian Murray, Andrew Keith

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

Small-scale DNA purification is performed on a Qiagen® Universal BioRobot®. Researchers provide overnight bacterial cultures that are processed by the facility. Sample numbers are consistently in the region of 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 (Clean Air Containment Services Ltd) and fumigation (Sanondaf – Containment Level II) 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, enzyme immunoassay against the four most common species of mycoplasma, or a colorimetric microplate assay to detect 16S ribosomal mycoplasma RNA.

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

Imran Ahmad (page 10)
Models of Advanced Prostate Cancer

Primary Research Papers

Kerr L, Salmond J, Ahmad I.
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Other publications

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Galbraith L, Leung HY, Ahmad I.
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Tom Bird (page 34)
Liver Disease and Regeneration

Primary Research Papers

Bird TG, Muller M, Boulter L, Vincent DF, Ridgway RA, Lopez-Guadamillas E, Lu WY, Jamieson T, Govaere O, Campbell AD, Ferreira-Gonzalez S, Cole AM, Hay T, Simpson KJ, Clark W, Hedley A, Clarke M, Gentaz P, Nixon C, Bryce S, Kiourtis C, Sprangers J, Nibbs RJB, Van Rooijen N, Bartholin L, McGreal SR, Apte U, Barry ST, Iredale JP, Clarke AR, Serrano M, Roskams TA, Sansom OJ, Forbes SJ.
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Karen Blyth (page 68)
Transgenic Models of Cancer

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McCarroll CS, He W, Foote K, Bradley A, McGlynn K, Vidler F, Nixon C, Nather K, Fattah C, Riddell A, Bowman P, Elliott EB, Bell M, Hawksby C, MacKenzie SM, Morrison LJ, Terry A, Blyth K, Smith GL, McBride MW, Kubin T, Braun T, Nicklin SA, Cameron ER, Loughrey CM.
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Shehab Ismail (page 44)
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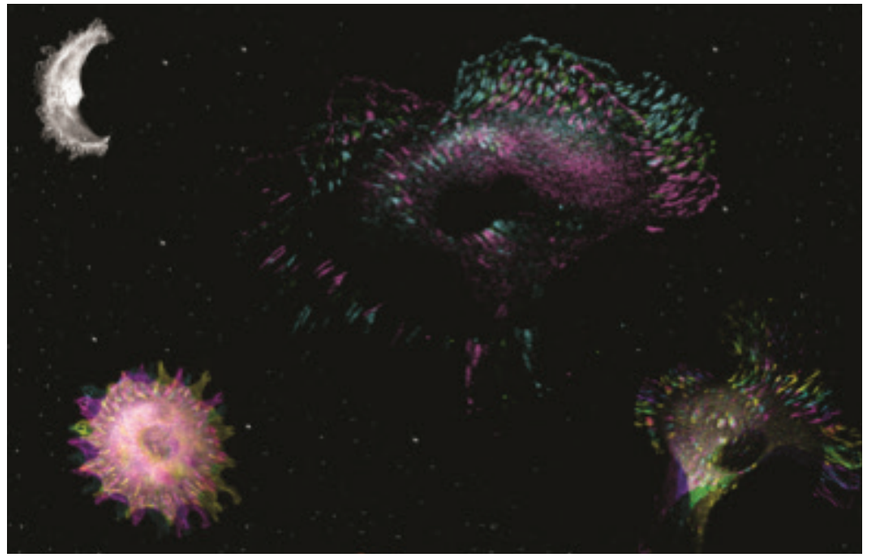

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David Lewis (pages 20 and 72)
Molecular Imaging & Translational Molecular Imaging

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Laura Machesky (page 46)
Migration, Invasion and Metastasis

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Gillian Mackay (page 66)
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Preclinical Precision Pancreas

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Other Beatson Institute publications

Primary Research Papers
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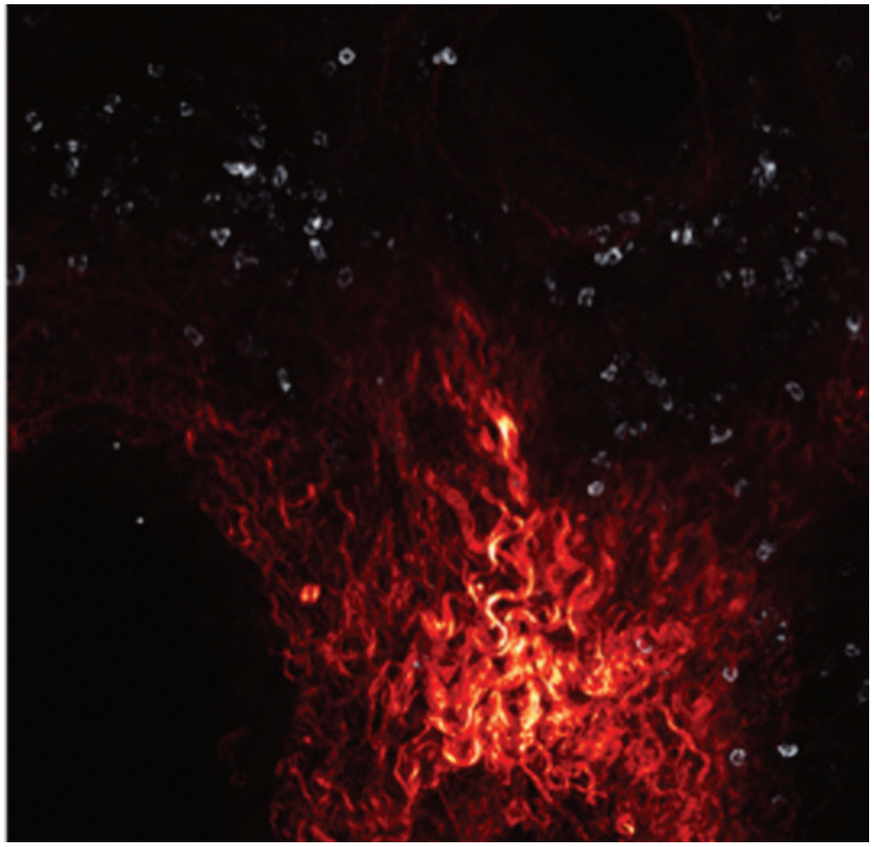

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John Paul Career Award

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

The winner of this year's award was Yasmin El Maghloob from Shehab Ismail's group. She has been investigating protein trafficking into the immune synapse.

Theses

Amato, Clelia (2018) Novel role and regulation of the WASP protein. [PhD thesis, University of Glasgow, Beatson Institute]

Bauer, Christin (2018) Bursicon: a novel regulator of intestinal homeostasis and systemic metabolism in adult Drosophila. [PhD thesis, University of Glasgow, Beatson Institute]

Davidson, Matthew (2018) Analysis of potential driver genes in oral squamous cell carcinoma. [PhD thesis, University of Glasgow, Beatson Institute]

Gay, David (2018) Investigating the cooperation of APC and KRAS mutations in colorectal cancer. [PhD thesis, University of Glasgow, Beatson Institute]

Some of our PhD students at their graduation

Halim, Silvia (2018) Interplay of cell proliferation and tissue remodelling in colorectal cancer. [PhD thesis, University of Glasgow, Beatson Institute]

Hodgson, Joseph (2018) The characterisation of a novel larval Drosophila melanogaster model of cancer cachexia. [PhD thesis, University of Glasgow, Beatson Institute]

Kowalczyk, Dominika (2018) Structural and biochemical characterisation of p14ARF – E3 ubiquitin ligase complexes. [PhD thesis, University of Glasgow, Beatson Institute]

Laprano, Nicola (2018) Metabolic alterations in a murine model of Barth syndrome. [PhD thesis, University of Glasgow, Beatson Institute]

Newcombe, Ashley (2018) Dual inhibition of MDM2 and BET is synthetically lethal in AML: pre-clinical testing and understanding the mechanism of drug synergy

Patel, Amrita (2018) Structural and biochemical characterisation of RING E3 mediated ubiquitination. [PhD thesis, University of Glasgow, Beatson Institute]

Port, Jennifer (2018) Investigating the therapeutic potential of NUAK1 for the treatment of colorectal cancer. [PhD thesis, University of Glasgow, Beatson Institute]

Riggio, Alessandra (2018) The role of Runx1 in genetic models of breast cancer. [PhD thesis, University of Glasgow, Beatson Institute]

Rudzka, Dominika (2018) Selection for invasive tumour cells reveals a role for MAPK signalling in cell elasticity regulation. [PhD thesis, University of Glasgow, Beatson Institute]

Salji, Mark (2018) Quantitative proteomics and metabolomics of castration resistant prostate cancer. [PhD thesis, University of Glasgow, Beatson Institute]

van der Reest, Jiska (2018) Proteome-wide analysis of cysteine oxidation reveals regulation of cellular metabolism by reactive oxygen species. [PhD thesis, University of Glasgow, Beatson Institute]

CONFERENCES AND WORKSHOPS

Cancer Research UK Beatson International Cancer Conference

Talk to the Niche - Understanding the Metastatic Niche

1-4 July 2018
Bute Hall, University of Glasgow
Scientifc Committee: Owen Sansom, Jim Norman, Kevin Ryan, David Bryant, Seth Coffelt, Robert Insall, Laura Machesky, Mike Olson, Sara Zanivan

One of the main challenges faced by clinicians engaged in treating solid tumours is that, at the time of diagnosis, malignant cells have already left the primary tumour and are either present in the circulation or have taken up residence in other organs as metastases. Our 2018 meeting focused on the nature of the metastatic niche – the components of the extracellular matrix that contribute to niche priming, how the immune system can foster metastasis and how these elements may be targeted therapeutically.

We were delighted to welcome Valerie Weaver (UCSF) to the opening session to give the 11th Colin Thomson Memorial Keynote Lecture, sponsored by Worldwide Cancer Research (WCR), in which she addressed how the dynamic interplay between extrinsic and intrinsic force regulates cancer progression. Valerie was awarded the WCR Colin Thomson Memorial Medal in recognition of her work. We also heard from David Lyden (Cornell, New York) about how tumour-derived exomes and exomeres promote metastasis and systematic disease, and from Ross Cagan (Mount Sinai, New York) on how personalised fly 'avatars' can be used to develop drug cocktails tailored to each patient.

There were a number of excellent presentations throughout the meeting, including selected short talks by Will Wood, Claus Jorgensen, Jacob Insua-Rodriguez, Clare Isacke, Massimiliano Mazzone, Joel Riley, Julio Aguirre-Ghiso, Janine Erler (sponsored by Breast Cancer Now), Sarah-Maria Fendt, Clothilde Théry, Angela Riedel, Alissa Weaver, Greg Hannon, Martin Miller, Thomas Tüting, David Adams, Christopher Halbrook, Laura Machesky, Jessica Perochon, Sara Zanivan, Nina-Marie Pedersen, Xavier Trepat, David Sherwood, Matt Jones, Paul Timpson, Michael Schmid, Mike Olson and Danijela Vignjevic. There was lots of great discussion and intellectual engagement during the meeting. Our particular thanks go to everyone who shared their unpublished work with the attendees.

The best poster prize was sponsored by AMSBIO and was awarded to Clelia Amato (Beatson Institute) for her work describing how WASP contributes to maintenance of front–rear polarity by spatially confining active Rac. The best short talk prize was sponsored by Portland Press and was awarded to Christopher Halbrook (University of Michigan) for his presentation describing how macrophage–epithelial metabolic crosstalk impairs chemotherapy in pancreatic cancer.
The meeting was generously co-sponsored by Cancer Research UK and Worldwide Cancer Research.

Protein Dynamics in Cancer 2019

For over 60 years, preserving genomic integrity has been considered the cornerstone of cancer prevention, with many mutated genes being identified as drivers of tumour progression. However, as many mutated genes ultimately encode for dysfunctional proteins, it has become clear that the preservation of protein integrity and the control of protein production are also essential in preventing malignant disease. Our 2019 meeting (Protein Dynamics in Cancer, 30 June–3 July) aims to detail and integrate the different ways in which protein dynamics both protect against cancer and contribute to tumour maintenance. As these pathways can also have perturbations and greater dependencies in cancer, it will be a key theme of the conference to better understand how these pathways can be targeted for cancer therapy.

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

Scottish Molecular Imaging Meeting

19 September 2018
The Scottish Molecular Imaging group aims to support a network of molecular imaging scientists within Scotland. Molecular imaging encompasses a wide range of modalities/techniques that provide detailed pictures of what is happening inside a whole organism at the molecular and cellular level. This meeting, hosted at the Beatson Institute, brought together researchers in Scotland who work in the field of molecular imaging to foster new collaborations and develop exciting new research ideas. Delegates heard from expert speakers from across Scotland who presented on a broad range of molecular imaging modalities. The meeting was kindly sponsored by Bartec, EM Imaging, MI Labs, MR Solutions, Lab Logic and Southern Scientific.

Glasgow–Edinburgh Cancer Immunology Meeting

12 October 2018
Researchers from Glasgow and Edinburgh met at Playfair Library Hall in Edinburgh for a full day of talks and discussion on cancer immunology. The purpose of this meeting was to bring together scientists with similar interests and to stimulate collaboration between our two cities. The meeting was attended by about 100 people. Professor Jeff Pollard gave the first talk of the day, highlighting the role of macrophages in metastasis. Several researchers from Glasgow presented their data, including Jen Morton, Yasmin El Maghloob (Shehab Ismail's lab), John Mackay (Leo Carlin's lab), Toshiyasu Suzuki (Seth Coffelt's lab) and Alan Hayes (Gerry Graham's lab). The day ended with a lively discussion on cancer immunology in Scotland and how to capitalise on our biological and technical strengths. More meetings are planned for 2019.

Open Evenings

Two very well-subscribed open evenings were held at the Institute this year, one in March (during British Science Week) for school students, which was attended by roughly 150 people, and the other in September for members of the public and Cancer Research UK supporters. At both events, our enthusiastic volunteers provided a series of very engaging talks, lab tours and demos for the visitors.

SEMINARS

The following seminars were held at the Cancer Research UK Beatson Institute during 2018.

January

Lorena Arranz, University of Tromso, Norway
Ashok Venkitaraman, MRC Cancer Unit, University of Cambridge

Charles ffrench-Constant, MRC Centre for Regenerative Medicine, University of Edinburgh

February

Volker Haucke, Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP) Berlin, Germany

Joan Brugge, Ludwig Center, Harvard Medical School, USA

Sabine Werner, Institute of Molecular Health Sciences, Zürich, Switzerland

Giovanna Mallucci, UK Dementia Research Institute, University of Cambridge

Andreu Casali, IRB Barcelona, Spain

March

Michiel Vermeulen, Radboud Institute for Molecular Life Sciences, Netherlands

Elke Ober, NNF Center for Stem Cell Biology, University of Copenhagen, Denmark

David Bhella, Centre for Virus Research, University of Glasgow

Volkan Sayin, NYU Langone Medical Center, New York, USA

Jean-Claude Martinou, University of Geneva, Switzerland

Barbara Tanos, The Institute of Cancer Research, London

April

Barry Taylor, Memorial Sloan Kettering Cancer Center, New York, USA

Igor Vivanco, The Institute of Cancer Research, London

Martin Miller, Cancer Research UK Cambridge Institute

Tony Ng, King's College London

May

Tatiana Petrova, CHUV and University of Lausanne Epalinges, Switzerland

Nadia Guerra, Department of Life Sciences, Imperial College London

Alexander Barrow, Department of Pathology and Immunology, Washington University, USA

Mike Murphy, MRC Mitochondrial Biology Unit, University of Cambridge Wellcome Trust/MRC Building

Frank Edlich, University of Freiburg, Germany

June

Ahmet Ucar, Manchester Breast Centre, The University of Manchester

Andrea McClatchey, MGH Center for Cancer Research, Harvard Medical School, USA

Ilaria Malanchi, The Francis Crick Institute, London

Payam Gammage, MRC Mitcochondrial Biology Unit, University of Cambridge

July

Garry Nolan, Department of Microbiology and Immunology, Stanford University School of Medicine, USA

Edward Roberts, University of California, San Francisco, USA

Bart Cornelissen, University of Oxford

August

Simon Babayan, Institute of Biodiversity Animal Health and Comparative Medicine, University of Glasgow

Guillaume Jacquemet, Turku Centre for Biotechnology, University of Turku, Finland

Bernadette Carroll, Institute for Cell and Molecular Biosciences, Newcastle University

Donal Wall, School of Life Sciences, University of Glasgow

Chiara Gorrini, The Campbell Family Institute for Breast Cancer Research, Toronto, Canada

Erica Sloan, Cancer & Neural-Immune Research Laboratory, Monash University, Australia

Crispin Miller, Cancer Research UK Manchester Institute, The University of Manchester

September

Michelangelo Campanella, Royal Veterinary College, London

Tony Whetton, Stoller Biomarker Discovery Centre and the Manchester Precision Medicine Institute, The University of Manchester

David McEwan, University of Dundee

Leila Akkari, Netherlands Cancer Institute

October

David Andrews, Sunnybrook Research Institute, Toronto, Canada

John Mattick, Genomics England

Sylvie Urbe, University of Liverpool

Zoi Diamantopoulou, Cancer Research UK Manchester Institute

Taka Nojima, Sir Wm Dunn School of Pathology, University of Oxford

Ian Cannell, New York Genome Center, USA

November

Rob Bristow, Manchester Cancer Research Centre

Sanjiv Sam Gambhir, Stanford University School of Medicine

Emilio Casanova, Medical University of Vienna, Austria

Susumu Hirabayashi, Imperial College London

Gillian Griffiths, Cambridge Institute for Medical Research

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 rigour, 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

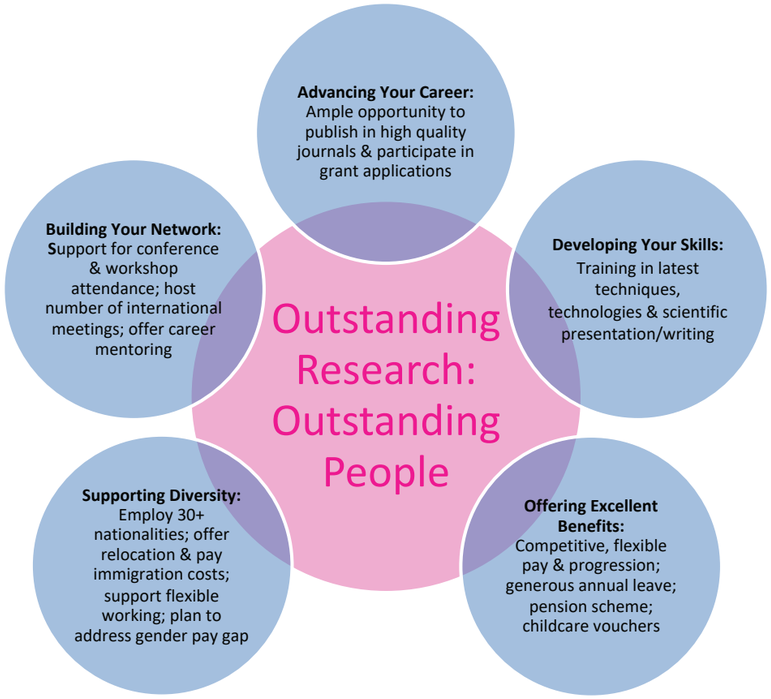
We see postdocs as pillars of the research and intellectual activities of their own groups and of the Institute as a whole. Our postdoctoral training, which is overseen by a senior member of the Institute (Professor Laura Machesky), is designed to promote the development of outstanding and dedicated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

Clelia Amato receiving her AMSBIO Best Poster Prize award from Owen Sansom for her work describing how WASP contributes to maintenance of front–rear polarity by spatially confining active Rac.

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, Jacqui Clare, Karen Connor, Lynn Wilson, Patricia Wylie

Human Resources
Angela Stuart FCIPD, Elaine Marshall ACIPD

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 2018, the team implemented a new HR system with the aim of further streamlining many HR processes, including recruitment and management reporting. HR also rolled out e-learning for all staff and students to provide everyone with an understanding of their collective and individual responsibilities for equality and diversity; data protection; health and safety, systems security and unconscious bias.

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), Barbara Laing, Sarah Price, Catriona Entwistle, Shona McCall/ Rebecca Gebbie (PAs to the Director)

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

The Research Management supports scientists at the Beatson Institute by editing publications, including the Scientific Report, manuscripts and grants; organising and taking minutes at a range of scientific and operational meetings; maintaining an up-to-date website and publications database; overseeing all aspects of the graduate student training programme; and providing reports and answering queries for both internal and external audiences, including Cancer Research UK, about the Institute's research and outputs. The team also assists researchers in identifying and applying for external grant funding, and has a role in providing training and advice on good practice in research. This has involved helping draft and implement publication and data management policies for the Institute.

Cancer Research Technology
Tracy Weightman PhD

Cancer Research Technology (CRT) is an oncology-focused technology transfer and development company wholly owned by Cancer Research UK with 130 employees, based primarily in London and Cambridge. Since a substantial amount of the funding for the Beatson Institute comes from Cancer Research UK, CRT manages all intellectual property–related matters on behalf of the Institute and the charity. To facilitate this, there is a CRT Business Manager based full-time at the Institute.

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
Wellcome Trust

Karen Blyth
Breast Cancer Now

Martin Bushell
AstraZeneca, Celgene

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

Drug Discovery Unit
Celgene, Daphne Jackson Trust, Pioneer Fund

Danny Huang
European Community, Nuevolution

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

Laura Machesky
Medical Research Council, Pancreatic Cancer Research Fund

Jim Norman
West of Scotland Women's Bowling Association

Kevin Ryan
Worldwide Cancer Research

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

Alexei Vazquez
Deutsche Forschungsgemeinschaft, European Community

Institute of Cancer Sciences, University of Glasgow

David Bryant
EssenBio, Royal Society

Seth Coffelt
European Community, Medical Research Council, Naito Foundation, Tenovus, Wellcome Trust, William Forrest Charitable Trust

Daniel Murphy
British Lung Foundation, European Community, Medical Research Council, Worldwide Cancer Research

Stephen Tait
BBSRC, Breast Cancer Now

Beatson scientists and their dogs assembling to support the Stand Up To Cancer campaign

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.

Apollo Scientific
E Atkins, donation collected from guests at Golden Wedding celebration
Margaret G Brown
R Byiers
Charities Aid Foundation
Charities Trust
CTT Charity Payments
Senga Dempster
Mr Terry Fitzpatrick
James Inglis Testamentary Trust
Eastwood High School
ELECTA CHAPTER No.27 O.E.S
Enterprise Rent a Car Foundation Fund
Friends of Glasgow University Library, in memory of Mrs Jean Smith
GE Healthcare
Late Sarah Greechan
Avril Haddow
IFF Research
Barbara Jordan, in memory of her husband, Mr Thomas Jordan
A M Kidd
Freda Learmond
Jean Waugh Lightbody
Mrs Margaret Lindsay
Janet Lyke, in lieu of Christmas presents
Anna MacMillan
Lord Michael Martin
Rookmeen McCabe, donation raised at Charity Night
Catherine McCafferty, donation raised at Martin Family reunion party
Bernadette McGuire
Grace McLaughlin, in memory of her Mother, Jeannie Houston
Fiona McNeill, donation from family in lieu of sending Christmas cards
Mosshead Primary School
M Muir
Order of the Eastern Star, Priory Chapter No 21
Sarah Percy
Radnor Park Church of Scotland
Tom Slimming
Ann Soutar, donation from Walter Soutar Memorial Fund and sponsorship for climbing Ben Nevis
St Rollox Bowling Club
Ken Stirling, in memory of his wife, Mrs Margot Stirling
John Teevan, in memory of his Mother
Jacqueline Thomson, in memory of her Dad
Thornhill Gardening Society
J Walker
West of Scotland Women's Bowling Association
M A White
Jeanie Whiteford
Elizabeth M Whitelaw

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

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

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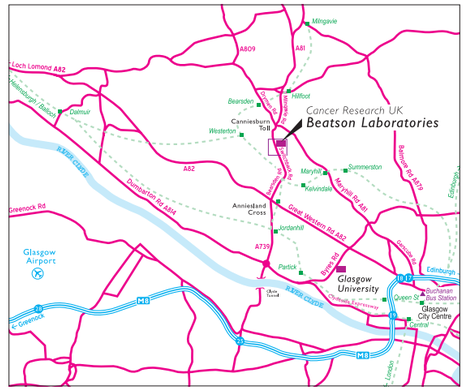
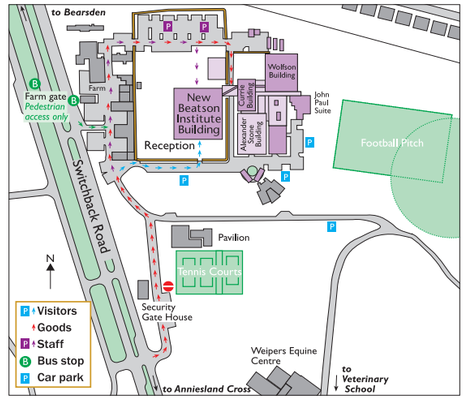
www.cruk.org





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