Mitochondria, the powerhouses of the cell, are required for cancer cell death. This image shows mitochondrial shape changes during cancer cell death, where each colour represents mitochondrial shape at a specific time point during cell death.

Image supplied by Joel Riley, a post-doc in Stephen Tait’s group.
DIRECTOR’S INTRODUCTION

In August of this year, Professor Owen Sansom was appointed as the next Director of the Cancer Research UK Beatson Institute. As its newest Director, Owen here sets out his vision for the Institute’s strategy over the next five to ten years, along with some of the research highlights there were during 2017.

Vision and strategy
Over the past ten years, the Beatson Institute has built an excellent reputation for basic cancer research, including world-class metabolism studies and renowned in vivo modelling of tumour growth and metastasis. This is something that current and former members of the Institute can be very proud of. My goal for the future is for this discovery work to continue apace but for it to also be applied to preclinical and clinical studies. This will include working with the well-established clinical pipeline in Glasgow, exemplified by initiatives such as PRECISION-Panc, which began this year. To do this, we will work closely with colleagues in the wider Glasgow Cancer Centre, particularly clinical academics and clinicians focused on the treatment of cancer patients.

As a core-funded Institute, I believe we also need to continue to do high-risk/high-gain projects, and an important component of this will be the advanced technologies established both within the Institute and the University of Glasgow, which underpin much of what we do. Our research themes will remain broadly the same (cancer growth and metabolism, and cancer metastasis and recurrence) but with a re-emphasis on:

- Cancer vulnerabilities caused by their aberrant metabolism;
- Interplay between the tumour microenvironment, metastasis and recurrence;
- Biology of early disease, aimed at developing a ‘precision prevention’ approach.

Our aim will be to identify non-clinical and clinical leads for each of our themes, and for there to be a programme of recruitment, including for a number of senior clinicians in the areas of particular clinical interest in Glasgow, such as colorectal cancer and hepatocellular carcinoma.

Highlights
This has been our most successful year in winning external grant funding and finding ways to expand our research base, including functional imaging, precision medicine and informatics.

Two large initiatives, which researchers at the Institute are participating in, began this year. The Grand Challenge team (led by Josephine Bunch, National Physics Laboratory) is focusing on using state-of-the-art mass spectrometry imaging to map metabolism in vivo, something that aligns very closely with previous work at the Institute. Meanwhile, PRECISION-Panc (led by Andrew Bankin, Owen Sansom and Jeff Evans) aims to utilise functional stratified medicine approaches to deliver new treatment options for pancreatic cancer. Much of the preclinical work will be done within the Institute, in particular by Dr Jennifer Marton, who now leads the Preclinical Precision Pancreas Laboratory.

In addition, Pancreatic Cancer UK awarded us a Future Leaders Academy, enabling us to appoint four PhD students, along with a fifth funded by Pancreatic Cancer Scotland, to our graduate studentship programme, which this October saw its largest intake to date (23 students in total). We continue to monitor the progress of our research groups. Professors Robert Insall and Laura Machesky’s quinquennial reviews, held in May, were very positive, while Dr Sara Zanivan was promoted to Senior Staff Scientist in March and now heads up both her research group and the Proteomics team. We also held a number of advisory mid-term reviews, for junior group leaders Dr Alexei Vazquez and Dr David Bryant and for clinician scientist Dr Tom Bird. We are very grateful to all of the external reviewers who participated in these important meetings.

I was pleased by the progress of some of our intermediate clinicians this year - Colin Steele (a former clinical research fellow in my group) was awarded an NHS Education Scotland/ICSO Postdoctoral Clinical Lectureship, while Nigel Jamieson won a CRUK Intermediate Clinical Fellowship. I was also delighted to be elected a Fellow of the Academy of Medical Sciences. In addition, two of our researchers, Drs Saadia Karim and David Vincent, were recognised with a CRUK Flame of Hope Special Commendation for all of their research engagement activities over the years, while Dr Emma Shanks was awarded CRUK’s Communications and Brand Ambassador Prize as an inspiring communicator of its research.

Arrivals and departures
In January, Dr David Lewis joined us from the CRUK Cambridge Institute to establish preclinical functional imaging at the Institute. In support of this, the Beatson Cancer Charity has provided generous funding (via the Glasgow Cancer Centre) that will allow the establishment of a facility at Garnethill for probe synthesis. This includes the appointment of a senior chemist and a physicist to support the group.

Martin Drysdale resigned as Director of the Drug Discovery Unit (DDU) in July to take up a prestigious role at the Broad Institute in the US, and in the interim period our respective heads of chemistry and biology, Justin Bower and Heather McInnerny, have been acting as DDU leads. They have recently been appointed as joint heads of the DDU, and we will work with them very closely in the coming years to develop exciting new targets for cancer treatment. Mike Olsson will also be leaving the Institute in April 2018 to lead a department in Ryerson University (Toronto), bringing cancer and physical sciences together after winning a highly competitive Canada Research Chair.

In memory
The year ended on an incredibly sad note with the loss of two of our most outstanding and unique contributors to cancer research in Glasgow - Professor Tessa Holyoake and Dr Gabriela Kaina. The very warm tributes paid to them in this report (pages 11 and 12) demonstrate how well regarded they were and how much they will be missed as both friends and colleagues.
This year, there were a number of highlights within the Institute of Cancer Sciences (ICS) at the University of Glasgow, with its primary goal of delivering cutting-edge translational cancer research for patient benefit.

We saw the start of PRECISION-Panc, a dynamic and coordinated research platform to define, understand, test and implement stratified therapeutic strategies for pancreatic cancer (PDAC). The initiative is centered on discovery and preclinical and clinical development, and is supported by a 5-year award of £10m from Cancer Research UK along with substantial additional funding from industry partners.

For discovery (Lead: Andrew Blair), the consortium holds the world’s most accurate and comprehensive molecular catalogue of pancreatic cancer data, generated as part of the International Cancer Genome Consortium from a prospective observational cohort of patients with pancreatic cancer with extensive clinico-pathological, treatment and outcome information. These in-depth analyses have uncovered the detailed molecular pathology of PDAC and its potential underlying vulnerabilities. The initial focus for clinical studies will be on detecting defects in the DNA damage response pathway, apparent in approximately 20% of PDAC, and extend into overlapping vulnerabilities through targeting of the immune system.

For preclinical studies (Leads: Owen Sansom, Jen Morton), therapeutic targets identified by the consortium have developed a number of model systems including patient-derived xenografts (PDXs) and 25+ genetically engineered mouse models (GEMMs) with a physiologically relevant tumour microenvironment and inflammatory response. These models are excellent for addressing the precise functions of individual genes and non-mutations in vivo, and for the preclinical evaluation of putative cancer therapeutics.

PRIMUS (Pancreatic Cancer: Personalised Multi-Arm Umbrella Study) is the PRECISION-Panc clinical development platform (Leads: Jeff Evans and Juan Valle, Manchester). The ultimate aim is to develop a multi-drug, multi-sub-study, biomarker-driven family of clinical trials that use state-of-the-art genomic profiling to match patients to sub-studies testing targeted investigational therapeutics, coordinated by the CRUK Glasgow Clinical Trials Unit (CTU). The first trials are not dependent on rapid turnaround time for molecular testing, but include mandatory tissue sampling for patient recruitment, and the initial focus will be on streamlining the processes for rapid analyses during the course of these initial trials (PRECISION-Panc Master Protocol - Lead: David Chang). These trials include a safety study of a CXCR2 inhibitor in combination with an anti-POD-1 antibody, sponsored by AstraZeneca, which has completed recruitment and a randomised phase II study in untreated patients with metastatic PDAC comparing FOLFOX plus nab-paclitaxel with Gemcitabine plus nab-paclitaxel combinations, which has opened to recruitment.

In 2017, the Translational Pharmacology Laboratory (led by Fiona Thomson) was also selected by the CRUK Centre for Drug Development (CDD) as one of its four Biomarker Centres of Excellence to develop and perform biomarker studies within the CDD’s clinical trials portfolio, while the CRUK Clinical Trials Unit (Director: Bob Jones) underwent a successful quinquennial review. The CTU develops, coordinates and delivers national and international multi-centre studies. A particular highlight this year was the publication of the final disease-free survival of the SCOT study (an international phase III randomised, non-inferiority trial comparing 3 versus 6 months of FOLFOX plus nab-paclitaxel combinations, which has completed recruitment and a pooled analysis of six randomised, phase III trials that were conducted concurrently. This pooled analysis (New Engl J Med 2018; 378:1177-88) will define the new standard of care internationally for the duration of adjuvant chemotherapy in resected stage III colon cancer. The CTU also supports the TASTER Clinical Trial and Experimental Medicine Programme in Chronic Myeloid Leukaemia (Mara Ciapetta and David Vetrie). Management of CML requires life-long tyrosine kinase inhibitors (TKIs) that can cause significant side effects. While TKIs induce remission, they are rarely curative due to persistence of TKI-resistant leukaemic stem cells (LSC) that evolve to drive TKI resistance and disease progression. Recently, research teams at the Paul O’Gorman Leukaemia Research Centre have identified survival factors that can be exploited therapeutically to eradicate TKI-resistant LSC and manage urgent clinical need in CML. This forms the basis of a new Experimental Medicine Programme funded by CRUK, combining a phase II clinical trial (TArgeting Stem cell Resistance, TASTER) with a preclinical-medicine–based scientific programme, investigating the safety and efficacy of HDM2, EZH2 or BET inhibitors in combination with TKI in patients with all phases of CML. Exploratory objectives include investigation of how LSC clonal architecture changes during drug treatment and whether these changes can be computationally modelled to predict drug response. The scientific programme will also determine whether TKI-resistant LSC clones exist at low levels in CML drug-naïve diagnostic samples and, using in silico models as predictors at the point of diagnosis, will determine the efficacy of standard-of-care or the novel therapies.

A number of our early-career researchers were successful this year. Ross Caruthers was awarded a CRUK Clinician Scientist Fellowship that has allowed him to establish a new research group investigating elevated DNA replication stress in glioblastoma and neural stem cells and exploiting its therapeutic potential, while Peter Bailey, Senior Lecturer in Cancer Systems Biology, and Patricia Roxburgh, Senior Clinical Lecturer and Honorary Consultant in Medical Oncology with an interest in early-phase clinical trials and ovarian cancer, joined the ICS as principal investigators. Lisa Hopcroft was awarded Fellowships by Leuka and the Kay Kendall Leukaemia Fund, Gillian Horne was awarded the Thomas Smellie prize for the best PhD thesis by a clinician, and Evangelos Giampaolos was awarded the prize for the best non-clinical PhD thesis. During 2017, Iain McInnes also left to undertake a prestigious position at Imperial College, London.

Publications listed on page 96
This section features some of the key research findings made by scientists at the Beatson Institute and Institute of Cancer Sciences in the past year.


In this paper, Emmanuel Dornier and colleagues establish a role for glutamine metabolism in cancer cell invasion. In a series of elegant cell culture and in vivo experiments, they show that glutaminolysis drives membrane trafficking to promote cancer invasion. Nat Commun 2017; 8: 2255

This paper describes work done by PhD student Evangelos Giam pepeliasios and others to determine whether caspase-independent cell death (CICD) might be a better way to kill cancer cells than mitochondrial apoptosis, where caspase activity can lead to unwanted effects such as DNA damage. In fact, cells undergoing CICD display a potent pro-inflammatory phenotype dependent on NF-kappaB activity. Interestingly, engagement of CICD stimulates caspase-independent cell death as an anti-cancer therapy warrants further investigation.

This paper describes work done by PhD student Christine Gundy and colleagues to understand more clearly how Rab-coupling protein (RCP) influences metastasis in vivo, something that this group and others have already shown in vitro. The authors identify a receptor tyrosine kinase, EphA2, as a cargo of an RCP-regulated endocytic pathway, which leads to cell:cell repulsion, driving tumour cells apart. Importantly, using mouse models of pancreatic cancer, they also show that RCP and EphA2 are both required for metastasis in vivo.

Secreted CLIC3 drives cancer progression through its glutathione-dependent oxidoreductase activity. Nat Commun 2017; 8: 14206

This paper describes work done by PhD student Christine Gundy and colleagues to understand more clearly how Rab-coupling protein (RCP) influences metastasis in vivo, something that this group and others have already shown in vitro. The authors identify a receptor tyrosine kinase, EphA2, as a cargo of an RCP-regulated endocytic pathway, which leads to cell:cell repulsion, driving tumour cells apart. Importantly, using mouse models of pancreatic cancer, they also show that RCP and EphA2 are both required for metastasis in vivo.

In this work, the authors describe how the chloride intracellular protein 3 (CLIC3) drives angiogenesis and cancer progression through its glutathione-dependent oxidoreductase activity. Having found that CLIC3 is secreted by cancer-associated fibroblasts, they use both in vivo and 3D culture systems to demonstrate that it promotes the invasive behaviour of both endothelial and cancer cells via its modulation of transglutaminase-2. Clinically, the authors also show that CLIC3 is abundant in ovarian cancers, with higher levels correlating with poorer outcomes in patients.
Current treatments for chronic myeloid leukemia (CML) successfully target differentiated cells but not leukemic stem cells (LSCs), which can lead to disease relapse. Thus, this paper investigates potential metabolic vulnerabilities in LSCs that might be exploited therapeutically. Using stable isotope-assisted metabolomics and functional assays, PhD student Elodie Kuntz and co-authors compare the metabolic signatures of stem cells and differentiated cells from CML patients with their normal counterparts and show that LSCs cells rely on upregulated oxidative metabolism for tumour growth in clinically relevant mouse xenotransplantation models of human CML, by comparing the standard CML treatment (imatinib) with tigecycline, an antibiotic that inhibits mitochondrial protein translation. The concept of LSC eradication by inhibiting mitochondrial metabolism now needs further investigation in the clinic.


Work from these authors and others has established that cancer cells utilise serine and glycine to support their growth. In this important follow up study, they show that dietary restriction of these amino acids can reduce tumour growth in clinically relevant mouse models of both intestinal cancer and lymphoma. In addition, increased survival is further improved by antagonising the anti-oxidant response. However, KRAS-driven models are less responsive to serine and glycine depletion, reflecting an ability of activated KRAS to increase the expression of enzymes that are part of the serine synthesis pathway, thus promoting de novo serine synthesis.

Nomura K, Kleijnen M, Kowalczyk D, Hock AK, Sibbet GJ, Vousden KH, Huang DT.


Binding between the tumour suppressor p53 and MDM2 inhibits p53’s transcriptional activity and targets it for degradation. Inhibitors that disrupt this binding are able to activate a p53 response in tumours, however, this can also lead to toxicity in normal tissues due to p53’s basal control not being properly maintained. In this paper, Koji Nomura and colleagues take a novel structural approach to design MDM2 mutants that lack E3 activity – and are thus unable to target p53 for degradation – but retain an unaltered RING domain structure – and so are still able to limit p53’s transcriptional activity. The authors suggest that this approach could widen the therapeutic window of p53 activation in tumours.


Bromodomain Protein BRD4 is a Transcriptional Repressor of Autophagy and Lysosomal Function. Mol Cell 2017; 66: 517–32 e9

In this study, the authors investigate mechanisms of autophagy regulation. Using RNAi screening and transcriptome analysis, they identify the epigenetic reader BRD4 as a transcriptional repressor of autophagy and lysosome function. However, BRD4 only suppresses some types of autophagy and not others. Furthermore, in the case of starvation, chromatin-bound BRD4 is displaced via AMPK signalling, leading to autophagy gene activation and cell survival. Thus, while this work details one mechanism of autophagy regulation, it also highlights that there are likely many additional control points relevant to human diseases, including cancer.

Dr Gabriela Kalna (1966 – 2017)

It is with the heaviest of hearts that we report the sad passing in November of our dear friend and much-valued colleague Dr Gabriela Kalna.

Nine years ago, the Beatson Institute had a real problem with statistics and mathematics. We had teams of excellent scientists generating ever-more data, as the equipment we used got better and more productive, but we still handled data the same way and it was slowing down our progress. A variety of different areas, from mass spectrometry through microscopy to metabolomics, were becoming swamped. We also needed better advice about informatics. With some trepidation – cancer scientists are often frightened of mathematics – we advertised for help. We didn’t need to worry; our first round of interviews revealed Gabriela Kalna.

Gabriela earned her PhD in Numerical and Optimization Methods at The Comenius University in Bratislava and was at the time working as a lecturer in the department of Mathematics (now Mathematics & Statistics) at Strathclyde University. Interviews for her position were a walkover – she was so much better than the other candidates and she was hired without discussion. A short while later she arrived, and immediately improved every aspect of mathematics and statistics in the Institute. Her success was based around three different gifts. Firstly, she would put her mind to almost any problem, taking papers home and pepperin us with questions until she could find the right way to handle the issue; the more abstract and unusual the question the happier she was. Secondly, she was an extremely good general mathematician – her solutions usually solved their target problems, and many of them are still being used now. Thirdly, and perhaps most important of all, she had no problem communicating with even the most mathematically inept students and faculty; her door was always open for anyone who was passing by with a problem, she dealt patiently even with people who insisted on doing statistically foolish things, and everyone became a little bit less frightened of numbers. Under Gabriela’s auspices the computational biology and bioinformatics team grew and are now an integral part of the multidisciplinary science at the Institute.

It was a tragedy for all of us when she became unwell in 2016. In typical Gabriela style, she carried on working though her health deteriorated with haste - “What else should I do?”, she said, “I like maths and I hate daytime TV”. “Inspirational” is a strong word but is befitting of her character and desire to carry on as normal, even coming to work on the bus as she was no longer able to drive. Everyone was pleased, however, when she seized the opportunity to take some of the special trips she had not quite managed to do – a journey to Florence was a highlight, as was the holiday with her husband Karol to the Czech Republic and her home country of Slovakia. She had a love of castles and made a point of visiting several in Scotland and Wales - taking in their coffee shops as well as the history! We will not forget how she equanimously dealt with the awful disease we are all working to beat. And now, hopefully, we commemorate her in the most practical way, by all being a little bit better at what we do – statistically smarter, and a little bit closer to mathematically acceptable. We miss her clever, chatty and interesting presence and know that the Institute is a better place for her being here. Our thoughts are with her husband Karol and children Juriin and Vik; and her friends, of whom she had many.
In this year’s report, we would like to remember Tessa Holyoake, Professor of Experimental Haematology in the Institute of Cancer Sciences at the University of Glasgow and Director of the Paul O’Gorman Leukaemia Research Centre (POG), who died peacefully at her cottage on Loch Tummel on 30 August 2017, aged 54.

Professor Tessa Holyoake was a world-renowned expert in chronic myeloid leukaemia (CML) and one of the most exceptional scientists and clinicians of her generation, who collaborated with many of the research groups at the Beatson Institute.

Born in 1963 in Aberdeen, she was educated at Albyn School and later went on to study Medicine at the University of Glasgow, graduating in 1985. She completed a PhD in Glasgow in 1996 before embarking on a research fellowship in Vancouver, where she lived for two years with her husband Andy.

Tessa Holyoake made a number of seminal observations that have transformed our understanding of CML and its treatment. Most notably, she was the first to identify the existence of cancer stem cells in CML in 1999 during her research fellowship in Vancouver. Later, she demonstrated the resistance of these stem cells to CML-specific therapies such as imatinib. More recently, she made a further world-leading contribution to her field, by identifying key CML stem cell survival pathways that can be manipulated to develop potential new treatments. As a direct result of Professor Holyoake’s research, CML patients who have had poor responses to standard therapies will be offered alternative treatment in clinical trials in an attempt to achieve remission.

In 2009, she won the Scottish Health Awards Cancer Care Award, in 2011, the Lord Provost of Glasgow Health Award, and in 2015, the Scottish Alba Saltire Society Fletcher of Saltoun Award for her contribution to science, the Scottish Cancer Foundation Inaugural Prize and Evans/Forrest Medal. She was made a Fellow of the Academy of Medical Sciences in 2013. In March 2017, she was awarded the prestigious Rowley Prize by the International CML Foundation in recognition of her groundbreaking work. She was elected to the Royal Society of Edinburgh in 2007, and in July 2017, she was awarded a RSE Royal Medal by Her Majesty the Queen in recognition of her outstanding contribution to the field of Life Sciences. She is remembered for her world-leading contributions to CML, and as an inspiration by family, friends, colleagues and patients.
CANCER GROWTH AND METABOLISM
MODELS OF ADVANCED PROSTATE CANCER

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’s) 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 Ppary activation in metastatic prostate cancer

Using a murine forward mutagenesis screen (Sleeping Beauty) in a PtenNull background, we were able to identify the gene peroxisome proliferator-activated receptor gamma (Ppary), which encodes a lipid uptake and adipogenesis. In our model, upregulation of Ppary was associated with an activation of lipid signalling pathways, including upregulation of lipid synthesis enzymes (fatty acid synthase (FASN), acetyl-CoA carboxylase (ACAC), and ATP citrate lyase (ACLX)), resulting in aggressive prostate cancer.

As a proof of principle, we were able to demonstrate that inhibition of Ppary suppressed tumour growth in vivo, with downregulation of the lipid synthesis programme. We showed that elevated levels of Ppary strongly correlate with elevation of FASN in human prostate cancer and that high levels of Ppary/FASN and Pisk/PAR/T 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 Ppary/FASN and PTEN levels to identify patients with aggressive prostate cancer who may respond favourably to Ppary/FASN inhibition (low PTEN/High Ppary 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.

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 insertion transposon mutagenesis screen (PiggyBac) and then validating the top genes of interest in patient-derived organoid cultures (Gao et al. Celi 2014; 159: 176–87). 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.

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Figure 1

Data from dbio portal (www.cbioportal.org) demonstrating Ppary gene amplification on 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, ACLX).
We investigate the impact of chromatin structure and epigenetics on cell proliferation, ageing and cancer. We hypothesise that age-associated changes in chromatin structure, function and regulation contribute to the dramatic age-associated increase in the incidence of cancer. While age is the biggest single risk factor for most cancers, the reason for this is currently poorly understood. We are also exploiting epigenetics to develop novel drug combination therapies to combat cancer.

Figure 1
A model depicting the many factors proposed to contribute to the age-associated increase in cancer incidence. These include congenital mutations and mutations acquired in early growth and development, genetic mutations acquired during ageing and age-associated changes to dynamic molecular, cellular and tissue systems, e.g. the epigene, the metabolism, the proteome, stem cell niches, the immune system and others.

Figure 2
Prevalence of ‘chromoasis’ confers epigenetic stability, healthy ageing and suppression of age-associated disease. Prevalence of ‘chromoasis’ homoeostasis or ‘chromoasis’ mechanisms are predicted to actively maintain the dynamic epigenome at steady state over the life course, thereby suppressing age-associated disease. Diverse interventions – genetic, dietary, exercise and drug – that prolong longevity, healthy ageing and suppression of disease are predicted to enhance chromoasis. Such preventative strategies can be an alternative to the current paradigm of targeted therapies for advanced disease. Strategies to enhance chromoasis – and other cellular homoeostatic mechanisms – may prevent multiple diseases of ageing and be more successful than targeting advanced disease.
Our lab utilises state-of-the-art metabolomics capabilities to study metabolic transformations and to identify metabolic vulnerabilities in cancer. An early hallmark of cancer tissues is metabolic reprogramming. First noted by Otto Warburg, who found that cancer cells rely on glycolysis under aerobic conditions. More recent research has shown that metabolic alterations in cancer involve many additional pathways, potentially increasing the number of clinical targets. In fact, most, if not all tumour suppressors and oncogenes regulate metabolism. Furthermore, tumours are typically placed in a metabolically stressful environment, leading to essential metabolic adaptations. Our major interest is in metabolic enzymes that also function as tumour suppressors or oncogenes, or that regulate the essential metabolic requirements of cancer cells.

Pyruvate carboxylation enables growth of SDH-deficient cells by supporting aspartate biosynthesis. The tricarboxylic acid (TCA) cycle is a key source for mitochondrial NADH and the core metabolic route for production of many biosynthetic precursors. Despite their pivotal metabolic role, oncogenic mutations in three TCA cycle–related enzymes, succinate dehydrogenase (SDH), fumarate hydratase (FH) and isocitrate dehydrogenase (IDH), have been identified. While these discoveries implicated a causal link between altered metabolism and neoplastic transformation, they left the question of how these cancer cells fulfill energetic and anabolic demands largely unresolved. SDH is a hetero-tetrameric, nuclear-encoded protein complex responsible for oxidation of succinate to fumarate in the TCA cycle and for feeding electrons into the mitochondrial respiratory chain for ATP production. Inactivating mutations in the human genes for any of the SDH subunits, or the SDH complex assembly factor (SDHAF2), are associated with susceptibility to develop neuroendocrine neoplasms, gastrointestinal stromal tumours and renal cell carcinoma. We and others have previously demonstrated that the loss of SDH causes succinate accumulation in cells, which activates hypoxia-inducible factors at normal oxygen tension and inhibits α-ketoglutarate–dependent histone and DNA demethylation, thereby establishing, respectively, a pseudo-hypoxic and hypomethylator phenotype in tumours. Although SDH is the first discovered TCA cycle enzyme with tumour suppressor properties, the molecular mechanisms that enable the survival and growth of SDH-defective cells remain largely unexplored. We generated SDH-deficient immortalised kidney mouse cells, and by unsupervised metabolomic screenings and isotope tracing approaches, we identified metabolic pathways essential to support their proliferation. We found that SDH is loss sufficient to ensure a complete block of the TCA cycle and to drive Warburg-like bioenergetic features of aerobic glycolysis in proliferating cells. We demonstrated that ablation of SDH activity commits cells to convert extracellular pyruvate needed to sustain maximal glycolytic flux and support the diversion of glucose–derived carbons into aspartate biosynthesis via pyruvate carboxylase activity (Fig. 1). This study unveiled a metabolic vulnerability for potential treatment of SDH–associated neoplasms (Carstens et al., Nat Cell Biol. 2015; 17: 1317–26).

Glutamine synthetase activity fuels nucleotide biosynthesis and supports growth of glutamine–restricted glioblastoma. Glutamine and glutamate constitute a metabolic hub in cellular physiology. An increased demand for glutamine by transformed cells has been recognised for almost a century and has been linked to its role as an abundant circulating respiratory fuel. Notably, glutamine carbons can support anabolism through entering the TCA cycle via glutaminolysis. In certain cancer models, the inhibition of glutaminase, which deaminates glutamine to glutamate, reduces proliferation and tumourigenicity. Glutamine addiction has been proposed as a mark of glioblastoma, the most aggressive glioma. Using isotope tracing (13C– and 15N–labelled glutamine) in vitro and in vivo, we dissected the differential metabolic roles of glutamine-derived carbon and nitrogen atoms in sustaining anabolism and growth in six human glioblastoma cell lines, primary glioblastoma stem–like cells and normal astrocytes. Additionally, glutamine-related metabolism was investigated in vivo utilising isotope tracing in both primary orthotopic murine xenografts and glioblastoma patients. These studies led to the discovery of a metabolic crosstalk between glioblastoma cells and normal astrocytes in the brain (Fig. 2). In contrast to the current view that in cancer cells that undergo aerobic glycolysis, accelerated anabolism is sustained by glutamine–derived carbon, which replenish the TCA cycle (anaplerosis), we have demonstrated that in glioblastoma cells, almost half of the glutamine–derived carbon is secreted as glutamate and does not enter the TCA cycle. Furthermore, the inhibition of glutaminolysis did not block proliferation of glioblastoma cells. Instead, the conversion of glutamate to glutamine by glutamine synthetase (cataplerosis) confers glutamine prototrophy and fuels de novo purine biosynthesis in glutamine–derived cells. In both orthotopic glioblastoma mouse models and in patients, 13C–glucose tracing showed that glutamine synthetase produces glutamine from TCA cycle–derived carbons. Finally, while glutamine is only marginally supplied by the circulation to the brain, the glutamine required for the growth of glioblastoma tumours is either autonomously synthesised by glutamine synthetase–positive glioma stem cells or supplied locally by astrocytes (Tardio et al., Nat Cell Biol. 2015; 17: 1556–68).

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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 initiates the cascade by adenylating Ub’s C-terminus in the presence of Mg²⁺ and ATP, followed by the 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. E1 and E2 activity is required for E3 binding, and E3 recruits E2–Ub conjugate via its RING domain, recruiting an E2–Ub conjugate via its RING domain and promoting the formation of the closed E2–Ub complex to facilitate Ub transfer to the target substrate. Ub ligase (E3) functions as an activator. It binds the E2–Ub complex and promotes the formation of the closed E2–Ub complex to facilitate Ub transfer to the target substrate. The crystal structure of the pCBL RING domain bound to UbICBl reveals that phosphoTyr371 interacts with Tyr68 from UbICBl (c). UbV.XR functions as an activator. It dimers and binds to a surface on the IAP RING domain dimer that is remote from the E2–Ub binding site. UbV.XR contacts the closed E2–Ub complex and further stabilises the dimer to activate the ligase activity. The crystal structure of the XIAP RING domain dimer bound to UbV.XR is shown with the E2–Ub binding site in each RING domain indicated by an arc. Therefore, they responded more quickly to cellular stress than cells expressing wild-type MDM2. Our work reveals a ligase-independent role of MDM2 in p53 regulation and suggests that targeting the MDM2 E3 ligase activity could widen the therapeutic window of p53 activation in tumours, since rapid p53 induction can be achieved while basal p53 control by MDM2 is maintained.

Selective targeting of the catalytic domain of RING E3s
The catalytic domain of RING E3s, commonly known as the RING domain, contains ~75–100 amino acid residues that form two loops stabilised by two Zn²⁺ ions. 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. Development of a general platform for targeting the RING domain would enable us to address the biological functions of these enzymes and to investigate whether inhibition of the ubiquitin ligase activity could be a suitable approach for targeting RING E3s. In collaboration with Professor Sachev’s lab at the University of Toronto, we have utilised a phage–display ubiquitin variant (UbV) library to screen for UbVs that bind selectively to the RING or Ub-box domain. The UbV library contains native Ub sequence that was randomised to generate billions of ubiquitin variant sequences. We identified three UbV-E4B, UbV-pCBL and UbV-XR that bind selectively to the RING or Ub-box domain of monomeric UbV-E4B, phosphorylated active CBL, and dimeric XIAP, respectively. We showed that UbV-E4B and UbV-pCBL inhibit the Ub-pCBL complex (Fig. 2b). Furthermore, cell-based analyses showed that UbV-E4B inhibits UbV-E4B-mediated p53 ubiquitination and UbV-pCBL inhibits CBL-catalysed EGF-dependent ubiquitination of XIAP, a dependent manner, a condition that leads to Tyrs37 phosphorylation of CBL. Inhibition of EGF-induced ubiquitination resulted in EGF stabilisation, decreased EGF accumulation in early endosomes, and prolonged downstream signalling events.

In contrast to UbV-E4B and UbV-pCBL, UbV-XR binds XIAP dimers and activates the ligase activity. The crystal structure of XIAP–UbV-XR showed that UbV-XR binds to a region in XIAP dimers in the RING domain that is remote from the E2–Ub binding site (Fig. 2c). Structural modelling and biochemical analyses revealed that UbV-XR contacts the E2–Ub complex to assist stabilisation of the E2–Ub complex in the closed active conformation, thereby enhancing the ligase activity. When UbV-XR was introduced into HEK293T cells, it bound XIAP and enhanced SMAC ubiquitination upon induction of apoptosis. Collectively, our work demonstrates the versatility of the Ub technology in the identification of inhibitors and activators of RING E3s.

Publications listed on page 97
Lipids are a diverse class of biomolecules that are involved in tumour onset and progression. From a metabolic perspective, they are among the most abundant cellular ‘building blocks’ that cells need for growth. From a cell biology perspective, they are important signal transducers. In fact, the most frequently deregulated pathway in cancer, the PI3K-AKT pathway, features a PI(3,4,5)P3 lipid as its central messenger. Despite their clear relevance, technical limitations have until now prohibited the elucidation of fundamental aspects of lipid metabolism and signalling. To address this, we harness novel lipidomics and stable isotope tracing strategies to better understand the role of lipids in cancer, with an emphasis on studying the effect of the tumour microenvironment on cancer cells.

Acetate consumption and metabolism by tumour cells

Fatty acids are the bulk components of cellular membranes and are therefore in high demand by growing cancer cells. The precursor for fatty acids is acetyl-CoA, of which the acetyl group is mostly derived from glucose-derived carbon. However, in hypoxic (i.e. low oxygen) conditions, a common occurrence in solid tumours, most glucose carbon is shunted towards lactate, causing a reduction in the available carbon for acetyl-CoA, and hence fatty acid production. Exactly how cells cope with this has remained unclear. Previously, we reported a drastic increase in the apparent production of nuclear-cytosolic acetyl-CoA from acetate in hypoxic conditions (Kamphorst et al., Cancer Metab. 2015; 3: 37). This observation supported the findings made by others, including Zach Schug and Eyal Gottlieb, that the enzyme responsible for making acetyl-CoA from acetate, acetyl-CoA synthetase 2 (ACSS2), promotes tumour growth during metabolic stress (Schug et al., Cancer Cell. 2015; 27: 57–71). Acetate was therefore considered to be an alternative substrate for acetyl-CoA production during oxygen limitation.

While acetate is considered a ‘backup’ nutrient in hypoxic cancer cells, how much acetate actually consumes and how it is dispersed among downstream pathways (fatty acid synthesis, mitochondrial oxidation, protein acetylation) remained unstudied, but may reveal cancer cell vulnerabilities. To address this, we developed a method to measure both free and bound acetate directly, based on derivatisation by alkylation and gas chromatography–mass spectrometry (Tumanov et al., Cancer Metab. 2016; 4: 17). This method is fast and sufficiently sensitive to permit analysis of physiologically relevant acetate levels. We investigated heavy (13C) acetate uptake and its incorporation into fatty acids in a panel of cancer cell lines, both in atmospheric and low-oxygen conditions. We found that there is a direct relationship between acetate uptake and usage with ACSS2 expression levels. In other words, the ACSS2 expression level directly determines how much exogenous acetate is used for fatty acid synthesis. Further quantitative analysis revealed that the majority of exogenous acetate is used for the high acetyl-CoA demanding flux of fatty acid biosynthesis, and to a lesser extent for mitochondrial oxidation in cells that express the mitochondrial ACSS isoform (ACSS1). Measurements of labelling in acetate bound to histones revealed that exogenous acetate is only very sparingly used for histone acetylation.

Although exogenous acetate activated by ACSS2 does not appear to be an efficient substrate for histone acetylation, ACSS2 has previously been reported to be expressed in both the cytosol and nuclei of cells. To investigate ACSS2 localisation in cells exposed to various conditions, we performed immunofluorescence experiments. Interestingly, not only did total ACSS2 expression go up when cancer cells were exposed to hypoxic and low-serum conditions, so did the nuclear localisation of ACSS2. Thus, although exogenous acetate does not substantially contribute to histone acetylation, the acetate-activating enzyme ACSS2 becomes more nuclear. We solved this apparent conundrum by showing that ACSS2 not only captures exogenous acetate, but that it also plays an important role in recapturing acetate that is released by cells. In the nucleus, histone deacetylases continuously remove histone acetyl marks and ACSS2 reactivates the resultant acetate to acetyl-CoA so that it can be reused for histone acetylation, and maintenance of histone acetylation promotes cell survival. With help from Karen Blyth we were able to show that nuclear ACSS2 expression is also especially prominent in the poorly perfused, hypoxic regions of mouse breast cancer tumours (Fig. 3). In short, nuclear ACSS2 retains endogenously produced acetate to maintain histone acetylation (Bulusu et al., Cell Rep. 2017; 18: 647–58). We suggest that this is especially important in hypoxic, nutrient-deprived tumour regions, to retain as much carbon as possible and to promote cancer cell survival and growth. As a next step we plan to further study acetate metabolism in pancreatic cancer.

Developing a comprehensive lipidomics strategy

It has been estimated that mammalian cells contain up to 10,000 individual lipids. Arguably more important is that even in the most comprehensive recent lipidomics studies, only half of all measured lipids could be assigned a molecular structure and function. It is therefore apparent that much lipid biology remains to be discovered, and considering the important roles that known lipids play in cancer, we hypothesise that many lipids that currently remain uncharacterised contribute to tumour progression. Unfortunately, the ability to explore these unknown lipids is limited when using standard mass spectral-based lipid profiling approaches, as they are unable to cope with the structural diversity of lipids (Fig. 2). Recognising this issue, we are working towards a practical approach using two separate extraction procedures and two modes of separation, enabling the extraction and analysis of lipids over the entire polarity range (Tumanov et al., Curr Opin Biotechnol. 2017; 43: 127–33). Preliminary experiments using this strategy have revealed the presence of bioactive lipids in the tumour microenvironment of pancreatic tumours. A major focus of the lab is to characterise their involvement in tumour progression and to study the effect of modulating their synthesis and degradation.

Publications listed on page 99
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. Recent improvements in hormonal therapy and chemotherapy have brought about modest impact on patient survival. There remains an urgent need to understand treatment-resistant prostate cancer better in order to develop more effective personalised therapies.

We have a highly comprehensive cross-disciplinary programme of translational research aimed at tackling treatment (hormonal and/or taxane chemotherapy) resistance. Our research efforts are revealing novel targets for therapy. Timely validation studies on the identified targets are enabling us to launch ‘therapy’ discovery campaigns and initiate proof-of-concept clinical trials. Our preclinical and clinical expertise facilitates a seamless transition from laboratory findings to the design of clinical studies, as well as the development of clinical cohort studies.

Target discovery to overcome treatment resistance

We applied three pairs of human isogenic hormone-responsive and -resistant prostate cancer cell models and grew them as orthotopically implanted tumours in a nude mouse model. Collaborating with Dr Sara Zaninović, quantitative proteomic analysis was performed to study the proteome of the tumours. We identified two candidate proteins that were potentially upregulated in hormone-(or castration)-resistant tumours. We are now testing the functional impact of the candidate proteins on prostate cancer growth and metastasis. If successful, we will further evaluate the value of these candidates as targets for therapy.

Using a CRISPR screen in an orthotopic model, we hope to identify novel genes that may enhance the treatment efficacy of hormone treatment (androgen deprivation therapy) or docetaxel chemotherapy. Hits from the screen are now being validated and further pursued as candidate targets. A forward genetic screen has been launched as part of Dr Imran Ahmad’s CRUK Clinician Scientist Fellowship. This project builds on an earlier screen, which yielded highly informative data, resulting in the nomination of PPARγ as a potential therapeutic target.

Preclinical and clinical validation of novel therapeutics

SPECTRE is a proof-of-concept efficacy clinical trial based on our recent publication (Patel et al., 2017), in which we highlighted the importance of tumoural cholesterol uptake by castration-resistant prostate cancer in testosterone de novo synthesis.

We have previously proposed ERK5 as a potential target for therapy. Using a genetically modified mouse model of androgen-driven prostate cancer, we can confirm that reduced ERK5 expression suppressed prostate carcinogenesis. This data directly supports the value of ERK5 as a target for therapy. Our plan is to further investigate whether the observed effect on tumour growth depends on the kinase activity of ERK5 or simply depends on its expression status.

PPARγ has a wide-ranging metabolic impact on glucose and lipid metabolism and has been implicated in our earlier forward genetic screen (Ahmad et al., Proc Natl Acad Sci USA 2016; 113: 8290–5). Comprehensive analysis of how PPARγ metabolically and phenotypically contributes to prostate carcinogenesis is now underway.

Novel treatment strategies

Through a number of collaborative projects, we are exploring a number of promising treatment strategies.

From a repurposing screen, we have nominated a drug to combine with docetaxel for enhanced efficacy. We are currently considering the possibility of a proof-of-concept Phase III clinical study.

Other collaborative projects include approaches to exploit caspase-independent cell death (in collaboration with Dr Stephen Tait); palladium pro-drug therapy (in collaboration with Dr Asier Unciti-Broceta (University of Edinburgh); and a nanoparticle-based drug delivery complex (in collaboration with Dr Christine Delves, University of Strathclyde).

Taking advantage of our clinical expertise, we are developing translational studies focusing on patients who have relapsed from previous radiation-based therapy and those patients with evidence of significant cancer with regional nodal metastasis. We are particularly interested in understanding the tumour microenvironment in recurrent progressive tumours.

Publications listed on page 99
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 develop 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 a new tool for guiding cancer surgery and novel vectors for tomographic imaging of tumour initiation and treatment response monitoring in transgenic mouse models.

Resecting brain tumours with radioactive amino acids and light

Accurate surgical resection in glioma is essential because the extent of resection correlates with survival and over-resection can cause severe morbidities such as paraplegia. We applied a new biomedical imaging technique, Cerenkov luminescence imaging (CLI), to guide the surgical removal of brain tumours. CLI is a novel optical intraoperative imaging modality that has demonstrated clinical feasibility in breast cancer surgery. What is lacking, and why our work is important, is proof that CLI has an advantage over current approaches for intraoperative imaging. We directly compared a novel CLI probe, the radiolabelled amino acid analogue \[^{18}F\]fluoro-ethyl-tyrosine (FET), with the gold standard intraoperative method, 5-ALA fluorescence imaging, in multiple animal models of glioblastoma. 5-ALA was approved in 2014 by the European Medicines Agency for image-guided surgery of high-grade glioma due to an improvement in progression-free survival. Targeting glioma with CLI and FET exploits the metabolic reprogramming that is a hallmark of malignant transformation. Our results show that CLI with FET is more accurate and quantitative than 5-ALA fluorescence imaging when guiding brain tumour resection. Given the superiority of FET CLI and the rapid clinical translation of CLI, this technique has the potential to have a clinically meaningful impact in glioblastoma surgery. FET CLI could also be used for intraoperative imaging of low-grade glioma where there are no tools for visualising the tumour during surgery.

Imaging oncogenesis: novel multi-transgenic vectors for somatic delivery of radionuclide reporter genes

Oncogenesis, the transformation of normal cells into premalignant lesions and cancer, is optimally modelled using genetically engineered mouse models. The best of these recapitulate the genetic and microenvironmental heterogeneity which is often found in clinical cancer patients. However, it is frequently difficult to visualise oncogenesis as tumours develop at autochthonous sites with varying latency. This is the first example of a radionuclide reporter gene being used to monitor real-time spontaneous tumour development. This is important, as unlike bioluminescent approaches, it enables sensitive three-dimensional imaging of tumour development in vivo that is unaffected by overlying tissue–depth or tissue pigmentation. Unlike adenoviral- or adeno-associated virus–transduction–based approaches, lentiviral vectors stably integrate into the genome of infected cells. Thus they are useful for delivering transgene expression to both the transduced cell as well as to subsequent progeny. We exploit that feature to both initiate and permanently label tumour development from normal murine somatic cells with inherited ‘silent’ tumour-initiating genes. This approach has significant advantages over conventional germine transgenic approaches. It enables in vivo measurements with extremely high signal-to-noise ratios, since background is practically absent from non-labelled cells. The approach is versatile as the vector can be readily customised to modify reporter readout or the capacity to add or subtract specific gene expression from the base/ inherited conditional genotype without additional transgenic mouse strain development or breeding. It should also be useful for inducing tumours in organs other than the lung, such as the pancreas and prostate, thus facilitating sensitive tomographic imaging at these sites. The long-term goals of this research are not only to visualise 3D tumour development in preclinical models of cancer but to enable rapid and accurate non-invasive monitoring of cancer therapy.

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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 β-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 glycolysis 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 AKT/PI3K/AKT1 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 decreasing ATP levels while simultaneously depriving cells of AMP- generating capacity, suggesting that suppression of NUAK1 may be an effective means to selectively kill cancer cells with high levels of MYC expression.

Additionally, we have now found that NUAK1 plays a key role in protecting cells from toxic levels of reactive oxygen species (ROS). ROS are naturally produced as by-products of mitochondrial electron transport chain activity, and the elevated metabolic demand of cancer cells can thus increase ROS production. Paradoxically, hypoxia can also elevate ROS production and is moreover a common feature of most tumours. 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 colonic neoplasia. All of this provides strong evidence to support targeting NUAK1 in human colorectal cancer, this observation challenges dietary advice commonly given to patients who already have cancer, in that popular consumption of antioxidant supplements may actually benefit the cancer cells more than the patient.

Paradoxically, canonical activation of NUAK1 requires STK11 (aka LKB1), an erstwhile tumour suppressor associated with Peutz–Jeghers 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 precursors 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 318 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 actually seek ways to amplify signaling 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 Marston, 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 2017
A major development was a successful application, spearheaded by our colleague Jennifer Marston, to establish a Pancreatic Cancer UK–funded ‘future leaders in pancreatic cancer’ postgraduate academy of the PhD students, resulting in Dr Cyril Whyte commencing his studies in my group. At the other end of the PhD journey, Jennifer Port and Tiziana Monteverde both completed their studies and submitted their respective theses in September. Tiziana subsequently moved to the University of Manchester Institute, joining the group of Michela Garofalo, while Jennifer secured a scientific writing position in the Netherlands. We embarked on an exciting project to develop new in vivo models for mesothelioma, butted by collaboration with the MRC Toxicology groups in Leeds, along with support from clinicians in Edinburgh and Glasgow. This programme aims to recapitulate the inflammatory ecosystem associated with asbestos exposure, combined with state-of-the-art manipulation of genes associated with human mesothelioma, in order to shed much-needed light on early-stage disease progression. New work performed primarily in our own group was published in Oncogene, while we made significant contributions to other works published in Nature Communications and Nature Scientific Reports. Additionally we published a translational review on the subject of mesothelioma along with two invited commentaries on important new discoveries in mesothelioma and lung cancer.

Publications listed on page 102
TUMOUR CELL DEATH

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 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.

The role of autophagy during tumour development

There are many pathways in cells that regulate cell viability. One group of processes that promote cell survival and maintain cellular fidelity are collectively known as autophagy – literally, ‘self eating’. Autophagy, and more specifically the form called macroautophagy (hereafter referred to simply as autophagy), is a mechanism of cellular traffic that delivers cytoplasmic material to lysosomes for degradation (Fig. 1). As such, autophagy is a major mechanism for the removal of damaged proteins and organelles, thereby promoting cellular fidelity. In addition, autophagy can be modulated in response to various forms of stress and can mitigate the effects of this stress to promote cell viability and also cell survival.

It is now well established that autophagy has an important role in both tumour suppression and tumour development. However, the way in which autophagy is controlled in response to various stimuli is currently incompletely resolved, and this area is a major focus of our lab. We hope that the knowledge gained will enable the bespoke modulation of autophagy in a way that potentiates the effectiveness of chemotherapeutic responses in tumours without detrimental effects on normal tissue.

The transcriptional control of autophagy

As an approach to identify new autophagy regulators, we performed an RNA interference (RNAi) screen in Drosophila cells. Drosophila cells were chosen as a model system due to their relatively low level of functional redundancy and due to the ease with which human counterparts of Drosophila proteins can be identified. Through this screen, we identified the product of the Drosophila gene fs(1)h as a regulator of autophagy. The human orthologues of fs(1)h are members of the bromodomain and extraterminal (BET) family of proteins. There are four BET proteins in human cells: BRD2, BRD3, BRD4 and BRDT. Our further analysis revealed that BRD4 was also an autophagy regulator, and more specifically we found the protein was a repressor of genes involved in both autophagy and lysosome function.

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BRD4 is a chromatin reader protein which binds to acetylated histones. The protein then recruits proteins which can modulate gene expression, such as methyltransferases. We were therefore interested to understand how this mechanism of gene regulation might be affected under a physiological autophagic response. Amino acid deprivation elicits an evolutionarily conserved autophagic response, and we found that under these conditions, BRD4 dissociates from chromatin, resulting in the repression of genes involved in autophagy and lysosome function. This response involves the histone deactylation SIRT1 and the energy-sensing AMP-activated protein kinase (AMPK) (Fig. 2).

In most situations, BRD4 has been reported to be involved in the activation of gene transcription, but in the case of autophagy and lysosome genes, we found BRD4 to be a repressor. This indicated that BRD4 must bind a repressor of gene transcription to mediate this effect. Through analysis of the literature, we learnt that the methyltransferase G9a can act as both an activator and repressor of gene transcription, and our subsequent studies revealed that G9a was indeed a repressor of autophagy and lysosome genes via BRD4.

The main purpose of our study was to identify autophagy regulators that may modulate autophagy in a specific manner. To this end, we examined if BRD4 was a repressor of autophagy in response to additional autophagic stimuli. This revealed that in addition to amino acid deprivation, inhibition of BRD4 augments autophagy induced by glucose starvation, hypoxia, oncogenic Ras and the protein aggregates associated with Huntington’s disease. In contrast, inhibition of BRD4 has no effect on the autophagic clearance of bacteria or mitochondria.

BET domain proteins are considered promoters of tumour development in a variety of cancers. In particular, a chromosomal translocation involving BRD4 and a gene called NUT produces a fusion protein called BRD4-NUT, which is considered a driver of a specific type of cancer called NUT midline carcinoma. As a result, several BET inhibitors have been developed for the treatment of this and other cancers. We were therefore interested to know whether BRD4-NUT and also BET inhibitors have an effect on autophagy. In line with what we observed upon knockdown of BRD4, knockdown of BRD4-NUT caused a marked induction of autophagy. Similarly, treatment of cells with various BET inhibitors also caused induction of autophagy. Since in many cases autophagy is cytotoxic, this result opens up the exciting possibility of combing BET inhibitors with inhibitors of autophagy to give an enhanced therapeutic response.

Application of CRISPR reveals new roles for autophagy

The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) system has revolutionised the ability to investigate gene function in cells and animals. The system can be used to disrupt or mutate endogenous genes very efficiently. We applied this system to delete essential autophagy genes, enabling us to understand the role of autophagy in cancer cells under various settings. Against common belief that the majority of human cancer cells are dependent on autophagy, we found that acute CRISPR-mediated disruption of either Atg5 or Atg7, which are essential autophagy genes, was consistent with cell viability in a panel of human tumour cell lines. These findings have important implications for targeting autophagy in human cancer, although the survival of these cells under conditions of tumour-associated stress is yet to be determined.

Using these autophagy-deficient cell systems, we have also been able to assess the role of autophagy in therapeutic situations. In this regard, we found that the drug Veramipin – a cardiac drug which is being considered for cancer treatment – was a potent inducer of autophagy. This drug also causes apoptosis, and treatment of autophagy-deficient cells with Veramipin resulted in an enhanced cytotoxic effect, indicating that combinations of Veramipin with autophagy inhibitors is worthy of further investigation.

Publications listed on page 104
MITOCHONDRIA AND CANCER CELL DEATH

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, issues of toxicity and resistance limit the effectiveness of anti-cancer therapies. To address these, our research centres on understanding how mitochondria regulate cancer cell death and inflammation, with the ultimate goal of improving cancer treatment.

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

How a cancer cell dies matters
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 recent 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 response, we have found that CICD is much more effective than apoptosis at reducing tumour growth – often CICD led to complete regression (Fig. 1).

Figure 2
Caspase-independent cell death: an anti-cancer double whammy
Following widespread mitochondrial permeabilisation, cancer cells die regardless of caspase activity. Inhibiting caspases, leading to caspase-independent cell death (CICD), has multiple beneficial effects. First, it prevents caspase-associated toxicity. Second, cancer cells undergoing CICD are immunogenic, i.e., they require NFKb-dependent cytokine upregulation. By dying in this manner, CICD triggers host anti-tumour immunity that can kill remaining tumour cells.

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 recent 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 response, we have found that CICD is much more effective than apoptosis at reducing tumour growth – often CICD led to complete regression (Fig. 1). These beneficial effects are entirely dependent on intact immunity, consistent with CICD being an immunogenic cell death.

Mitochondria drive 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-kB transcription factor pathway. This, in turn, is required for inflammatory signalling during CICD. Mechanistically, mitochondria activate NF-kB by releasing proteins that downregulate cIAP1/2, resulting in NIK and NF-kB activation. Similar to others, we have found that permeabilised mitochondria, by releasing mitDNA, can also activate cGAS/STING, triggering an interferon response. As such, while mitochondrial apoptosis is largely viewed as a non-inflammatory type of cell death, the central event that initiates it – MOMP – is itself pro-inflammatory. Key questions currently being investigated include 1) are there additional inflammatory signals initiated by mitochondria? and 2) how do caspases suppress these effects? Finally, in addition to targeting these effects to improve cancer treatment, we are investigating roles for these inflammatory effects in different areas of health and disease.

Caspase-independent cell death – an anti-cancer double whammy
We have previously found that sub-lethal caspase activity can promote DNA damage and genomic instability. Moreover, other studies have shown that caspase-dependent effects may contribute to tumour growth as well as the toxicity of chemotherapy. Coupled to our recent findings discussed above, this suggests that the benefits of inhibiting caspase function in cancer therapy would be multi-fold. Not only will it block unwanted toxicity but, by engaging anti-tumour immunity, caspase inhibition enhances therapy-induced killing (Fig. 2). Based on this, we are investigating the potential benefit of targeting CICD in a range of cancer types.

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Figure 2
Caspase-independent cell death: an anti-cancer double whammy
Following widespread mitochondrial permeabilisation, cancer cells die regardless of caspase activity. Inhibiting caspases, leading to caspase-independent cell death (CICD), has multiple beneficial effects. First, it prevents caspase-associated toxicity. Second, cancer cells undergoing CICD are immunogenic, i.e., they require NFKb-dependent cytokine upregulation. By dying in this manner, CICD triggers host anti-tumour immunity that can kill remaining tumour cells.

Figure 1
Caspase-independent cell death is more effective than apoptosis at inhibiting tumour growth
Control, apoptosis-proficient CT26 colorectal cancer cells were rendered CICD-proficient through shRNA knockdown of APAF-1. Cells were implanted into syngeneic, immunocompetent BALB/c recipient mice. Following tumourigenesis, mice were treated with the BH3-mimetic ABT-263 to engage apoptosis (blue) or CICD (red). Tumour volume was measured over time; individual tumour growth (SI) is shown. Inducing CICD inhibited tumour growth in all cases, where complete tumour regression was observed in 50% of mice.

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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 view is that the tissue of origin influences the biochemical pathways utilised by tumours to grow in two ways. On the one hand, by imposing environmental constraints, the tissue of origin exposes metabolic vulnerabilities of the tumour. On the other hand, enzymes normally restricted to a defined population of differentiated cells, and required for tissue physiological functions, can be hijacked by cancer cells to enhance their metabolic fitness.

Glutamine and glutamate metabolism in brain and liver cancer

Glutamine and glutamate are instrumental to physiological processes, such as neurotransmission in the brain and ammonia homeostasis in the liver (Fig. 1). 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 glioblastoma xenografts implanted orthotopically.

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 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 glutamine synthetase is deleted conditionally. 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.

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 cells, as well as their response to the environment, and their epigenotype and genotype, very little attention has been focused on 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 glycæa. 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 designed, developed and produced a cell culture medium with 53 nutrients and metabolites at the concentration normally found in human blood. This is among the first examples of cell culture medium formulated on physiological criteria. The newly formulated medium allows the culture of mammalian cells with reduced supplementation of FBS. It has been successfully tested in a variety of cell culture systems, including murine normal, stem and cancer cells, as well as 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, thus favouring more translational biomedical research.
Infernet, European Union H2020 and tissues from different GEMMs for models (GEMMs). (a–d) Serum genetically engineered mouse increased serum formate levels in intestinal cancer, (b) lymphoma, (c) glioblastoma, and (d) one-carbon units.

**MATHEMATICAL MODELS OF METABOLISM**

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 the cancer cell, including but not limited to cell proliferation and cell migration. Our group investigates cancer metabolism from an evolutionary point of view. We hypothesize that given specific microenvironmental conditions and metabolic constraints, there is an optimal mode of cell metabolism to achieve a metabolic objective. This metabolic mode will offer an evolutionary advantage and therefore will be selected for during the time course of cancer development. 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.

**Increased formate overflow is a hallmark of 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 (Fig. 1). Formate can also be recycled back to ribose-5-phosphate via cytosolic one-carbon metabolism. In cells with defective mitochondrial one-carbon metabolism, the cytosolic pathway is reined, compensating for the loss of mitochondrial formate production. When both cytosolic and mitochondrial pathways are compromised, cells can utilise exogenous formate or endogenous formate as alternative sources of one-carbon units.

**Figure 1** Increased serum formate levels in genetically engineered mouse models (GEMMs). (a–d) Serum formate in wild-type (WT) pre-neoplastic (P) and neoplastic (Py) tissues from different GEMMs: (a) intestinal cancer, (b) mammary carcinoma, (c) lymphoma, and (d) sarcomatoid ductal adenocarcinoma (PDAC).

**Figure 2** Formate overflow promotes cancer cell invasion in glioblastoma

(a) Cartoon illustrating the experimental setup to analyse cancer cell invasion using coated Boyden chambers. (b–d) Addition of extracellular sodium formate increases invasiveness in a concentration-dependent manner in (b) U87, (c) LN229 and (d) NCH601 cells. (e–f) Reduced invasiveness by MTHFD1 knockdown can be rescued with extracellular formate in (e) LN229 and (f) NCH601 cells.

In 2017, we developed the experimental methods to investigate formate overflow in vivo. We have established an experimental protocol based on 13C-methanol tracing that allows us to determine the rate of serine catabolism to formate in tissues. Using this protocol, we uncovered a basal serine catabolism to formate in normal tissues, with tissue-specific rates in increasing order of their serine levels and oxidative profile. To test the rate of serine catabolism in cancer tissue, we analysed intestinal adenomas from APCmin+ mice and mammary carcinomas of PyMT mice. Both the intestinal adenomas and the mammary carcinomas exhibit significantly increased rates of serine catabolism to formate compared to normal adjacent tissue and other non-tumour-bearing organs. In addition, we have observed that plasma formate levels are significantly increased in tumour-bearing mice compared to wild-type mice in different genetically engineered mouse models of cancer (Fig. 1). This indicates that the tumour-specific high serine catabolism causes the elevated plasma formate levels.

In parallel, we have conducted phenotypic studies to determine the potential selective advantage of formate overflow. Using multiple cancer cell lines, we have shown that genetic knockdown of MTHFD1, the enzyme responsible for formate production in the mitochondria, does not result in any significant change in cell proliferation. Yet we have recently observed that inhibition of formate production by genetic knockdown reduces invasion and this phenotype can be rescued by exogenous formate (Fig. 2). We will continue these studies to decipher the mechanism of the formate-dependent induction of invasion. We conclude that some cancers are characterised by significant oxidative metabolism, and we identify formate overflow as the hallmark of such oxidative cancer types. Furthermore, we propose cell invasion as a key selective advantage of formate overflow.

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CANCER METASTASIS AND RECURRENCE
Liver cancer is the second most common cause of cancer-related death worldwide. The focus of my group is to understand what makes the liver regenerate in health, and how abnormalities in specific signalling pathways lead to liver cancer. We study the main functional liver cell, the hepatocyte, and what makes some cells regenerate in health and in response to injury, whilst others are prevented from regenerating and others transform to cancer. We are developing models of human liver cancer to study how early cancers escape the normal controls governing regeneration, aiming to identify novel therapeutic targets.

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. 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 them 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 of the cells at the level of individual cells within this environment. We are now studying ways to interfere with such spreading senescence as a means to develop treatment for states of regenerative failure e.g. fulminant liver failure, alcoholic hepatitis and liver failure following surgery.

Transformation of regenerative hepatocytes into malignancy

Whilst the Wnt/β-catenin pathway plays a role in regeneration, it is a 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 recently developed a number of models of HCC, utilising of targeted genetic manipulating at a clonal level in 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 can then 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 recapitulate human disease well, including spread to other organs. Using the advanced facilities within the Institute, we are able to map tumours as they develop using preclinical imaging and also to isolate and characterise the mutant cells at each stage of tumour development. 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 at risk of HCC, and 50,000 adults in the UK are estimated to have cirrhosis as a result of a trebling in disease incidence in the last 30 years. Hand in hand with chronic liver disease, primary liver cancers are becoming more common also. Liver disease is both preventable and reversible, as is the risk of HCC, but even if HCC develops, it is potentially curable providing it is detected at an early stage. However, deaths from liver cancer are predicted to continue to increase until we are able to identify people at risk of liver disease and HCC, and provide 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 are at risk of HCC. We hope to provide a rationale for inclusion of these biomarkers in routine clinical practice to facilitate the early treatment and cure of HCC. We collaborate with experts in public health and statistics to gather and analyse additional data collected from across Scotland and the UK and have already shown that by the application of novel statistical analysis for individual patients, we are able to detect HCC in its early forms. We are working to improve the accuracy of this approach and move towards clinical trials.

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One of the universal features of solid tumour types is that a loss of tissue organisation is the strongest predictor of poor outcome. Our lab studies the molecular mechanisms that control how cells organise to form normal tissues, and how this goes awry during tumour formation. We aim to understand this process such that we can identify potential novel targets for therapeutic intervention in cancer.

MOLECULAR CONTROL OF EPITHELIAL POLARITY

Our group studies the gain and loss of collective cell polarity and invasion in prostate, and more recently ovarian, tumours. Our research is focused on two intersecting streams: 1) understanding the molecules that regulate collective cell polarity, and 2) developing the computational and 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 to provide bioinformatics solutions to studying 3D cultures via live imaging.

In collaboration with the Functional Screening 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 a rapid fashion, the factors that regulate tissue formation and its disruption in cancer.

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 to be redundant have highly divergent and sometimes opposing roles in invasion, and show that there may indeed be specificity of signalling between family members. In addition, we are focusing on how alternative splicing of ARFome proteins can contribute to divergent functions of such alternatively spliced isoforms. This is an important preambule 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 Shank’s lab Functional Screening 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 Podxl-driven invasion.

Our future aim is to understand which of these in vivo 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 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.

Figure 2
3D cultures of cells to form cysts (also called spheroids or organoids) allows us to model the basic structure of epithelial organs. This allows us to understand and manipulate factors that are altered in cancer patients and model their effect on tissue organisation.
The immune system can both benefit and antagonise the growth of cancer. 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 work in prinicple. 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, both as a site of primary tumour development and as a target 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. Moreover, localisation and regulation of leukocytes within the pulmonary capillaries 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 also pose the first line of defence against many pathogens 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 matrixes – chemoreactive 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 gain more information about the way that they interact and to uncover potential pathways to modify.

By looking across multiple, relevant cancer models we hope to be able to do two things: 1) uncover general mechanisms by which pulmonary neutrophils and their regulation contribute to the cancer microenvironment; and 2) uncover cancers with the strongest or most manipulable interaction with pulmonary neutrophils. In addition to continuing our work on neutrophil regulation by interactions with other leukocytes, we have spent the last year establishing several productive collaborations with other teams at the Institute to better understand the neutrophils’ role in cancer biology (e.g. with Daniel Murphy’s lab to investigate lung cancer, Fig. 1A, B; and with Seth Coffelt’s lab, Fig. 1C; and with Jim Norman’s lab on metastasis to the lung).

Cancer has parallels with inflammation in terms of the cells, mediators and mechanisms involved. Using our knowledge of lung inflammation and tractable models of acute and chronic inflammation, we continue to investigate these parallel mechanisms and potential perturbations.

We had a great time discussing this at the Beatson’s Understanding Tumour Immunology and Metastasis workshop that we co-organised with Seth Coffelt and Jim Norman. It was a really interactive and successful meeting, and we would like to extend a huge thanks to all of our colleagues at the Institute and outside who helped us run the meeting and contributed with talks, posters and discussion. We have also enjoyed contributing to collaborative studies outside the lung, some of which were published this year, e.g. with Cristina La Cerio’s group at Imperial College in their work on acute myeloid leukaemia and with Sara Zarvin’s group at the Institute studying cancer cell–blood vessel interactions.

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The spread of cancer (or metastasis) from its primary site of origin to distant locations is the major cause of death among cancer patients. Our lab is interested in the involvement of immune cells in metastasis, with the intention of designing novel immunotherapies for patients with metastatic disease.

This was our first full year at the Institute. We expanded our team with the addition of three new people, because we were fortunate to receive funding from the Naito Foundation of Japan, the Wellcome Trust, Pancreatic Cancer UK, the Royal Society and Tensovus. During this year, we continued to build a research programme focused on the role of immune cells in metastasis. We are particularly interested in γδ T cells, which are a rare population of T cell receptor-expressing cells that drive breast cancer metastasis by controlling the function of neutrophils. Our ultimate goal is to understand how γδ T cells participate in metastasis and to uncover the mechanisms regulating the behavior of these cells so that novel immunotherapies may be developed for patients with metastatic disease. While most of our work uses mouse models of breast cancer, new collaborations at the Institute over the past year have allowed us to expand our questions about γδ T cells to other cancer types. Looking forward into 2018, the data generated from these collaborations will lead to new insights into metastasis-associated γδ T cell biology.

γδ T cells in cancer

In the breast cancer setting, one outstanding question regarding γδ T cells is how these cells are regulated. Our investigations to address this question led us to the NKG2D receptor. We have found that pro-metastatic γδ T cells express high levels of NKG2D. This receptor is normally associated with NK cells; it recognises cancer cells and induces NK cell killing mechanisms. So we were intrigued by the observation that pro-metastatic cells express a receptor that mediates cancer cell killing, and we are currently exploring the function of NKG2D on γδ T cells. We are also interested in developing new treatment strategies for metastatic breast cancer. For these studies, we are using a model of BRCA1-deficient mammary carcinoma. Tumours arising in these mice contain very few myeloid and lymphoid cells when compared to BRCA1-proficient tumours. Current efforts are underway to boost T cell infiltration into these immunologically ‘cold’ tumours. All our work in breast cancer is done in collaboration with Karen Blyth and her team.

γδ T cells in colorectal cancer

γδ T cells are a major immune cell population in normal gut tissue, but whether these cells play a role in colorectal cancer initiation and progression is not well understood. Together with Owen Sansom and his lab, we are determining the importance of γδ T cells in several mouse models that recapitulate distinct molecular subtypes of colon cancer. We are crossing γδ T cell-deficient (Tdc−/−) mice with these various models, where we will investigate tumour formation and metastasis. Interestingly, we have found that colorectal tumours lose expression of an epithelial-derived molecule, called BTN1L, which is required for the maintenance of Vγ7-expressing γδ T cells. BTN1L latches Vγ7 cells specifically to the gut, therefore, Vγ7 cells are likely to be absent from these BTN1L-deficient tumours. We are working with the hypothesis that the loss of Vγ7 cells potentiates tumour formation. This work is funded by a Wellcome Trust Seed Award and is being done in collaboration with Adrian Hayday (Francis Crick Institute).

γδ T cells in pancreatic cancer

Our lab was fortunate to be part of the Pancreatic Cancer UK Future Leaders Academy in 2017, which allows us to investigate γδ T cells in this deadly disease. Our long-term goal for this project is to develop γδ T cell–specific immunotherapies for pancreatic cancer. In collaboration with Jen Morton and her team, using mouse models of metastatic pancreatic cancer, we have found that γδ T cells are virtually absent from normal mouse pancreatic tissue, but they are prevalent in pancreatic ductal carcinoma. The preferential tropism of γδ T cells to tumour tissue opens a window of opportunity to inhibit the migration of these cells and determine whether tumour progression and metastasis is slowed. Our research efforts will focus on the trafficking of tumour-infiltrating γδ T cells, as well as their communication with neutrophils.

γδ T cells in other cancer types

Over the coming year, we will make use of the unique mouse models at the Institute to address the function of γδ T cells in various cancer types. Together with Tom Bird, we will investigate γδ T cells in hepatocellular carcinoma, and Daniel Murphy and his lab will help us study γδ T cell function in lung cancer.

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CELL MIGRATION AND CHEMOTAXIS

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 different disciplines, 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 strategy 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. 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. In the past we have found that chemotaxis in Dictyostelium cells (an easily studied experimental model organism) works by a different mechanism than that which is usually described. Pseudopods are constantly generated in random directions, then the ones that point in the best directions are selected and maintained. We have now shown that a similar process drives chemotaxis in cancer cells, using several different cultured melanoma lines. We have used chemotaxis chambers of our own design to show that melanoma cells are exquisitechemotactically sensitive. They can navigate up a gradient of serum with unprecedented accuracy, irrespective of their stage – early melanomas 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.

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, 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. We are now finding that SCAR is phosphorylated at two sites in living cells, including tumours. This is a unique facility. We have now found that SCAR is phosphorylated at two particular 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 signaling 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.

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 are now finding that SCAR is phosphorylated at two particular 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 signaling 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 98
Primary cilia, found on almost all human cell types, are involved in the regulation of several signalling pathways and are reported to be lost in several cancer tissues. Lymphocytes are one of the few cell types that do not form cilia. However, they do form a structure called the immunological synapse, which shares similarities with cilia, at the interface between professional and non-professional antigen-presenting cells, including tumour cells.

GDP dissociation inhibitor (GDI)-like solubilising factors (GSFs) are a family of proteins, including PDE6D, UNC119a and UNC119b, which solubilise lipid-modified proteins and share structural homology with the Rho GDP dissociation inhibitors, a class of proteins known to bind prenylated Rho proteins. PDE6D binds to and is involved in the trafficking of prenylated proteins, whereas UNC119a and UNC119b are specific for myristoylated proteins (Wright et al. Genes Dev 2011; 25: 2347–60; Zhang et al. Nat Neurosci 2011; 14: 874–80; Zhang et al. Vision Res 2012; 75: 19–25).

Arl2 and Arl3 are small G-proteins that belong to the Ar (ADP ribosylation factor)-like small G-protein subfamily. They have a 52% sequence identity and share several interactors. Amongst Arl2 and Arl3 interactors are PDE6D, UNC119a and UNC119b, where the interactions are guanosine triphosphate (GTP) dependent and do not involve lipid moieties. Arl2 and Arl3 function as allosteric release factors for lipilated proteins bound to PDE6D and UNC119a/b in a GTP-dependent manner (Ismail et al. Nat Chem Biol, 2011; 7: 942–9; Ismail et al. EMBO J, 2012; 31: 4085–94). Furthermore, it has been reported that the ciliary protein Arl13b can act as a specific guanine nucleotide exchange factor (GEF) for Arl3 (Gotthardt et al. eLife 2015; 4: pii: e1859).

Last year we put forward a sorting model for prenylated protein INPP5E delivery to the cilia. The model depends on the affinity of cargo for PDE6D, the presence of an active Arl3 found exclusively in cilia, and the specific release of ciliary cargo by active Arl3.

Using INPP5E and Rheb as examples for ciliary and non-ciliary proteins, respectively, we show that lipidated cargoes are solubilised by binding to GSFs in the cytosol. If a cargo binds to GSFs with a low binding affinity, the complex will be disrupted by active Arl2GTP in the cell body. In the case of ciliary proteins, which bind to GSFs with strong binding affinities, the soluble complex can diffuse into the cilia, where it is released by Arl3, which is in turn activated by the ciliary protein Arl13b. The released cargo is then retained in cilia by associating with the ciliary membrane.

We are currently investigating this trafficking mechanism and its role in the formation of the immunological synapse between lymphocytes and cancer cells. Furthermore, using small molecules we are trying to manipulate the positioning of signalling proteins in the cilia and immunological synapse to alter their signalling output.

Publications listed on page 98
MIGRATION, INVASION AND METASTASIS

Cells in embryos migrate during morphogenesis and then when tissues are established, this migration is much reduced. However, these migration programmes can be reawakened in tumours when they become invasive and metastatic. Tumours are chaotic and contain elements of developmental programmes gone wrong, selected for as the tumour struggles to overcome challenges such as nutrient and oxygen deprivation. Metastasis presents additional challenges, as tumour cells are required to survive in hostile environments and may remain dormant for months or years before a metastasis grows to a detectable size. Our research programme has three main projects, each addressing cell migration and cancer metastasis at different levels, from fundamental molecular mechanisms to specific developmental and cancer models.

Uncovering new mechanisms behind cell motility decisions

Cell motility is key to tumour cell dissemination and immune cell homing to tumours. We study basic mechanisms of how cells move and how this affects cancer invasion and metastasis. The mechanisms by which normal and cancer cells turn actin assembly into motility are still not completely understood. One of our challenges is to find missing pieces of the puzzle, such as new regulators of the cytoskeleton.

The Rho family of GTPases comprises 21 Ras-related proteins in mammals that act as molecular switches to control cell migration, adhesion and growth. The RHOTPAKE RAC1 is specifically implicated in control of actin assembly at the leading edge of cells in sheet-like protrusions called lamellipodia. RAC1 is mutated in melanoma and its activity is essential for Ras-mediated oncogenic transformation. PhD students Loic Fort and Jose Batista (Robert Insall’s group) have discovered and characterised a new regulator of the connection between RAC1 and lamellipodia phosphorylation CYRI (Fig. 1). Mammals express two forms, CYRI-A and CYRI-B, and these proteins are highly conserved, as they are found in amoebas, some plants and more higher eukaryotes. Postdoc Jamie Whitelaw and students Anh Le and Savvas Nikolaou are currently studying how CYRI-A and -B control the migration of melanoma and pancreatic cancer cells. Additionally, they are exploring how CYRI proteins are regulated and how they contribute to feedback loops for actin-based motility.

From melanoblast migration to melanoma metastasis

Melanoma cells can revert to embryonic migration pathways and thus escape from the primary tumour. Melanoblasts, the embryonic precursor cells of melanocytes, are highly migratory during development and thus can serve as a model to understand how melanoma cells migrate. Unlike many other embryonic migratory cells, they move individually (not collectively) to populate the skin and hair follicles. RAC1 and CDC42 have key roles driving migration of mouse embryonic melanoblasts. Student Emma Woodham and postdoc Nikki Paul, together with Prof Gord Brabletsch (BIRC, University of Copenhagen, Denmark) showed that melanoblasts lacking CDC42 show defects in polarity, migration and integrin-based adhesion. Postdoc Karthic Swaminathan has discovered a role for the Scar/WAVE complex in melanoblast migration and melanoma tumour development and progression, suggesting that the Scar/WAVE complex may be an important melanoma target downstream of RAC1.

Pathways controlling the invasion and metastasis of PDAC

N-WASP is established as a key driver of formation of invadopodia and of cancer cell invasion in vitro (Yu, Zech et al., J Cell Biol. 2012; 199: 527-44), but much less is known about its potential role in vivo. MRC-funded clinical research fellow Hayley Morris found that loss of N-WASP accelerated tumour progression of APC-driven colorectal cancer in a mouse model. Her findings suggest that N-WASP could have a tumour suppressive role in colorectal cancer, even though it is a promoter of invasion and metastasis in other models. Postdoc Amelie Juin is studying the role of N-WASP in pancreatic ductal adenocarcinoma (PDAC) (Fig. 2). Amelie has discovered a role for N-WASP in tumour invasion and metastatic spread to secondary sites. Using cells cultured from the tumours, she uncovered how N-WASP drives matrix remodelling by tumour cells and potentiates chemotactic signalling loops that mediate cell egress from the primary tumour. Our efforts to target the actin-binding protein fascin in pancreatic cancer continue, with new compounds and tools being developed with the Institute’s Drug Discovery Unit. Postdoc Nikki Paul, together with Richard Bayliss and Selena Burgers in Leeds, has developed and characterised nanobodies to target fascin (funded by Cancer Research Technology and the Pancreatic Cancer Research Fund). These nanobodies will be useful tools to probe fascin function and to gain structural information about how inhibitors bind to fascin and alter its actin-binding activity.

Tumour cells are influenced by the surrounding stroma, consisting of extracellular matrix and other cell types – both in the primary tumour and in distant sites that they might colonise. In collaboration with bio-engineer Manuel Salmeron-Sanchez (Engineering, University of Glasgow), we are exploring the biophysical properties of tumour extracellular matrix that influence migration and proliferation vs dormancy of cancer cells. We hypothesise that increased local stiffness, combined with microenvironmental signalling, drives adhesion and actin-mediated reawakening of dormant tumour cells. PhD student Vassilis Papalazarou is exploring how the cytoskeleton and adhesion directly signal to metabolic and transcriptional programmes to push the cell into a state of active migration, growth or dormancy.

Publications listed on page 100
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 are, therefore, focussed on understanding how primary tumours are able to prime organs for metastasis, how this priming may be assessed and how the metastatic niche may be targeted therapeutically. We have recently discovered that certain oncogenes influence the release of metabolites and other factors, such as exosomes, which lead to metastatic niche priming. In particular, we have elucidated how tumour metabolites and exosomes drive alterations to endosomal trafficking in lung fibroblasts to change the deposition of collagens and other extracellular matrix proteins in this metastatic target organ.

We are currently determining how to use non-invasive imaging approaches to assess the extracellular matrix of primed metastatic organs, and we are exploring pharmacological approaches to reducing metastatic niche formation to oppose cancer recurrence following surgery.

Expression of mutant p53 and other pro-metastatic oncogenes generates 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 adenocarcinoma in the pancreas can influence integrin trafficking in lung fibroblasts to alter the deposition of extracellular matrix (ECM) proteins, such as collagen VI, in the lung. This altered microenvironment provides migratory cues which lead to priming of the lung as a metastatic niche. We are investigating how pancreatic and colon adenocarcinoma can colonise 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. Finally, we are determining how to use non-invasive ECM imaging to assess the priming of metastatic organs, and to explore pharmacological approaches to reducing metastatic niche formation to oppose cancer recurrence following surgery.

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 alters markedly during the progression of mammary tumours in mice. Levels of glutamate in the serum reflect tumour burden and acquisition of primary tumour invasiveness, and plasma aspartate and y-aminobutyric acid (GABA) concentrations increase as metastases form in the lung. Our 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 ECM deposition in metastatic target organs. Furthermore, we are determining how these metabolites modulate the immune landscape of tumours and metastatic target organs.

‘Nuclear capture’ of endosomes activates transcriptional programmes to favour metastasis. By designing a novel screen to identify receptors that are transported from the cell surface to the nuclear membrane, we have discovered a mechanism whereby endosomes are ‘captured’ at the nuclear surface by interaction of a nuclear localisation sequence in the cytodomain of the receptor tyrosine kinase EphA2 with the nuclear import machinery. This process promotes juxta-nuclear actin polymerisation, leading to activation of MRTF/SRF transcription factors and generation of a transcriptional programme favouring dissemination and metastasis of pancreatic cancer. Antisense oligonucleotide (ASO) drugs are being developed to target mutated oncogene products, such as KRas, and we are investigating how this pathway may be exploited to target the delivery of ASOs to cancer cells that are actively metastasising.

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A major function of the actin cytoskeleton is to provide the structural underpinning that gives a cell shape and mechanical strength. The actin cytoskeleton is dynamic, undergoing constant rearrangement and reorganisation in response to external factors, including soluble factors and the physical microenvironment. Alterations to the cytoskeletal architecture have significant consequences on the entire cell – such as morphology, cytokinesis, adhesion and motility – and also at the subcellular level. Research in our lab is focused on identifying important regulators of actin cytoskeleton dynamics that contribute to processes which are important for cancer. In particular, we have focused on kinase effectors that act downstream of Rho GTPases. Through these efforts, we aim to uncover proteins that could be potential cancer drug targets.

Gene expression associated with migration through physical constraints
The metastatic spread of cancer cells is a stepwise process that starts with dissociation from primary tumours and local invasion of adjacent tissues. The ability to invade local tissues is the product of several processes, including degradation of extracellular matrices (ECM) and movement of tumour cells through physically restricting gaps. To identify properties contributing to tumour cells squeezing through narrow gaps, invasive MDA-MB-233 human breast cancer and MDA-MB-435 human melanoma cells were subjected to three successive rounds of selection using cell culture inserts with highly constraining 3µm pores. For comparison purposes, flow cytometry was also employed to enrich for small-diameter MDA-MB-233 cells. RNA-sequencing (RNA-seq) using the Illumina NextSeq 500 platform was undertaken to characterise how gene expression differed between parental, invasive pore-selected or small-diameter cells. Gene expression results obtained by RNA-seq were validated by comparing with RT-qPCR.

Transcriptomic data generated will be used to determine how alterations that enable cell passage through narrow spaces contribute to local invasion and metastasis.

MRCK inhibitor development
In collaboration with the Institute’s Drug Discovery Unit, funding was obtained from Worldwide Cancer Research to characterise inhibitors of the MRCK proteins. The myotonic dystrophy-related CDC42-binding kinases MRCKα and MRCKβ contribute to the regulation of actin–myosin cytoskeleton organisation and dynamics, acting in concert with the Rho-associated coiled-coil kinases ROCK1 and ROCK2. The absence of highly potent and selective MRCK inhibitors has resulted in relatively little knowledge of the potential roles of these kinases in cancer. We discovered the azaindole compounds BDP9800 and BDP9066 as potent and selective MRCK inhibitors that reduce substrate phosphorylation, leading to morphological changes in cancer cells along with inhibition of their motility and invasive character. In over 750 human cancer cell lines tested, BDP9800 and BDP9066 displayed consistent anti-proliferative effects, with greatest activity in haematological cancer cells. Mass spectrometry identified MRCKα S103 as an autophosphorylation site, enabling development of a phosphorylation-sensitive antibody tool to report on MRCKα status in tumour specimens. In a two-stage chemical carcinogenesis model of murine squamous cell carcinoma, topical treatments reduced MRCKα

S103 autophosphorylation and skin papilloma outgrowth. In parallel work, we validated a phospho-selective antibody with the capability to monitor drug pharmacodynamics. Taken together, our findings establish an important oncogenic role for MRCK in cancer, and they offer an initial preclinical proof-of-concept for MRCK inhibition as a valid therapeutic strategy.

Inhibition of pancreatic ductal adenocarcinoma invasion and tumour growth by the ROCK inhibitor AT13148
The high mortality from pancreatic cancer demands that new therapeutic avenues be developed. The orally available small-molecule inhibitor AT13148 potently inhibits the ROCK1 and ROCK2 kinases that regulate the actomyosin cytoskeleton. We previously found that ROCK kinase expression increases with human and mouse pancreatic cancer progression, and conditional ROCK activation led to accelerated mortality in a genetically modified LSL-KrasG12D; LSL-p53R172H; Pdx1-Cre (KPC) mouse pancreatic cancer model. We now show that treatment of KPC mice and human TRICCS patient-derived pancreatic tumour cells with AT13148, as well as the ROCK-selective inhibitors Y27632 and H1152, acted comparably in blocking ROCK substrate phosphorylation. Furthermore, AT13148, Y27632 and H1152 induced morphological changes and reduced a) cellular contractile force generation; b) motility on pliable discontinuous substrates; and c) 3D collagen matrix invasion. AT13148 treatment reduced subcutaneous tumour growth, and blocked invasion of healthy pancreatic tissue by KPC tumour cells, in vivo, without affecting proliferation, suggesting a role for local tissue invasion as a contributor to primary tumour growth. These results indicate that AT13148 has anti-tumour properties that may be beneficial in combination therapies, or in the adjuvant setting, to reduce pancreatic cancer cell invasion and to slow primary tumour growth, which might have the additional benefit of enabling tumour resection by maintaining separation between tumour and healthy tissue boundaries.

Publications listed on page 104

MOLECULAR CELL BIOLOGY
**COLORECTAL CANCER AND WNT SIGNALLING**

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. 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**

Understanding the differences between APC and β-catenin mutations in terms of signalling provided mechanistic insights, but one question that still puzzled us was that if an activating mutation of β-catenin was within an intestinal stem cell, then this would be long lived and therefore cells should have ample time to accumulate β-catenin and 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 modeled 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 copies 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 colorectal tumours in man.

**Inhibiting Wnt signalling in vivo**

Given the strong link between deregulated Wnt signalling and colon cancer, we tested the impact of Wnt inhibition on the normal intestine using a clinically relevant Wnt inhibitor, WNT974. This inhibitor blocks the protein porcupine, which is required for Wnt ligand secretion, and is well tolerated in mouse and man. Within the intestine of treated mice, we found that there was a marked reduction in LGR5+ intestinal stem cells. This caused intestinal crypts to be functionally monogenic (i.e. with only one stem cell) rather than polyclonal (up to 8-10 stem cells). The consequence of this was that there was reduced stem cell competition, and that if mutations occurred they rapidly repopulated the entire crypt. Therefore, this suggests that one of the key evolutionary reasons for high levels of Wnt signalling in the intestinal crypt is to drive stem cell competition and prevent accumulation of deleterious or cancer-causing mutations (Huett et al., Nat Commun 2018, 9:1132).

**Demonstrating that HUWE1 is a bona fide tumour suppressor gene**

In human cancers, many genes are mutated, often at relatively low frequencies. Assigning these as functional modulators of cancer development or treatment is therefore very important. In CRC, mutations of HUWE1 (an E3 ubiquitin ligase) occur in approximately 10% of cases, and as they are on the X chromosome, a single mutation should result in loss of function. To investigate whether HUWE1 can act as tumour suppressor gene, we determined in combination with APC loss in vivo and found a dramatic increase in tumour initiation. Mechanistically, this was associated with a marked increase in the DNA damage marker γH2AX, which is a target of HUWE1 ligase activity (Dankaerts et al., EMBO Mol. Med. 2017, 9(1)). Other HUWE1 targets, c-Myc and MCL-1, were also upregulated. Importantly, this increase in DNA damage correlated with a sensitivity to cytotoxic agents, and patients who carry HUWE1 mutations respond well to chemotherapy. Together, our results therefore indicate HUWE1 mutations are driver mutations in CRC and may alter the response to therapy.

Publications listed on page 105
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 innovative strategy to hamper cancer growth and metastasis.

Our research primarily focuses on the role of CAFs in breast and high-grade serous ovarian cancers because these tumours contain a suitable proportion of stroma, which is densely populated by CAFs. Furthermore, CAFs have been shown to play key functional roles in the progression of both diseases. Importantly, ovarian cancer cells lack recurrent somatic mutations and this limits the availability of targeted therapies against the cancer cells. Therefore, CAFs may offer a valid alternative therapeutic opportunity in this tumour type.

We aim to decipher how CAFs contribute to tumour progression and metastasis, with the ultimate goal of identifying strategies to target these cells for therapy.

In particular, we study how CAFs promote invasive behaviour of the cancer cells and support their uncontrolled proliferation and survival, and how CAFs influence endothelial cell (EC) behaviour. ECs are a key cellular component of the blood vessels. ECs 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 uncontrolled growth 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. We also study the role of cell metabolism in the regulation of CAF function.

Our group has a strong expertise in mass spectrometry (MS)-based proteomics, and we integrate this innovative technology in our research to provide new levels of understanding of CAF biology.

CAFs can originate from the normal fibroblasts resident at the site where the primary tumour develops. Under stress conditions, such as chronic stimulation by factors secreted by the cancer cells, redox stress and hypoxia, the normal fibroblasts become activated. This activation induces extensive reprogramming of gene expression and protein levels, such that CAFs are characterised by a) being highly contractile, and b) secreting soluble factors and ECM components that promote the progression of cancer. This highlights the importance of a better understanding of how CAFs alter the tumour microenvironment and how the surrounding stromal and cancer cells react to these changes. To tackle this question, we make extensive use of state-of-the-art MS–proteomics approaches, which have previously shown to be a powerful technology to investigate cellular secretomes (Zanivan et al., Molec Cell Proteomics 2013; 12: 5999–6011) and molecular mechanisms underpinning EC behaviour and whether this impacts on tumour metastasis. We have therefore discovered that high ECM stiffness increases levels of heterotypic cell–cell adhesions on the surface of the ECs. In particular, we have characterised an unprecedented mechanism through which high stiffness promotes extravasation into the ECM. This mechanism underpins other studies that have shown that the CCN1/N-cadherin pathway facilitates the binding of the cancer cells to ECs, which is the first step of cancer cell intravasation into the blood vessel for the formation of distant metastasis (see Fig. 1). We are currently investigating the role of stromal CLIC3 in HGSOC to understand whether it is a potential novel target for this type of cancer, particularly for blocking metastasis, which is the major cause of death for this tumour type.

Recently, we have started investigating how hypoxia, which is typical in aggressive cancers, influences CAF functions. Using 3D co-cultures of CAFs and ECs, we found that hypoxia exacerbates the pro-angiogenic function of CAFs. This is important because excessive tumour angiogenesis can cause the formation of leaky blood vessels, which can worsen hypoxia and facilitate cancer cell extravasation into the blood flow to form distant metastasis. Extensive proteomic analysis of hypoxic breast cancer CAFs has pinpointed possible mechanisms underpinning this hypoxia–induced function, which are currently investigating further.

TUMOUR MICROENVIRONMENT AND PROTEOMICS

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 innovative strategy to hamper cancer growth and metastasis.

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Tumour stifferness favours cancer cell invasion

The CLIC3/TGM2 pathway that we have discovered promotes endothelial and cancer cell invasion by stiffening the tumour ECM. These results support previous findings that high stiffness promotes tumour invasion and metastasis. While several works have described how stiffness promotes invasive behaviour of cancer cells, it is largely unknown how stiffness controls EC behaviour and whether this impacts on tumour metastasis. We have discovered that high ECM stiffness increases levels of heterotypic cell–cell adhesions on the surface of the ECs. In particular, we have characterised an unprecedented mechanism through which high stiffness promotes extravasation into the ECM. This mechanism underpins other studies that have shown that the CCN1/N-cadherin pathway facilitates the binding of the cancer cells to ECs, which is the first step of cancer cell intravasation into the blood vessel for the formation of distant metastasis (see Fig. 1). We have therefore discovered a new function of tumour stiffness on the vasculature and discovered a pathway that can be targeted to reduce metastasis by blocking stiffness–induced intravasation of cancer cells.

Publications listed on page 110
The Drug Discovery Unit continues to make progress across its portfolio of exciting drug discovery projects that not only mirrors the focus of the outstanding research undertaken within the Institute, but also the CRUK focus of working on high-risk but potentially high-reward targets. In particular, our continued progress against KRAS is very exciting, and through our collaboration with the Frederick National Laboratory for Cancer Research in the US, we look forward to progressing to the next stage. In addition, through existing collaborations with Professor Mike Olson (Beatson Institute) and Professor Anthony Chalmers (University of Glasgow), our MRCK inhibitors are showing great promise in two independent in vivo models, one of skin squamous cell carcinoma and another of radiation-induced glioblastoma invasion.

**KRAS**
KRAS mutations are associated with many human cancers, and in particular are associated with the vast majority of pancreatic ductal adenocarcinomas (PDAC) and a significant number of other tumour types including colon and lung (30–40%). In the GTP-bound active state, KRAS signals from the plasma membrane through a functionally diverse set of downstream effector proteins (including PI3K, RAF and RALGDS) to pathways that control cellular growth, apoptosis, survival and differentiation. We aim to block the interaction between KRAS and its effectors to inhibit oncogenic KRAS signalling.

Our KRAS project maintains a highly competitive position within the small molecule inhibitor field. Signing a two-year agreement with the USA National Cancer Institute’s RAS Initiative (at the Frederick National Lab) has enhanced our resource within multiple areas of the project, enabling us to progress our high chemical series more aggressively whilst also working on additional backup series, presenting different opportunities and molecules profile. Results embedded within the Frederick Labs in biochemistry, cell biology, biophysics and protein production provides support for the development of novel assays, enabling us to explore the most effective approaches to measuring KRAS activity in a clinically relevant setting. Another key benefit of the collaboration is the direct access to the network of RAS cancer research across the USA, through this central hub of the RAS Initiative. This collaboration is funded jointly by CRUK and the CRT Pioneer Fund (CPF).

We have developed a medicinal chemistry strategy to optimise (Fig. 1) our existing KRAS ligands, resulting in new, significantly more active compounds. The team have made full use of our in-house X-ray crystallography data and computational chemistry expertise to effectively design and prioritise compounds for synthesis.

Optimisation has led to compounds with increased affinity as they fit better with the KRAS protein and make additional key interactions with amino acid residues in the pocket. This binding affinity has been improved significantly against KRAS GDP and KRAS GMP, with very tight binding observed and 33nM in our KRAS G12D GMP and KRAS G12D GDP surface plasmon resonance (SPR) binding assays. Importantly, tight binding has also been confirmed in additional, orthogonal binding assays, providing highly valuable information useful for further optimisation. Multiple, parallel approaches have been employed to develop biochemical assays for SOS-mediated nucleotide exchange (NEA) and effector binding (HTRF, FRET and pulldown). Significant improvements in potency were demonstrated across all biochemical assay systems as a result of the optimisation strategy and in line with our expectation. Excitingly, data for our best compounds show an effect on downstream signalling in cells at low micromolar concentrations.

We have characterised appropriate cell assay systems (both in wild-type, G12C/G12D mutant colorectal and pancreatic cancer cell lines and engineered RAS-less MEF cells) to measure target modulation and efficacy readouts. Assays looking at HAS/RAF interaction or levels of downstream signalling markers of the MAP kinase pathway (e.g. pERK) suggest that our current compounds are permeable and able to bind to RAS (with low micromolar EC₅₀ potency) in cells. Importantly, we are in the process of developing 3D cell culture systems, where we have observed improved responses to our compounds when compared to cells grown in monolayers, in terms of cell survival and proliferation.

**MRCK**
In close partnership with Professor Mike Olson and Anthony Chalmers, the Drug Discovery Unit has made excellent progress in its aim of developing and characterising inhibitors of MRCK. The myotonic dystrophy kinase-related CDC42-binding kinases, MRCKα and MRCKβ, regulate actin–myosin contractility and have been implicated in cancer invasion and metastasis. In concert with the closely related ROCK1/ROCK2 kinases, MRCK is known to phosphorylate downstream substrates such as MLCK and MYPT1 to facilitate the cytoskeletal changes which contribute to cancer cell motility and invasion. Preclinical studies in which MRCK knockdown was shown to reduce invasion of cancer cells in vitro suggest there are likely to be clinical areas in which MRCK inhibitors would have therapeutic benefits.

Using a focused fragment-based MRCK biochemical screen, in combination with MRCK structural biology, our lab has developed selective and potent MRCK inhibitors. In particular, our lead compound BDP-00009066 (MRCKα Kᵢ = 23pM) was designed starting from a ligand-efficient fragment BDP-0003246 (MRCKα Kᵢ = 4.49µM). Iterative rounds of medicinal chemistry and structure-based design using BDP-0003246 led to the identification of BDP-00009066, a potent and selective MRCK inhibitor with sufficient pharmacokinetic properties to enable its use to further explore the role of MRCK as a cancer drug target. Generation of such inhibitors has allowed us to validate the hypothesis that MRCK is involved in cancer cell invasion in different indications. First, we have studied the effects of MRCK inhibition in glioblastoma (GBM). GBM is an aggressive, incurable primary tumour which is characterised by highly infiltrative cells. Patients are treated with surgery, radiotherapy and chemotherapy, but due to the invasive nature of the disease, outcomes remain poor and recurrence rates are high. Whilst radiotherapy extends life expectancy, recent research has indicated that it can also promote a more invasive phenotype in cells which survive treatment. Our studies have shown that MRCK activity is upregulated by irradiation at the invasive edges of GBM tumours. Using BDP-00009066, it has been demonstrated that inhibiting MRCK activity is effective at reducing radiation-induced migration of GBM cells in vitro and in vivo. In a clinically relevant intracranial G7 cell mouse model (Fig. 2), BDP-00009066 was shown to prevent the invasion of GBM cells into the contralateral brain hemisphere (Birch et al., in preparation), and studies are ongoing to determine whether such effects of this compound lead to improved survival rates.
In addition to studies in glioblastoma, BDP-00009066 has also been used to show that MRCK inhibition has utility in squamous cell carcinoma (SCC). BDP-00009066 treatment of SCC cells led to reduced cell motility and 3D invasion at sub-micromolar levels in vitro, and MRCK activity was elevated in mouse skin tumours in a chemical carcinogenesis model. When evaluating the in vivo efficacy of BDP-00009066 in this SCC mouse model, we found that topical application of the compound resulted in a significant reduction in papilloma size (Unbekandt et al., Cancer Res. 2018; 78: 2096–114), highlighting further therapeutic action of this inhibitor.

These studies have revealed exciting opportunities for BDP-00009066 as a potential chemotherapeutic agent. As a consequence, two patent applications for our MRCK inhibitors were filed in 2017, adding further support that our compounds represent considerable advances in probing the effects of MRCK inhibition in in vivo models of cancer cell invasion.

Publications listed on page 96
BEATSON ADVANCED IMAGING RESOURCE (BAIR)

Light microscopy allows us to gather information about important regulatory mechanisms in tumours and key cells of the microenvironment whilst preserving spatial and temporal information. No other technique allows us to simultaneously record multiple important molecules and cells with subcellular sensitivity and resolution in living samples whilst maintaining the context of the microenvironment, be it 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. We try to assist from experimental design right through to the finished figures. We train scientists in all stages of modern microscopical research, from advice on sample preparation, basic and advanced microscope 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 light microscopy 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.

We held our annual imaging competition earlier in the year and had fantastic entries again. This year Nikon provided a digital SLR camera for first prize, won by Amélie Juin! Some of the stunning pictures that exhibit the excellent technique of our researchers can be seen throughout the report. We also had a great time hosting the annual Scottish Microscopy Group one-day meeting at the Institute in November. Margaret was the local organiser, ensuring the smooth running of the meeting, and David and Ewan both helped run demos and make sure everyone was at the right place at the right time. We welcomed colleagues from all over Scotland (and also from elsewhere in Europe) for a day of talks, posters, networking and seeing the latest developments. We would like to thank all that helped out on the day and in the run-up, the commercial exhibitors for sponsoring the meeting and showing us their latest products and everyone who attended. And Nikki Paul from Laura Macchesey’s team won the image competition!

Core themes and new methodologies

Although basic transmitted light and epifluorescence microscopy may not appear to have advanced much in recent years, the way that we use them has substantially. We have stable long-term timelapse systems capable of automated, unattended imaging and we have also increased our fleet of IncuCyte incubator-based imaging systems again this year due to demand, seeing the latest IncuCyte model (S3) installed just before Christmas. These can multiplex up to six imaging plate-based experiments and perform week-long recordings, making the most of the stable incubator environment and, for example, allowing researchers to look at the effects of several mutations or perturbations at once. The newest system allows additional flexibility by scheduling more complicated timelapses independently for each plate and allowing us to use all three objectives in the same experimental run. David has enjoyed running a regular user group meeting dedicated to these instruments. We continue to work with our colleagues in Functional Screening and Histology to make sure that researchers are using the best combination of high-content/throughput methodologies to extract as much meaningful data as possible from each experiment.

Again, confocal microscopy is a mainstay of the BAIR and our three confocal laser-scanning instruments, two spinning disc microscopes and two multiphoton systems (one of which is embedded in the Biological Services Unit, BSU) have all been heavily used by researchers at the Institute. We capture highly resolved fluorescence data in several ways that optimise speed (spinning disc), resolution and number of channels (laser-scanning), depth (multiphoton) and other readouts, e.g. fluorescence lifetime (spinning disc FLIM, multiphoton FLIM). Excitingly, we have once again made more use than ever of our Zeiss LSM 880 ‘Airyscan’, which uses a spatially arrayed multi-GaAsP detector to increase signal and resolution beyond the usual diffraction limit to 140 nm laterally and 400 nm axially. The addition of beam shaping optics to the excitation pathway essentially allows us to scan four lines at once, increasing imaging speed fourfold with a small decrease in resolution.

Our second Zeiss LSM 880, ‘Airyscan FAST’, is equipped with multiphoton excitation (approx. 700–1300nm) and is embedded in the BSU. This is now in full service and has been used for intravital microscopy of brain, liver and prostate cancer this year and ex vivo imaging of lung cancer tissue with improved resolution at depth. It is an upright system, capable of fast superresolution imaging and synergises exceptionally well with the existing BSU inverted confocal fluorescence microscopy (SHG) imaging system.

First harmonic generation (SHG) imaging works well on this system as well as our other multiphoton systems, allowing us to perform label-free imaging of the extracellular matrix, an important component of the tumour microenvironment. Ewan has enjoyed applying grey-level co-occurence matrix (GLCM) analysis to a whole host of new SHG images from several groups to quantify geometric differences in collagen extracellular matrix.

Publications listed on page 95
Bioinformatics and Computational Biology

The Bioinformatics and Computational Biology unit provides support for a variety of research projects that require computational approaches, advanced statistical analyses and mathematical modelling. Although there is a significant and growing demand for high-throughput data analysis, we strive to ensure that even the smallest task receives our full attention. We offer advice relating to experimental design, the appropriate use of statistical tests, and the clear presentation of results for use in theses and publications.

Our team focuses on exploratory data analysis, and our ultimate goal is to provide insights that enhance our understanding of cancer biology. We carry out routine processing of RNA and DNA sequencing data, differential expression analysis, and splicing and copy number variations. Our data analysis and modelling tasks are 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 or in collaboration with our colleagues from the areas of mathematics, statistics, computer science and biology. One of our routine tasks involves submitting lists of genes and metabolites for functional annotation, clustering, enrichment, ontology and pathway analysis using Ingenuity Pathway Analysis and GeneGo MetaCore. We also employ the Oncomine Research Premium Edition database to satisfy the demands of researchers who wish to make use of publicly available datasets. Many tasks (such as motif incidence and isoform identification) require the use of online databases, and so we regularly write customised data mining scripts that download the relevant data and extract the appropriate information.

Over the last year, we have performed our first analyses of RNA immunoprecipitation sequencing (RIP-Seq) data and small RNA–Seq data (with the latter being used to investigate Piwi-interacting RNA). Our revised search strategy, which utilises publicly archived RNA-Seq data, has been used to procure evidence of differential alternative splicing patterns between normal and tumour samples. Our aim is to quantify the occurrence of both known and novel splicing events in genes of specific interest to researchers.

The last year has also seen considerable progress in the development and application of our in-house library of computational tools. First, we have used simulations from our mathematical model of cell migration and phagocytosis to show that chemotactic responses can promote the engulfment of particles that are coated with a chemotacticant, this work is supported by experimental data from our collaborators. Second, we have developed an R workflow that employs machine learning (using support vector machines) to identify groups of proteins that are predictive in classifying subjects into a pre-defined set of disease states; this work is complemented by our own interactive visualisation tool that allows us to easily explore and assess the space of predictive proteins. Finally, we have developed a number of workflows in R, in some cases, KNIME that, for a very specific set of experimental designs, automate the analysis process. This is particularly useful for researchers who want a quick and easy way to perform the same analysis on more than one data set of the same type (for example, in the case of repeated experiments).

To ensure that appropriate statistical methods are used and presented in publications, we offer advice on experimental design, statistical techniques and data presentation. Moreover, personalised training is available to researchers (on request) for implementing and applying specific methods, programming in R, and using Bioconductor. Our team also participates in delivering part of the postgraduate MSc in Cancer Sciences programme.

Proteomics

The Proteomics facility is working with cutting-edge MS-proteomic technologies and robust platforms for sample preparation and data analysis to answer basic questions of cancer biology, thus contributing to the progress of cancer research.

The proteomics team has a strong expertise in using high-resolution, Orbitrap-based mass spectrometry (MS) in combination with highly accurate quantification approaches and data analysis. We work in collaboration with groups at the Institute and outside, and we actively develop MS-based proteomic platforms to address a variety of questions to help researchers better understand the mechanisms that regulate various aspects of cancer.

To achieve this, the facility is well equipped with three LC-MS systems, of which one is a new-generation Orbitrap instrument Q Exactive HF. All our instruments are coupled online to Easy-nLC systems, and high-resolution chromatography is achieved by packing our nano-columns in house.

We house a number of dedicated software of which MaxQuant is the most used, for highly accurate quantitative analysis. Moreover, we use Skyline for the analysis of PRM data. Finally, we use Perseus and Scaffold for data compilation, analysis and dissemination.

During 2017, we have worked with many of the groups at the Institute and provided significant contributions to the success of their research. A key achievement is the development of a novel stable isotope-based method to quantitatively measure cysteine oxidation to detect substrates of oxidoreductases (Hernandez-Fernaud & Ruengeler et al., Nat Commun 2017; 8: 14206) ; Port et al. Cancer Discov 2018; 8 632–47 ; van der Reest & Lilla et al. Nat Commun 2018; 9:1581). 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.

Currently, we are expanding our quantitative approaches. In addition to SILAC-, dimethyl- and label-free-based methods for peptide and protein quantification, we are setting up TMT labelling approaches, which allow multiplexing up to ten samples in one. We are also working to improve the depth of the proteomes achieved starting with small amounts of sample using high pH reverse-phase LC fractionation. This will enable us to perform improved global proteomic, sub-proteomic and post-translational analyses of primary cells and 3D organotypic cell cultures.

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The Screening facility couples high-throughput functional genomics screening and drug repurposing approaches with high-content imaging (HCI) to translate fundamental cancer research towards new therapies.

In 2017, we extended our major collaboration with the Bryant lab to successfully utilise our HCI resources to automate image acquisition and image analysis of prostate cancer spheroids in 3D. A central issue around 2D approaches is a perceived lack of predictive ability of responses in higher-order, more organised 3D structures. However, the utility of 3D in vitro approaches and comparison with 2D approaches have not been extensively characterised. Our collaboration has provided insight into this concern and has led to advancements in our understanding of heterogeneity: this is important because it is not necessarily the most common phenotype that is the most relevant, and under-represented phenotypes may represent significant behaviours. Using HCI, we can provide the required statistical power to give relevance to these rare phenotypes.

During 2017, we ran a total of five screening campaigns. To date, we have generated over 5.3 million data points across 32K screening plates. We have conducted the following screens this year:

- Identification of novel therapeutic avenues for metabolomic and immunogenic subclasses of pancreatic cancer (Drug repurposing and HCI)
- Functional analysis of solute carrier family proteins to determine i) serine and ii) glycine transporters (Functional genomics and HCI coupled with metabolomics analysis)
- Identification of putative metabolomic biomarkers of fascin function (Functional genomics and HCI)
- Evaluation of HDAC inhibitors as anti-inflammatory chemopreventatives (Targeted drug screen and HCI)
- Identification of OXPHOS inhibitors for the treatment of chronic and acute myeloid leukaemias (Drug repurposing, spectrophotometrically determined viability)
- Epigenetic modifiers of immunogenicity in ovarian high-grade serous carcinoma (Focused epigenetic drug library, ELISA)
- Pooled CRISPR screen optimisation, execution and analysis: Identification of mechanisms supporting radioresistance under hypoxia and normoxia in oral cancer.

Further to this work, we have experienced a rapidly increasing demand for the imaging acquisition and automated ‘machine learning’-centred analysis we support. Machine-learned image analysis is a powerful tool, bringing robustness and throughput to the process, while alleviating researcher-dependent analysis. After liaising with several companies, we have evaluated (e.g. Fig. 1) and subsequently acquired new instrumentation, whilst implementing a modified infrastructure to allow us to deliver to demand. We propose to further enhance our capabilities by implementing additional infrastructure, e.g. bioinformatics-driven refinement of our image analysis pipelines, so that we can deliver a complete HCI package.

Publications listed on page 108

Figure 1
Technical comparison of a prostate cancer spheroid acquired on the Opera Phenix (left) and the Operetta (right). Clear enhancement of the spheroid physiology can be identified and subsequently quantified.
The Metabolomics facility employs state-of-the-art mass spectrometry techniques to measure small molecule (metabolite) changes in cancer cells. We have well-established targeted LC–MS methods, where we measure approximately 100 metabolites per sample. For metabolite profiling, we have expanded our capabilities and are now using both Thermo Scientific’s Compound Discoverer as well as Nonlinear Dynamics’ Progenesis QI software to explore novel changes in metabolic pathways in cancer cells. For a second year, we were involved in organising and delivering a practical metabolomics course at Cold Spring Harbor Laboratory in the USA.

In 2017, we purchased Thermo Scientific’s new Altis Triple Quad mass spectrometer for more targeted LC–MS/MS analysis. This can offer increased sensitivity and specificity for known metabolites. We have three other Thermo Scientific LC–MS systems (Q Exactive Plus, Q Exactive and Exactive) with their high-resolution, accurate mass Orbitrap technology, useful for metabolite profiling. These are complemented with our Agilent GC–MS/MS Triple Quad instrument.

With our targeted approach to metabolomics, we analyse a range of sample types, including cell extracts, medium, plasma, urine, cerebrospinal fluid and tumour and other tissues. Having purchased a metabolite library of 600 standards from Sigma, we are increasing the number of metabolites we can identify on our LC–MS platform. In one analysis, we can determine a broad range of metabolites of different classes, including amino acids, organic acids, sugars, phosphates (glycolysis and pentose phosphate pathways), nucleotides and cofactors (such as CoA, NADH). Experiments using stable isotope tracers (often labelled with 13C glucose in the medium) enable us to examine the intracellular kinetics and the proportional distribution of many metabolites produced from the tracer. We can calculate metabolite exchange rates between cells and the medium in which they are grown.

Metabolomics can be used to look for novel metabolic changes, by identifying compounds showing different abundances in cancer cells, using an untargeted approach. We are working with Thermo Scientific’s software team for Compound Discoverer in the USA, beta testing the newest version of their software. Using Compound Discoverer has enabled us to further develop our untargeted approach to metabolomics over the past year. We can link to other Thermo Scientific tools, including their mzcloud database of fragmentation spectra. This is very important for us as this fragmentation library has been prepared from Thermo Q Exactive mass spectrometry instruments and the fragmentation spectra are much more similar to our own fragmentation spectra than previously used in silico fragmentation databases. Our workflow has been developing throughout the past few years and we have now taken a large step forward and have demonstrated inter-batch comparison, by the use of pooled samples and internal standards. 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 several projects, many involving clinical samples.

With the help of Jurre Kamphorst’s and Alexei Vazquez’s groups, methods are being developed for GC–MS analysis using our Agilent Triple Quad mass spectrometer. The method of sample preparation is more complex than for LC–MS, as samples need to be derivatised to allow them to be volatile in the GC. We have methods in place for fatty acids, amino acids, acetate and formate.

We work closely with the groups of Eyal Gottlieb, Saverio Tardito, Jurre Kamphorst, Alexei Vazquez, Vignir Helgason and Karen Vousden and also support several other research groups within the Institute who have specific interests in cancer metabolism. We also have regular meetings with all the mass spectrometer users at the Institute, particularly those in proteomics and lipidomics, to share knowledge about mass spectrometry.

Publications listed on page 100
Pancreatic cancer is a major healthcare challenge, 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 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.

Personalised therapy
Pancreatic cancer is a genetically heterogeneous disease. Whilst mutations in KRAS, TP53, SMAD4 and CDKN2A are common, there are many genes mutated less frequently that may be clinically relevant targets in subsets of patients. We have been using genetically engineered mice to model these subsets of patients who may ultimately benefit from more personalised approaches to treatment based on their mutations. For example, 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. By layering further genetic aberrations onto this model we have been able to study the importance of various signalling pathways in tumourigenesis, and to use these mice to test new therapies and combinations. Using the state-of-the-art imaging technologies available in the Institute, we have been able to monitor tumour growth and response to therapy, for example, using high-resolution ultrasound imaging (Fig. 1). We are also using novel models to manipulate genes in different cellular compartments within the tumour, e.g. fibroblasts or different immune cells, so as to better understand the complex signalling network that exists within the tumour microenvironment.

Modulating the immune microenvironment
A distinguishing feature of pancreatic cancer is the dense desmoplastic stromal microenvironment that surrounds and supports the tumour cells, which can account for up to 90% of the tumour volume in human pancreatic ductal adenocarcinoma (PDAC). This microenvironment includes fibroblasts, stellate cells, immune cells, blood vessels and extracellular matrix proteins such as collagen and fibronectin. We and others have shown that each component of this stroma can play an important role in PDAC progression and influence tumour cell proliferation, metabolism, migration, response to chemotherapy and immune evasion (Fig. 2).

A major mechanism of immune evasion by tumours is via ligation of PD1, an immune checkpoint receptor on T cells, by ligands PD-L1 and PD-L2 on the surface of tumour and stromal cells. However, despite excitement around the use of immunotherapies in other cancers, unselected clinical trials testing immune checkpoint inhibitors in pancreatic cancer have been disappointing. We have previously found that ablating neutrophils homing to pancreatic tumours, via CXCR2 inhibition, could inhibit metastasis but also enhance T cell infiltration into the tumours, providing a therapeutic opportunity for PD1-blocking immunotherapy, which could increase survival in mice even with late-stage disease. More recently, we have been studying the effects of macrophage ablation via CSF1R. We have found that inhibition of monocyte/macrophage infiltration alone, via CSF1R targeting, is enough to extend survival in mice with late-stage pancreatic cancer. In this scenario, CSF1R inhibition is sufficient to alter the immune-suppressive microenvironment, inhibiting PD-L1 expression and reducing fibrosis (Fig. 3), and allowing activated T cells to accumulate without the need for immune checkpoint inhibition.

Genomics and immunotherapeutics
Recent data have suggested that mutation burden may also predict the efficacy of immune checkpoint inhibitors. Until recently we have focused on the role played by the microenvironment in immune evasion. We are now investigating how the intrinsic genomic landscape of the tumour, and potential genomic instability, influences host response to the cancer or efficacy of immunotherapy. In particular, we are modeling the effects of DNA damage repair genes which are mutated in pancreatic cancer, e.g. BRCA1 and 2, and ATM, to study the genomic landscape and to test response to therapies.

There are no predictive markers or signatures for the use of immunotherapy in tumours with stable genomes. However, expression analysis of human pancreatic tumours has identified four subtypes, and we believe that stratifying based on subtype may be beneficial. Thus, in the future we plan to extend genomic and transcriptomic analyses across a variety of models to define subtypes and investigate intra-tumour subtype heterogeneity, with a view to preclinically tailoring novel concepts in the appropriate models.
Our lab uses in vivo models to interrogate cancer processes in physiologically relevant systems. These preclinical models enable us to follow cancer progression and metastasis, recapitulate human cancers, and test novel ways to therapeutically target the disease.

A specific interest of our group is in the RUNX/CBFβ co-factor complex and how these proteins contribute to breast and other epithelial cancers.

Transgenic Models of Cancer

Epithelial cancers exist as a complex mix of tumour cells co-evolving and interacting with other cell types such as stromal fibroblasts, blood vessels and immune cells. These 3D entities ultimately outgrow the organ of origin, invade surrounding tissue and metastasise to distant sites. Thus, investigating aspects of the cancer journey in a 3D tissue culture setting has certain limitations, and an integrated understanding of the process demands biologically relevant in vivo models. To this end, our lab uses xenograft, allograft and sophisticated genetic models of various tumour types such as breast, ovarian and prostate cancers, and melanoma. These models allow us to interrogate the metabolism of both tumours and the host, track metastatic disease, and probe novel therapeutic approaches.

Modeling cancer in vivo

Electricalins exist as a complex mix of tumour cells co-evolving and interacting with other cell types such as stromal fibroblasts, blood vessels and immune cells. These 3D entities ultimately outgrow the organ of origin, invade surrounding tissue and metastasise to distant sites. Thus, investigating aspects of the cancer journey in a 3D tissue culture setting has certain limitations, and an integrated understanding of the process demands biologically relevant in vivo models. To this end, our lab uses xenograft, allograft and sophisticated genetic models of various tumour types such as breast, ovarian and prostate cancers, and melanoma. These models allow us to interrogate the metabolism of both tumours and the host, track metastatic disease, and probe novel therapeutic approaches.

We can exemplify how these models are vital in translating the seminal findings from the lab to a physiological setting in some key collaborative studies with our colleagues at the Institute and the University of Glasgow. For example, exploiting the vulnerability of cancer cells to serine and glycine starvation, we used dietary restriction of these non-essential amino acids to elicit reduced tumour growth in genetic and allograft models of intestinal cancer and lymphoma. This was a fruitful collaboration with Oliver Maddocks (University of Glasgow) and Karen Vousden (Beatson Institute), where we showed that the effectiveness of this therapeutic approach was highly dependent on the KRas genetic status of the tumour cells (Maddocks et al., 2017). We were also delighted to work with Stephen Tait’s lab to show how the tumour microenvironment influences metastasis (Reid et al., 2017). The RUNX genes, together with their binding proteins, form a transcriptional complex that regulates several key signalling pathways associated with cancer. For a long time, these proteins have been known to significantly contribute to the aetiology of human leukaemia. However, in the last few years we have come to appreciate that this family of genes is also altered in specific epithelial cancers, such as breast cancer (Fig. 1). What is particularly intriguing is the paradoxical role of these proteins in different types of breast cancer. RUNX1 is frequently amplified in breast cancer (Fig. 1), demonstrating that the RUNX genes are altered in over 87.3% of all breast cancers. Using data generated by The Cancer Genome Atlas (TCGA, Cell 2015) and mined using cBioPortal (www.cbioportal.org), we find that the RUNX/CBFβ genes are altered in over 12% of all breast cancers and occur as mutations, deletions and amplifications, demonstrating the context-dependent nature of these alterations. RUNX2 is the most altered, in 6% of 974 breast cancers.

Breast Invasive Carcinoma (TCGA, Cell 2015)

<table>
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<tr>
<th>OTHER GENES</th>
<th>ALL RUNX</th>
<th>ALL CBF-β</th>
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<tr>
<td>87.3%</td>
<td>12.7%</td>
<td>6.8%</td>
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<td>3.5%</td>
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Our analyses verify that this family of genes is also altered in specific epithelial cancers, such as breast cancer (Fig. 1). What is particularly intriguing is the paradoxical role of these proteins in different types of breast cancer. RUNX1 is frequently amplified in breast cancer, demonstrating that the RUNX genes are altered in over 87.3% of all breast cancers. Using data generated by The Cancer Genome Atlas (TCGA, Cell 2015) and mined using cBioPortal (www.cbioportal.org), we find that the RUNX/CBFβ genes are altered in over 12% of all breast cancers and occur as mutations, deletions and amplifications, demonstrating the context-dependent nature of these alterations. RUNX2 is the most altered, in 6% of 974 breast cancers.

RUNX1 is amplified in triple negative breast cancer, which correlates with reduced survival of patients; yet mutation and deletion of this gene is found in oestrogen-positive (ER+) breast cancers. Using in vivo breast cancer models, the lab has convincingly shown that RUNX1 does in fact act to restrict tumour development at early stages of disease progression. Furthermore, the RUNX2 sense promoter has been shown in the absence of RUNX2. Current studies are pursuing the mechanism of this further.

We have also uncovered a novel role for RUNX1 and RUNX2 in renal cell carcinoma, finding high levels of these proteins in patient biopsies and preclinical models (Fig. 2). In addition, we have observed that CRISPR/Cas9–mediated deletion of RUNX1 in human kidney cancer cell lines causes a reduction in cell growth, and we are now testing the relevance of this in our in vivo models.

As discussed above, tumours are comprised of a milieu of different cell types, and the microenvironment plays a significant role in epithelial cancer development. A long-standing project in the lab has been looking at the role of RUNX2 in the tumour microenvironment. We have discovered strong RUNX2 expression in the fibroblasts of the tumour stroma and find that fibroblast-specific deletion of RUNX2 can slow mammary cancer development. We are now investigating whether the expression of RUNX2 in the host stromal environment influences breast cancer development using syngeneic allograft experiments. In addition, we have been using a range of complex 2D and 3D in vitro assays to determine how cancer-associated fibroblasts that have been modulated for RUNX2 expression interact with and influence breast cancer cell proliferation, invasion and migration.

Publications listed on page 93
We use molecular genetic techniques to analyse gene function in the progression of cancer. We can introduce precise genetic alterations into stem cells using methods such as gene targeting or genome editing. These methods allow us to accurately model the specific changes detected in human cancers by engineering the same changes in the same loci in stem cells. Multiple genetic alterations can be introduced concurrently into one stem cell line, allowing us to make highly sophisticated models of human disease. They allow us to test how these modifications interact and contribute to the progression of the human disease.

Generating cancer models with stem cell technology

Stem cells have a number of unique properties which facilitate their use in modelling human disease. First, they are amenable to techniques that allow us to generate accurate genetic alterations, identical to those found in human disease. And second, once altered, they can be differentiated to almost any cell type. Consequently, the effects of a variety of genetic alterations can be analysed in the development of disease not only in the stem cells themselves, but in the cells from the tissues in which the cancer normally arises.

Towards more precise reagents for cancer modelling

Although mouse cells are generally good models of their human counterparts, there are occasional examples of genetic differences between mouse and human orthologous genes. One such example of this type of difference is the gene Tafazzin (Taz), which has 11 exons in humans but only 10 in the mouse. This gene has previously been shown to play a role in apoptosis and also underlies the human genetic disease Barth Syndrome.

The human Taz gene shows a number of differences in splicing between humans and mice. In particular, the human gene has an additional exon that is not present in the mouse (ENSEMBL Human transcript Taz-101 Exon 5; ENSG00000350674). This is especially notable as the remainder of the intron–exon structure is identical between mouse and human, with all the remaining 10 exons being an identical length in the two species.

One consequence of this marked difference between the evolution of human and mouse Taz genes is that attempts to replicate human disease causing mutations in the mouse may not give the anticipated results, as any point mutation introduced to the mouse gene would only be present in mouse transcripts and not the full range of alteratively spliced transcripts present in human, in particular those isoforms including the human exon 5, not present in the mouse.

Replacing the mouse gene with the human gene was accomplished using gene targeting in mouse embryonic stem cells. As the entire gene is only 758kb in the mouse and 1017kb in humans, in both cases the entire locus could be manipulated using conventional plasmid-based approaches. The replacement of the mouse gene by the human gene was done in three simple steps.

First, the entire coding region and promoter of the mouse Taz gene was deleted in embryonic stem cells. A small DNA vector was generated comprising short 5’ (green box in Fig. 1) and 3’ (orange box in Fig. 1) homology arms. Between these homology arms are a single loxP site and an FRT site. A hygromycin selectable marker was subsequently introduced into the vector at the loxP site by Flp recombination in E.coli. The deleted mouse gene following gene targeting. The entire coding sequence and promoter of the ‘Taz’ gene were removed and replaced by a hygromycin selectable marker. AloxP site (grey triangle) and an FRT site.(orange semicircle).

Second, recombination was enhanced using dual recombinase-mediated cassette exchange (dRMCE; Fig. 1C). Following retrieval, the human Taz gene inserted into the mouse X chromosome, this was achieved by dRMCE using theloxP and FRT sites. The red and light blue boxes indicate the homology regions used to recombine the human sequences. The positions of thelox sites (blue triangles) and the Neoycin cassette (purple box) are indicated.

Lastly, the entire human Taz gene was inserted into the targeted locus in the mouse X chromosome by dual recombinase–mediated cassette exchange (dRMCE, Fig. 1C). Following retrieval, the human vector was transferred into the ES cells generated in step 1, in which the mouse ‘Taz’ gene has been deleted and which have JaxPand FRT sites inserted at the site of the original mouse Taz gene.

Taken together, our data show that we have managed to replace the mouse Taz locus with the entire region from the corresponding human gene. This will allow us to test whether the human gene has identical functions in mouse cells as the endogenous gene. Furthermore we can now test the consequences of disease-relevant mutations in the context of the human genome.

Figure 1

A) Mouse wild-type Taz allele

B) Mouse Taz deleted allele

C) Human Taz locus inserted into the mouse X chromosome

Publications listed on page 108
PET Radiochemistry

The Cyclotron Facility at Gartnavel Hospital currently supports routine production of FDG (fluorodeoxyglucose) and a small number of other fluorine-18-labelled clinical tracers. In 2017, we recruited a new Senior PET Chemist, Dmitry Soloviev, who brings world-class expertise in carbon-11 labelling of metabolites for cancer imaging. Carbon-11 is a highly versatile radiolabel, allowing tracing of many endogenous metabolic pathways. We are building new capacity at the Radiopharmaceutical Unit of the West of Scotland PET Centre by providing a new radiochemistry platform that will facilitate the development of new carbon-11- and fluorine-11-labelled PET probes. We are upgrading the cyclotron and ancillary radiochemistry equipment to work with carbon-11-labelled gaseous products and are installing two identical, universal and automatic 11C/18F synthesisers (Synthra GmbH, Germany) in the R&D and GMP labelling suites (Fig. 1). Any radiotracer developed for preclinical research will be available for rapid translation to human studies at the Radiopharmaceutical Unit in Gartnavel Hospital. Installation of the two synthesisers is planned for 2018, and the first new tracers – [(F)H]acetate, [(F)F]fluoro-ethyl-tyrosine (FET) and [(F)C]methionine – will be available for preclinical studies in May. The list of available radiotracers will be gradually expanded according to the demands of the preclinical and translational imaging research projects. Parallel GMP production of the same tracers will be established for early-phase clinical trials.

Preclinical and Translational Imaging

PET imaging allows non-invasive assessment of specific biological processes, such as glycolysis, fatty acid synthesis, proliferation, redox, hypoxia, amino acid uptake, and protein and nucleotide synthesis (Fig. 2). Together with MRI, which provides functional and high-contrast soft tissue images, PET can monitor the effectiveness of novel cancer therapies and increase understanding of tumourigenesis at the molecular level. Our new facility will drive in vivo imaging research projects from preclinical models through to clinical implementation.

In 2017, the preclinical facility recruited new personnel and expanded equipment capabilities. A talented Senior Scientific Officer, Emma Johnson, has joined the group to support preclinical imaging studies, and we have installed an automatic gamma counter, cryomicrotome and multi-mouse anaesthetic platform for high-throughput tumour characterisation, complementing the non-invasive imaging available with our state-of-the-art NanoScan PET/MRI scanner. We redesigned, renovated and extended the laboratory to facilitate this increased workflow.
RESEARCH FACILITIES

Research Facilities support Beatson Institute research groups and University of Glasgow groups based on the Beatson site. This year there has been investment in major new equipment. The Flow Cytometry Facility acquired a BD FACSARia II Cell Sorter; the Histology Facility purchased a second Leica Bond RX autostainer for fully automated in situ hybridisations; the Molecular Technology Service has upgraded a Biomek FXP for RNA-seq library preparation; and Information Technology has significantly extended the capacity of the new main storage server. In addition, Laboratory Management have replaced several main pieces of core equipment, and Building Facilities have been active with a number of projects to upgrade laboratory areas.

Building Facilities
Alistair Wilson, Alex Kiemahan, John Trivet

Building Facilities manage the outsourced provision of catering and janitorial services. We provide maintenance support for the Institute buildings, manage alterations and refurbishments, and ensure that all statutory compliance issues with respect to buildings and systems are up-to-date. An online helpdesk is used as an effective means to log and react to calls for maintenance and repair.

This year, there have been projects to upgrade the PET imaging facility and create a connected suite of rooms to facilitate the workflow and accommodate additional equipment. There have also been a number of laboratory alterations to facilitate installation of new equipment and the creation of a new administrative office space.

Central Services
Margaret Laing (Supervisor), Elizabeth C retrospective, Dilkan Kahawela, Kirstie McPherson, Jonny Sawers, Lauren Ure, Linda Scott, Tracy Shields, Rose Steel, Robert Storey

Central Services perform a wide range of duties, including cleaning and sterilisation of reusable laboratory glassware, sterilisation of consumables and preparation of tissue culture solutions, bacterial culture media and Drosophila food. The team cleans and checks equipment such as centrifuge rotors, X-ray processors, water baths and pH meters. On a daily basis, it also stocks the tissue culture suites, and collects and autoclaves laboratory waste to make it safe.

Flow Cytometry
Tom Gilbey, Tim Harvey

Flow Cytometry combines the flow facilities of the Institute and the neighbouring Wolfson Wohl building to create a comprehensive service. We provide advice, assistance and education to researchers on all aspects of flow cytometry.

Pre-acquisition, users discuss their needs with facility staff to get advice on which instrument to use, the best combination of fluorochromes and the correct controls and gating strategy. Before working on their own, researchers are trained to use the analysers and templates are created to enable data acquisition. Due to their complexity, only facility staff members operate the cell sorters. Post-acquisition, facility staff can help researchers analyse their data using various software options.

The service has a number of cell analysers and sorters: BD FACScalibur, for simple flow experiments such as cell cycle and proliferation studies; BD FACSVerse, for most routine flow experiments, including analysis on 6- to 96-well plates; Attune Nxt (Applied BioSystems), for complex flow analysis, including analysis of rare events and red fluorescent proteins; BD Fortessa, recently acquired for complex flow analysis; BD FACSARia, for sorting red fluorescent proteins and identifying side populations in a sample; and BD FACSARia Fusion, enclosed in a class II safety cabinet for safely sorting samples such as virus-transfected cells and human primary cells.

Histology Service
Colin Nixon, Barbara Cadden, Brenda McGuire, Christine Whelanw, Fiona McGregor, Gemma Thomson, Mark Hughes, Sara Ghafoor, Shauna Currie Kerr, Vivenne Morrison, Wendy Lambie

The Histology Service processes a wide range of tissue samples and cellular material fixed in an array of different fixatives. Tissue samples are trimmed, processed and orientated in paraffin wax blocks for sectioning and staining. Three large-capacity, automated tissue processors allow large-scale, consistent processing, although specialised processing cycles can also be designed. Other material such as organotypic slice cultures, cell pellets, spheroids and agar plugs can also be processed to provide a wax block for sectioning and further investigation. Three large-capacity, automated tissue processors allow large-scale, consistent processing, although specialised processing cycles can also be designed. The facility has a Leica LMD6500 laser microdissection system that allows subpopulations of tissue cells to be obtained from histological slides under microscopic visualisation. Both DNA and RNA material can thus be retrieved from the tissue sections for downstream analysis. It also provides a fully automated, large-capacity Leica SCN400F slide scanner capable of capturing bright-field. When fixation is not required, we also offer a frozen section resource, allowing frozen tissue, embryos or cells to be sectioned and stained using routine histological, immunohistochemical or immunofluorescence methods. Both paraffin-embedded and frozen tissue can be sectioned for DNA/RNA investigation, PCR analysis and immunofluorescence staining.

We also offer a comprehensive immunohistochemistry service that includes access to a large repertoire of previously validated antibodies, three autostainers that allow consistent, high-quality staining, and training for researchers. New antibodies can be optimised to produce a working protocol that allows the antibody to be used on an autostainer or by a researcher. In addition, we provide in situ hybridisation using a reagent system designed to visualise cellular RNA targets in formalin-fixed, paraffin-embedded tissue sections and bright-field microscopy. Staining for this is performed on a Leica Bond RX autostainer, and specific probes can be purchased or designed to exact specifications by external companies such as Advanced Cell Diagnostics.

The facility has a Leica LMD6500 laser microdissection system that allows subpopulations of tissue cells to be obtained from histological slides under microscopic visualisation. Both DNA and RNA material can thus be retrieved from the tissue sections for downstream analysis. It also provides a fully automated, large-capacity Leica SCN400F slide scanner capable of capturing bright-field.
or high-quality digital images. This enables researchers to scan and store the data required, and to perform image analysis. The image analysis software also allows staining techniques to be scored using specifically designed algorithms. Finally, if required, mouse tissue microarrays (TMA) can be constructed using paraffin-embedded tissue blocks.

Information Services
Peter McHardy, Ian White
Information Services provides server support, hardware cover, an on-site helpdesk for 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 750 TB of online storage, with frequent 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 High Sierra 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, including iPhones, iPads and other smart phones.

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 local and production applications. The lab also allows us to provide virtual workstations for researchers with both high core counts and large amounts of RAM, making them ideal for mass spectrometry and computationally intensive applications. We currently provide virtual desktops for OS X users requiring access to Windows-based packages. We have rolled out wKaps for specific imaging, proteomics and metabolomics applications. Significant investment has been put into creating a managed infrastructure and procedures to allow us to run the service in a manner commensurate with ITIL. This is used as the foundation of our business continuity documentation and has led to the revision and ongoing improvement of many of our day-to-day working practices. Our intranet uses a content management system framework, allowing service managers and support departments the ability to easily upload forms and information for users.

A range of replacement hardware is stored on-site to allow fast repairs. A good selection of loan IT hardware, from USB drives to digital projectors, is held centrally. We provide video conferencing facilities, enabling conference calls between the Institute and other CRUK sites as well as many other locations. Audio-visual support services for large conferences have been provided at a number of external and 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 provides 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 with regard to fire risks is also managed. Health and safety processes are reviewed and monitored regularly, as are training needs, in order 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 staff attend a series of safety induction talks.

We also oversee equipment servicing, replacement and purchase. Servicing and maintenance of core equipment is essential and carefully managed and coordinated to ensure equipment breakdowns are kept to a minimum. Any equipment repairs are coordinated to ensure these are done 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. All outgoing orders are monitored 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 and to ensure best prices are used and orders comply with any requirements for import or regulatory requirements.

This year the stores facility welcomed Michael Dott to the team. The Stores facility stocks a wide range of consumables, with rapid re-stocking to ensure high-use materials are always available. This year the Stores team have worked hard, in conjunction with Finance, to improve the stock taking system, and the stock labelling system, to increase efficiency and to achieve better clarity on stock status. Items can be withdrawn on a self-service basis with automatic cost centre allocation. A porter service is run to deliver external orders to the researchers, while stores processes outgoing samples or materials for courier collection. We also negotiate free samples from suppliers to enable the scientific staff to assess new or alternative products. By maintaining a good relationship with suppliers, preferential pricing is obtained, especially for bulk orders. As a result of these 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.

Molecular Technology and Reagent Services
Billy Clark, Jillian Murray, Andrew Keith
The Molecular Technology Service provides routine sequencing of plasmids and PCR products on an Applied Biosystems 3130xl (16 capillary) Sequencer, which has 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 technologies, offering large-scale sequencing in a matter of hours. An Illumina NextSeq500 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. QC of libraries is done using a Qubit fluorometric quantification assay and an Agilent Tapestation 2200.

Small-scale DNA purification is performed on a Qagen 8000 Bioblotter. Researchers provide overnight bacterial cultures that are processed by the facility. 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. 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 data integrity and is increasingly requested by journals.

The Reagent Service ensures the servicing and fumigation of biological safety cabinets which is coordinated every six months with the engineer. The mycoplasma screening service offers testing of cells every three to four months. Newly imported cell lines are tested as soon as possible after arrival using a luciferase assay that detects mycoplasma enzymes. Hoechst staining to detect mycoplasma is coordinated every six months with the engineer. We also have a good relationship with suppliers to take advantage of any centralised agreements. We continue to provide a very popular large-scale DNA purification (maxiprep) service from bacterial cultures.

The facility prepares cell-derived matrices from T74 cells to order, stocks commonly used tissue culture medium and coordinates batch testing of serum. It provides a range of commonly used buffers, for example T1X BST 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 Medaclace (Integra Biosciences AG) to sterilise and dispense into the plates.
Imran Ahmad (page 16) Models of Advanced Prostate Cancer

Primary Research Papers


Peri-prostatic fat volume measurement as a potential predictive marker for progression of iRFP713 to track recombinase activity and tumour development in vivo. Cancer Res 2017; 7: 1837

Epigenetic aging signatures in mice livers are slowed by dwarfism, calorie restriction and rapamycin treatment. Genome Biol 2017; 18: 57

Other Publications

Marmorstein R, Adams PD. Epigenomics meets metabolism through histone-mediated histone H3.3 deposition by HIRA. Stem Cell Investig 2017; 4: 46

Tom Bird (page 42) Liver Disease and Regeneration

Primary Research Papers

Other Publications

Karen Blyth (page 80) Transgenic Models of Cancer

Primary Research Papers


Other Research


RESEARCH PUBLICATIONS (CONTINUED)

OJ, Blyth K, Veusden KH.


Other Publications


David Bryant (page 44)

Molecular Control of Epithelial Polarity

Primary Research Papers


Other Publications


Leo Carlin (pages 46 & 70)

Leukocyte Dynamics & Beatson Advanced Imaging Resource (BAIR)

Primary Research Papers


Seth Coffelt, (page 48)

Immune Cells and Metastasis

Primary Research Papers


Tumor matrix stiffness promotes metastatic cancer cell interaction with the endothelium. *EMBO J* 2017; 36: 2373–89

Other Publications

RESEARCH PUBLICATIONS (CONTINUED)

Justin Bower & Heather McKinnon (page 66)
Drug Discovery Unit

Gray CH, Konczal J, Mezina M, Ismail S, Bower J, Drysdale M.
A fully automated procedure for the parallel, multidimensional purification and nucleotide loading of the human GTPases KRas, Rac1 and Ral1. *Protein Expr Purif* 2017; 132: 75–84

Konczal J, Gray CH.
Streamlining workflow and automation to accelerate laboratory scale protein production. *Protein Expr Purif* 2017; 132: 160–3

Other Publications
MR@C Inhibitors UK Patent Application Number 1713318.2 filed 18th August, 2017
MR@C Inhibitors UK Patent Application Number 1713319.0 filed August 18th, 2017
MR@C Inhibitors UK Patent Application Number 1713318.1 filed August 18th, 2017

Jeff Evans (page 6)

Primary Research Papers
Transient tissue priming via ROCK inhibition uncouples pancreatic cancer progression, sensitivity to chemotherapy, and metastasis. *Sci Transl Med* 2017; 9: pii: eaai8504


Other Publications
Wilson RAM, Evans TRJ, Fraser AR, Nibbs RJ.

Eyal Gottlieb (page 20)
Tumour Metabolism

Primary Research Papers

Other Publications
Gottlieb E, Vossen KH.
One carbon, many roads. *Cell Death Differ* 2017; 24: 193–4

Danny Huang (page 22)
Ubiquitin Signalling

Primary Research Papers

Nomura K, Klejnot M, Kovalczik D, Hock AK, Sibbet GJ, Vossen KH, Huang DT.

Clinical features of serous retinopathy observed with cobimetinib in patients with BRAF-mutated melanoma treated in the randomized coBRIM study. *J Transl Med* 2017; 15: 146–54


Transient tissue priming via ROCK inhibition uncouples pancreatic cancer progression, sensitivity to chemotherapy, and metastasis. *Sci Transl Med* 2017; 9: pii: eaai8504


Danny Huang (page 22)
Laura Machesky (page 54)
Migration, Invasion and Metastasis

Primary Research Papers

Other Publications

Gillian Mackay (page 76)
Metabolism

Primary Research Papers


Jennifer Morton (page 78)
Preclinical Precision Pancreas

Primary Research Papers


RESEARCH PUBLICATIONS (CONTINUED)


Michael Olson  (page 58)  

Molecular Cell Biology  

Primary Research Papers  


Kevin Ryan (page 32)  

Tumour Cell Death  

Primary Research Papers  


Other Publications  


Other Publications  


Owen Sansom (page 60)  

Colorectal Cancer and Wnt Signaling  

Primary Research Papers  


**RESEARCH PUBLICATIONS (CONTINUED)**

Other Publications
Huels DJ, Sansom OJ. R-spondin Is More Than Just Wnt’s Sidekick. Dev Cell 2017; 41: 456–8


Pesse TJ, Durban VM, Sansom OJ. Defining key concepts of intestinal and epithelial cancer biology through the use of mouse models. Carcinogenesis 2017; 38: 953–65


Emma Shanks (page 74)

Functional Screening

Primary Research Papers


Douglas Stratdhe (page 82)

Transgenic Technology

Primary Research Papers


van de Lagemaat LN, Stanford LE, Pettis CM, Stratdhe D, Stratdhe KE, Elsegood KA, Fricker DG, Croning MD, Komiyama NH, Grant SG. Standardized experiments in mutant mice reveal behavioural similarity on 129S5 and C57BL/6J backgrounds. Genes Brain Behav 2017; 16: 409–18

Stephen Tait (page 34)

Mitochondria and Cell Death

Primary Research Papers


Other Publications

Other Publications


Saverio Tardito (page 36)

Oncometabolism

Primary Research Papers


Aleixei Vazquez (page 38)

Mathematical Models of Metabolism

Primary Research Papers


Other Publications


Sara Zanivan (pages 62 & 73) Tumour Microenvironment and Proteomics

Primary Research Papers


Other Publications


Reid SE, Zanivan S. Tumor stiffness extends its grip on the metastatic microenvironment. Mol Cell Oncol 2017; 4: e1372866


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 Jiska van der Reest from Eyal Gottlieb’s group. She has been using proteome-wide analyses of cysteine oxidation to reveal metabolic sensitivities to redox stress.

Theses


CONFERENCES AND WORKSHOPS

Cancer Research UK Beatson International Cancer Conference

‘Feeding the Beast’ – The Metabolic Landscape of the Tumour and its Host

2 – 5 July 2017
Bute Hall, University of Glasgow
Scientific Committee: Jim Norman, Eyal Gottlieb, Jurre Kamphorst, Oliver Maddocks, Saverio Tardito, Alexei Vazquez, Karen Vousden

Our 2017 conference focused on a topic that has huge potential to impact cancer treatment and prevention – the metabolic vulnerabilities of cancer cells, and how the interplay between tumour, stroma and systemic metabolism contributes to cancer progression. We were delighted to welcome Tak Wah Mak (Toronto) to the opening session to give the 10th Colin Thomson Memorial Keynote Lecture, sponsored by Worldwide Cancer Research (WCR), in which he addressed the controversial role of reactive oxygen species (ROS) in tumour development and responses to anti-cancer therapies. We also heard from Lewis Cantley (New York, sponsored by the Multidisciplinary Digital Publishing Institute) about new approaches to killing cancer cells using phosphoinositide 3-kinase inhibitors and by targeting the metabolic pathways that cancer cells utilise to suppress ROS.

There were a number of excellent presentations throughout the meeting, including selected short talks by Thales Papagiannakopoulos, Emmanuel Dormier, Ramon Klein Geltink, Constantinos Alexandrou, David Lewis, Celia Berkens, Naama Kanarek, Andres Mendez-Lucas, Liron Bar-Peled, Mattia Falcone (sponsored by WCR) and Anafath Najamudeen (sponsored by Advanced Celi Diagnostics). Transnetix supported the ‘Tumour Microenvironment’ session, and AMSBIO-Trevigen the poster session. The meeting was generously co-sponsored by Cancer Research UK and Worldwide Cancer Research.

Congratulations went to James Conway (Garvan Institute), who was awarded the AMSBIO-Trevigen best poster prize for his work entitled ‘Dual (FLIM)/PLIM imaging identifies hypoxic regions resistant to Pi3K-pathway targeted therapies in pancreatic cancer’, and Ramon Klein Geltink who won The Biochemist short talk prize for his presentation describing mitochondrial priming by CD28.

Talk the Niche 2018

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. Thus, following complete excision of the primary tumour, these disseminated cells lie dormant, only to later appear as frank metastases. It is clear therefore that we need new ways to identify primed metastatic niches, and to develop strategies for targeting these therapeutically, following resection of primary tumours. Our 2018 meeting (‘Talk to the Niche’ – Understanding the Biology of the Metastatic Niche, 1-4 July 2018) will directly address the nature of the metastatic niche. We will discuss 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 (see www.beatson.gla.ac.uk/conf for more details and to register). This meeting series is designed to be a relaxed and friendly one where delegates and invited speakers have a chance to interact on both a scientific and social basis.

Beatson Workshop

Understanding Tumour Immunology

3 - 4 September 2017
CRUK Beatson Institute, Glasgow
Scientific Committee: Seth Coffelt, Leo Carlin, Jim Norman

Tumour immunology is a topic of considerable interest to a number of groups at the Institute as well as within both the Glasgow and Edinburgh Cancer Research UK Centres. The focus of this meeting, led by junior group leaders Seth Coffelt and Leo Carlin, brought together a wide breadth of experts who investigate immune cell biology in both cancer and inflammatory disease with the intent of understanding immune cell behaviour throughout tumour development. The invited speakers spanned the gamut of innate and adaptive immune cells, and included keynote speaker Karin de Visser (NIK).

The meeting was generously sponsored by Zeiss (keynote speaker), Roche (Carola Ries’ talk, student travel bursaries), EACR (Miki de Palma’s talk, poster prizes), Eurogentec, Millenyi Biotec and BioLegend.

Scottish Microscopy Group Symposium 2017

We, and in particular the Beatson Advanced Imaging Resource (BAIR), were delighted to host this symposium at the Institute on 22 November 2017. The theme of the meeting was ‘resolution’ and consisted of presentations covering acoustic imaging, flow cytometry with microscopy, light microscopy, super-resolution microscopy and electron microscopy, including the Nobel Prize–winning technique of cryo-EM. There were also microscopy facility presentations, a public engagement lecture, demonstrations by Zeiss (Airyscan), Leica (TIRF) and OMEO (OMERO-figure), a trade show and an imaging competition.

Open Evenings

Two very well-subscribed open evenings were held at the Institute this year for school students, teachers, members of the public and Cancer Research UK supporters, one in March (during National Science and Engineering Week) and the other in September. 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 2017.

January
Andrew Tobin, Institute of Molecular Cell and Systems Biology, University of Glasgow

February
Jukka Westermarck, University of Turku and Åbo Akademi, Finland
Riccardo Fodde, Erasmus MC, Rotterdam, Netherlands

March
Santiago Zelenay, CRUK Manchester Institute
Pierre Mangin, EFS-Grand-Est, Strasbourg, France
Annemarie Meijer, Institute of Biology, Leiden University, Netherlands
Josephine Bunch, National Physical Laboratory, London

April
Fabricio Loayza, Netherlands Cancer Institute, Amsterdam

May
Distinguished Seminar Speaker:
Julian Downward, Oncogene Biology Laboratory, The Francis Crick Institute, London

June
Tracy Robson, Royal College of Surgeons in Ireland, Dublin
Victoria Cowling, Centre for Gene Regulation and Expression, University of Dundee

Distinguished Seminar Speaker
Jannie Borst, Netherlands Cancer Institute, Amsterdam

August
Robert Rottapel, Departments of Medical Biophysics and Immunology, University of Toronto, Canada
Thorin Brunner, University of Konstanz, Germany

September
Chris Maden, Faculty of Medicine, Lund University, Sweden
Brent Derry, Hospital for Sick Children, Toronto, Canada
Lluis Montoliu, National Centre for Biotechnology (CNB-CSIC), Madrid, Spain
Evan Reid, Cambridge Institute for Medical Research and Department of Medical Genetics, Addenbrooke’s Hospital, Cambridge

October
Richard Houlston, Institute for Cancer Research, London
Rob Sneldong, National Heart & Lung Institute, Imperial College London
Ernst Lengyel, University of Chicago Medicine, USA

November
Gerhard Attard, Cancer Research UK Clinician Scientist and Honorary Consultant, The Institute of Cancer Research and the Royal Marsden, Sutton

December
Eric O’Neill, CRUK/MRC Oxford Institute for Radiation Oncology
Aaron Schimmer, Princess Margaret Cancer Centre, Toronto, Canada
Daniel Longley, Queen’s University, Belfast
Francis Stewart, Center for Molecular and Cellular Bioengineering, Technische Universität, Dresden
The training and career development of students and staff is essential in our mission to support cancer research of the highest standard. Our aim is to continue to attract enthusiastic scientists and clinicians early in their careers to work with our established staff and to draw on their experience but also to spark new ideas in a stimulating research environment. As well as learning a very wide range of practical and technical skills, our junior researchers participate in all intellectual activities and present and discuss their own work at internal seminars and external meetings. We provide support and facilities of the highest standards and scientific interactions are encouraged by our international conference, workshops and seminars and by funding participation in external meetings.

PhD Studentships

The purpose of our clinician/graduate training programme is to give students and clinical fellows starting in research an opportunity to work in state-of-the-art laboratories. This enables them to assess and develop their research talents to the full, to decide whether a research career suits them and to use their period of graduate study as a springboard for their future career path. Our four-year studentships are designed to give graduates who show a strong aptitude for research the opportunity to complete substantial research projects resulting in very good publications. All students receive training in safe working practices, writing project reports, good practice in research and other transferable skills. Training also involves learning to be an independent scientist, and students participate fully in the intellectual life of the Institute, attending and giving seminars and actively contributing to scientific discussions. Students are also given the opportunity to present posters on their work at national and international conferences to enhance their network of scientific contacts.

Our students are registered at the University of Glasgow and are allocated primary and secondary supervisors who are jointly responsible for supporting and monitoring their performance and progress. The primary supervisor is responsible for developing the student’s 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 panel reviewers to assist them in reviewing their progress and training needs.

Postdoctoral Research Scientists and Fellows

We see postdocs as pillars of the research and intellectual activities of their own groups and of the Beatson Institute as a whole. The training programme is designed to promote the development of outstanding and dedicated junior scientists, and we hope that by the end of their tenure they will be ready to compete for an independent position. 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 a postgraduate student. At the discretion of their group leader, funding may be extended for two more years.

For further details on Studentships, Postdoctoral and other posts currently available, see our website www.beatson.gla.ac.uk.


The graduate students above are members of the Prostate Cancer UK Future Leaders Academy. From left to right: Mark Lawrence, Laura Lapcynyte, Declan Whyte, Sigrid Fey and Rachael Smith.
OPERATIONAL SERVICES

Finance and Human Resources
Director and Company Secretary:
Peter Winckles ACA DChA

Finance
Gary Niven CA, Richard Spankie CA, Nicki Koliatsas, Jacqui Clare, Karen Connor, Lynn Wilson, Patricia Wylie

Human Resources
Angela Stuart CIPD, Elaine Marshall CIPD

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 have continued to develop and improve the range of costing and management information required by operational managers. Key projects started during the year include the development of a five-year forecasting and scenario planning model and a restructure of the team to create a dedicated role focussed on budgeting and forecasting.

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 2017, the team commenced the Institute’s first employee engagement survey, the results of which are now informing the people strategy for the future. We also continued to roll out our First Line Manager Development Programme to continue our investment in developing line managers and improving performance.

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 (PA to the Director), Rebecca Gebbie, Eleanor Best, Barbara Lang, Sarah Price, Catriona Entwistle

The Administration team, headed by the PA to the Director, 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

The Research Management team consists of two former postdoctoral researchers who support 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
Peter Ray 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.
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
Support from Cancer Research UK is critical to the continuation of our scientific research and patient care, enabling us to pursue cutting-edge studies and translate our findings into real-world solutions.

Wellcome Trust
Karen Blyth
Breast Cancer Now
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
Pancreatic Cancer Research Fund
Michael Olson
Medical Research Council
Kevin Ryan
Astellas, Worldwide Cancer Research
Owen Sansom
Alexei Vazquez
Deutsche Forschungsgemeinschaft, European Community
Karen Vousden
European Community, Institute of Cancer Sciences, University of Glasgow
Peter Adams
BBSRC, Medical Research Council, Wellcome Trust
David Bryant
EssenBio, Royal Society
Seth Coifet
European Community, William Forrest Charitable Trust, Naito Foundation
Jurre Kamphorst
Rosetrees Trust
Daniel Murphy
Worldwide Cancer Research
Stephen Tait
BBSRC, Breast Cancer Now

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.

Amey Power Services Ltd
Maureen Andrews, in memory of her husband, Norman
J D Appleton
J & U K Bobbemon, in memory of R Neale
Margaret G Brown
Clyde Travel
Trina Corbett
Thomas Donaldson
Electa Chapter No.27 O.E.S
Jaimes Inglis Testamentary Trust
John S Forsyth
Laraine Fox
Ann Galloway, Annual Art Exhibition in Beauly
Ian Gordon and Ethical Financial Services (Scotland)
Anna Hamilton, in memory of her husband, Dessie
Louise Hector, in memory of Ann McLaughlin
Help Fife Animals
The Lady Bowlers of Hillpark Club
Anna Murray Hogarth
The Lady Bowlers of Hillpark Club
Sarah Percy
PMV Pharmaceuticals
James Port, on behalf of Mr Port’s wife, Elizabeth
Brian Potter and Mrs Potter
Patricia Rooney (Darrow)
Jacqueline Thomson, in memory of her father
Thornhill Gardening Society
Trinity Minor pic
West of Scotland Women’s Bowling Association
Mrs Jean Whiteford and Miss A Paterson
Elizabeth Wiggins, in memory of her father

Dr. David Lewis receives cheque from Mosshead Primary School pupils

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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 Nic Jones (Chair)
Director, Strategic Initiatives, MCRC

Mr Craig Anderson
Former Senior Partner, KPMG

Mr Kirk Murdoch
Chairman, Pinsent Masons, Scotland & Northern Ireland

Prof Anton Muscatelli
Principal of the University of Glasgow

Ms Roaslie Chadwick
Partner, Pinsent Masons

Mr Nigel Armitt
Chief Finance Officer, Cancer Research UK

Company Secretary
Mr Gary Niven
The Beatson Institute for Cancer Research

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

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

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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 (1099464), Scotland (SC041666) and the Isle of Man (1103).
Registered address: Angel Building, 407 St John Street, London E2 4AD

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