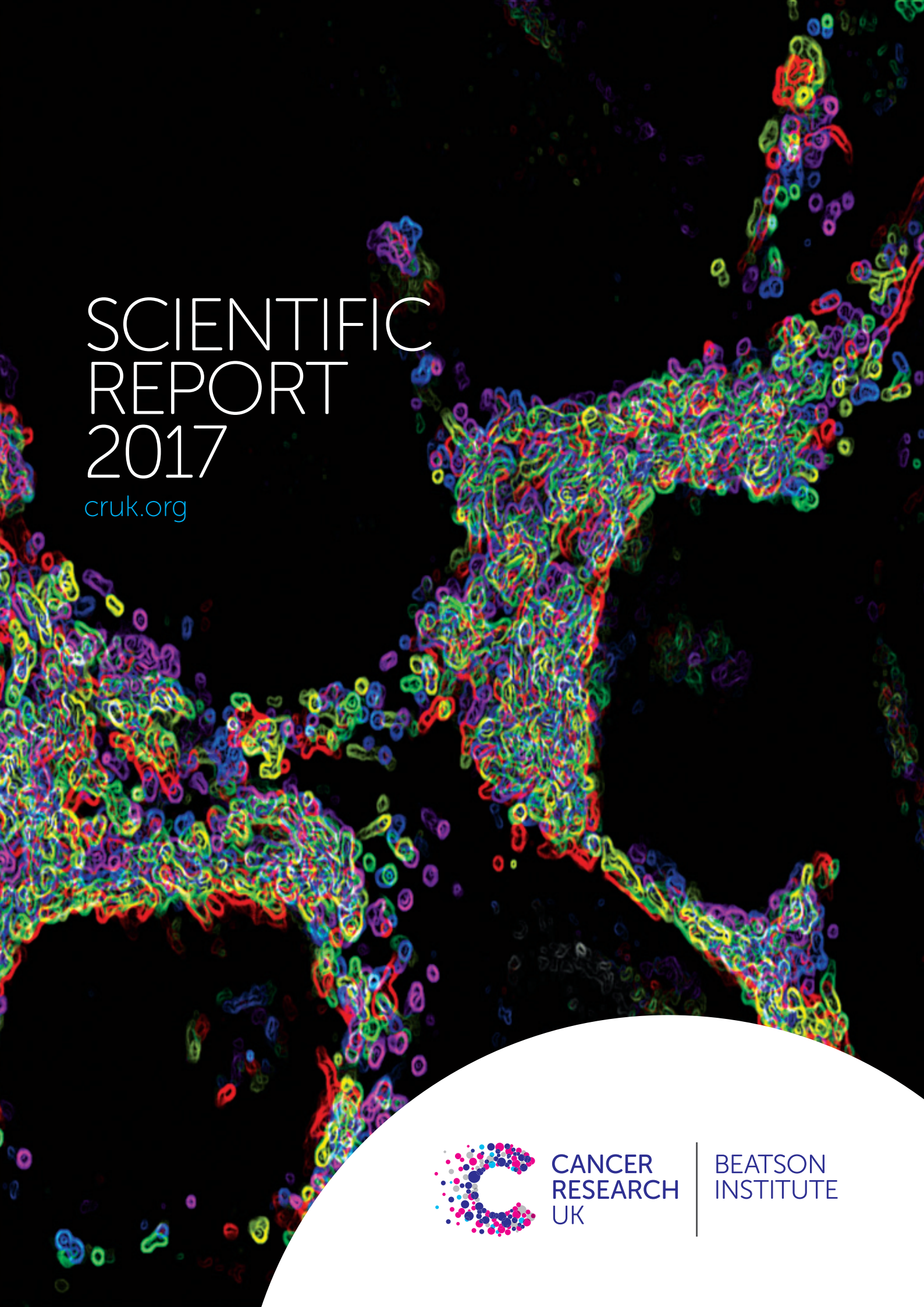


SCIENTIFIC REPORT 2017

cruk.org

CANCER RESEARCH UK

BEATSON INSTITUTE

COVER IMAGE

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.

SCIENTIFIC REPORT 2017

BEATSON INSTITUTE

University of Glasgow

CANCER RESEARCH UK | BEATSON INSTITUTE

Cancer Research UK Beatson Institute building

CONTENTS

DIRECTOR'S INTRODUCTION

Director of the Cancer Research UK Beatson Institute

Professor Owen Sansom FRSE, FMedSci

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 Biankin, 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 Morton, 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.

Researchers Drs Saadia Karim and David Vincent receive recognition for their research engagement activities

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/CSO 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 CRUK Flame of Hope Special Commendations 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 Gartnavel 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 McKinnon, 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 Olson 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 Kalna. 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.

INSTITUTE OF CANCER SCIENCES INTRODUCTION

Director of the Institute of Cancer Sciences

Jeff Evans FRCP Edin

This year, there were a number of highlights within the Institute of Cancer Sciences (ICS), University of Glasgow in line 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 centred 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 Biankin), 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 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 genomic sequencing and transcriptional profiling, as well as high-throughput screening, are being assessed both *in vitro* and *in vivo*. The consortium has developed a number of model systems including patient-derived xenografts 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 mutations *in vivo*, and for the preclinical evaluation of putative cancer therapeutics.

PRIMUS (Pancreatic CanceR Individualised 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-PDL-1 antibody, sponsored by AstraZeneca, which has completed recruitment and a randomised phase II study in unselected 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: Rob 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 oxaliplatin-based adjuvant chemotherapy for colorectal cancer; Jim Paul), which was included within a prospective, pre-planned, 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 (Mhairi Copland 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 unmet 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 precision-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 Carruthers 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 Giampazolias was awarded the prize for the best non-clinical PhD thesis. During 2017, Iain McNeish also left to undertake a prestigious position at Imperial College, London.

Publications listed on page 96

RESEARCH HIGHLIGHTS

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.

Dornier E, Rabas N, Mitchell L, Novo D, Dhayade S, Marco S, Mackay G, Sumpton D, Pallares M, Nixon C, Blyth K, MacPherson I, Rainero E, Norman JC.
Glutaminolysis drives membrane trafficking to promote cancer invasion. *Nat Commun* 2017; 8: 2255

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 increasing the extracellular levels of glutamate – which cells usually generate from glutamine but do not secrete – disrupts normal cell morphology and promotes an invasive phenotype. Furthermore, breast cancer cells – unlike normal ones – do release glutamate, which leads to an upregulation of matrix metalloprotease recycling and basement membrane disruption. The authors conclude that the key switch that makes a tumour more aggressive is it acquiring the ability to release glutamate extracellularly.

Gabrielsen M, Buetow L, Nakasone MA, Ahmed SF, Sibbet GJ, Smith BO, Zhang W, Sidhu SS, Huang DT.
A General Strategy for Discovery of Inhibitors and Activators of RING and U-box E3 Ligases with Ubiquitin Variants. *Mol Cell* 2017; 68: 456–70 e10

This study describes the generation of three ubiquitin variants, which the authors show, through various structural and biochemical studies, bind distinct E3 ubiquitin ligases and either inhibit or stimulate their enzymatic activity. Thus, they provide a general strategy for modulating E3 ligases, which are known to regulate a number of cellular processes and which are implicated in various diseases, including cancer. This could also be an important first step towards targeting these enzymes therapeutically.

Giampazolias E, Zunino B, Dhayade S, Bock F, Cloix C, Cao K, Roca A, Lopez J, Ichim G, Proics E, Rubio-Patino C, Fort L, Yatim N, Woodham E, Orozco S, Taraborrelli L, Peltzer N, Lecis D, Machesky L, Walczak H, Albert ML, Milling S, Oberst A, Ricci JE, Ryan KM, Blyth K, Tait SWG.
Mitochondrial permeabilization engages NF-kappaB-dependent anti-tumour activity under caspase deficiency. *Nat Cell Biol* 19: 1116–29

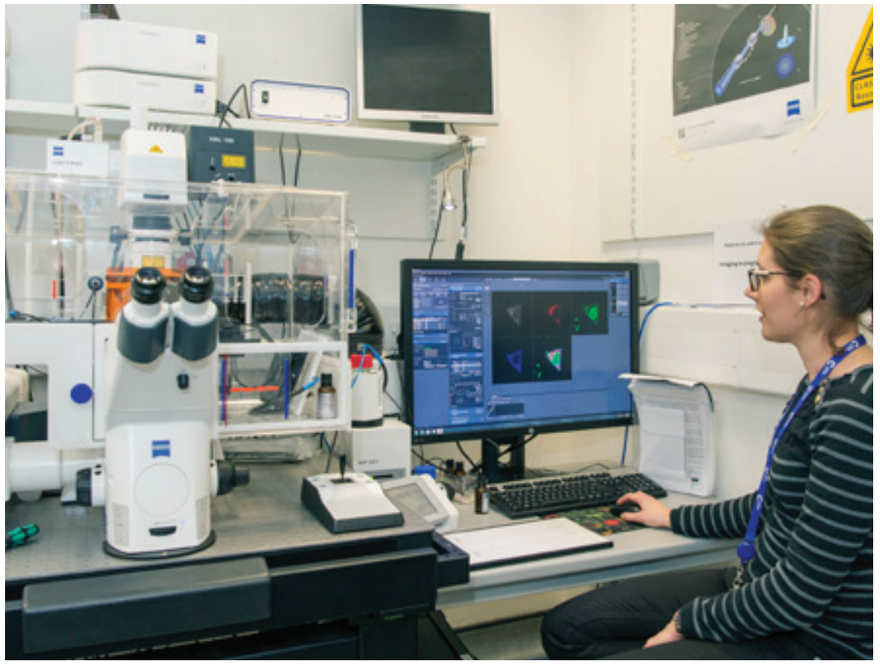
This paper describes work done by PhD student Evangelos Giampazolias and others to determine whether caspase-independent cell death (CICD) might be a better way to kill cancer cells than mitochondrial apoptosis, where caspase activity can lead to unwanted effects such as DNA damage. In fact, cells undergoing CICD display a potent pro-inflammatory phenotype dependent on NF-κB activity. Strikingly, engagement of CICD stimulates potent anti-tumourigenic effects *in vivo*, often promoting complete tumour regression in a manner dependent on intact immunity. In conclusion, the authors suggest that engaging caspase-independent cell death as an anti-cancer therapy warrants further investigation.

Gundry C, Marco S, Rainero E, Miller B, Dornier E, Mitchell L, Caswell PT, Campbell AD, Hogeweg A, Sansom OJ, Morton JP, Norman JC.
Phosphorylation of Rab-coupling protein by LMTK3 controls Rab14-dependent EphA2 trafficking to promote cell:cell repulsion. Nat *Commun* 2017; 8: 14646

This paper describes work done by PhD student Christine Gundry 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*.

Hernandez-Fernaud JR, Ruengeler E, Casazza A, Neilson LJ, Pulleine E, Santi A, Ismail S, Lilla S, Dhayade S, MacPherson IR, McNeish I, Ennis D, Ali H, Kugeratski FG, Al Khamici H, van den Biggelaar M, van den Berghe PV, Cloix C, McDonald L, Millan D, Hoyle A, Kuchnio A, Carmeliet P, Valenzuela SM, Blyth K, Yin H, Mazzone M, Norman JC, Zanivan S.
Secreted CLIC3 drives cancer progression through its glutathione-dependent oxidoreductase activity. *Nat Commun* 2017; 8: 14206

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.



Kuntz EM, Baquero P, Michie AM, Dunn K, Tardito S, Holyoake TL, Helgason GV, Gottlieb E.
Targeting mitochondrial oxidative phosphorylation eradicates therapy-resistant chronic myeloid leukemia stem cells.
Nat Med 2017; 23: 1234–40

Current treatments for chronic myeloid leukaemia (CML) successfully target differentiated cells but not leukaemic 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 their survival. Critically, they are also able to selectively eradicate LSCs both *in vitro* and in a xenotransplantation model of human CML by combining 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.

Maddocks ODK, Athineos D, Cheung EC, Lee P, Zhang T, van den Broek NJF, Mackay GM, Labuschagne CF, Gay D, Kruiswijk F, Blagih J, Vincent DF, Campbell KJ, Ceteci F, Sansom OJ, Blyth K, Vousden KH.
Modulating the therapeutic response of tumours to dietary serine and glycine starvation.
Nature 2017; 544: 372–6

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, Klejnot M, Kowalczyk D, Hock AK, Sibbet GJ, Vousden KH, Huang DT.
Structural analysis of MDM2 RING separates degradation from regulation of p53 transcription activity. *Nat Struct Mol Biol* 2017; 24: 578–87

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.

Sakamaki JI, Wilkinson S, Hahn M, Tasdemir N, O'Prey J, Clark W, Hedley A, Nixon C, Long JS, New M, Van Acker T, Tooze SA, Lowe SW, Dikic I, Ryan KM.
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)

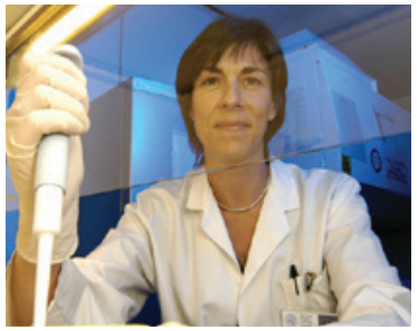
It is with the heaviest of hearts that we report the sad passing in November of our dear friend and much-valued colleague Dr Gabriela Kalna.

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 needn't have worried; 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 peppering 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 'Junior' and Viki, and her friends, of whom she had many.

Professor Tessa Holyoake (1963 - 2017)



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 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.

She was a key member of the fundraising campaign which raised in excess of £4 million towards building POG, which is recognised internationally as a centre of excellence for leukaemia research and houses the largest biobank of CML patient samples in the world. In addition to her role as Director of POG, she was a Consultant Haematologist at the Beatson West of Scotland Cancer Centre, where her main focus was to be an excellent and compassionate clinician.

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 (RSE) 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.

The name Beatson used in our title is in recognition of the early work of Sir George Beatson, who in 1912 established a research department at the cancer hospital in Glasgow. This department became independent from the hospital in 1967 when The Beatson Institute for Cancer Research was founded by the then Director, Dr John Paul. Dr Paul also raised sufficient funds to move the Institute in 1976 to our present location at Garscube Estate in Glasgow.

In 1990 Glasgow University researchers moved to adjacent refitted accommodation. More recently, other teams with University affiliations have moved here to share laboratory facilities with us and, in 2013, to the adjoining Wolfson Wohl Cancer Research Centre. The resulting Institute of Cancer Sciences provides a cutting-edge research environment situated in the beautiful, leafy green Garscube Estate on the north-western edge of Glasgow.

Sir George Beatson
1848 - 1933

Cancer Research UK
Beatson Institute

CANCER GROWTH AND METABOLISM

www.beatson.gla.ac.uk/imran_ahmad

MODELS OF ADVANCED PROSTATE CANCER

Group Leader

Imran Ahmad

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

Research Scientist
Laura Galbraith

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 Pparγ activation in metastatic prostate cancer

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

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

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

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

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

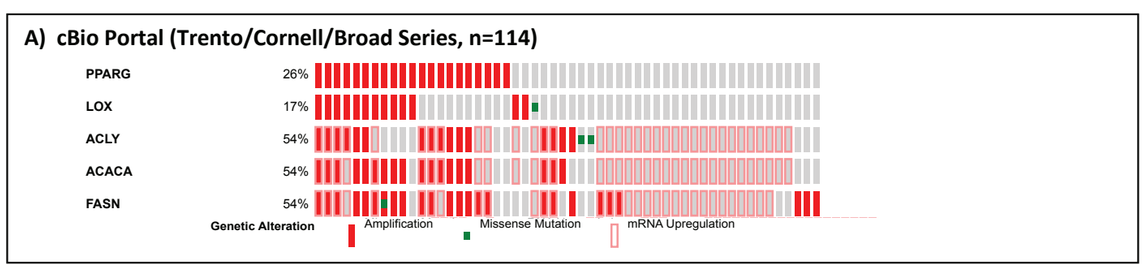
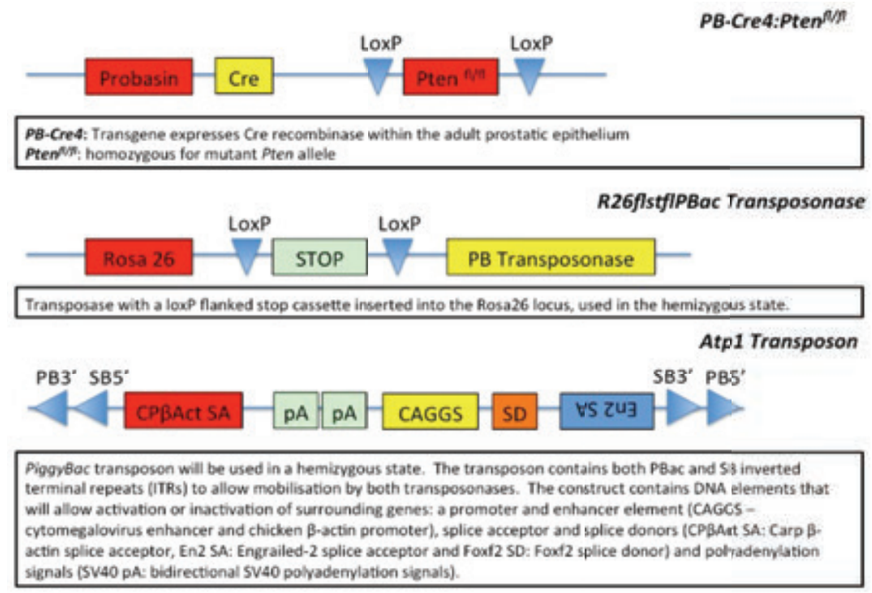


Figure 2
Genetic modifications of the *PiggyBac* mice.



Identification and Validation of New Therapeutic Targets in Castrate-Resistant Prostate Cancer

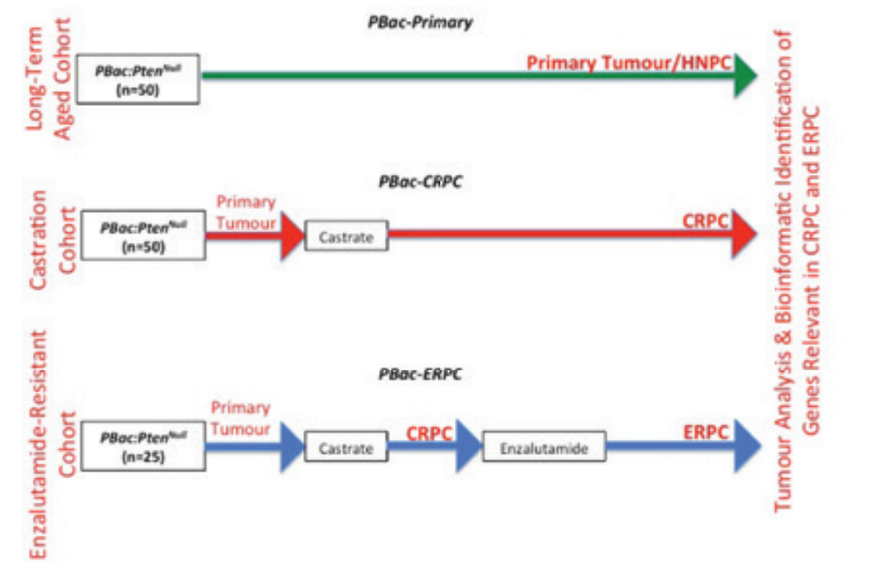
Androgen receptor aside, current treatment for advanced prostate cancer remains non-targeted. The development of targeted therapies has been hampered by a paucity of genes and pathways identified to be responsible for prostate cancer progression.

We aim to identify novel genes and pathways in castrate- and enzalutamide-resistant prostate cancer (CRPC and ERPC, respectively). We are using an unbiased insertional transposon mutagenesis screen (*PiggyBac*) and then validating the top genes of interest in patient-derived organoid cultures (Gao *et al.* Cell 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.

Publications listed on page 92

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



EPIGENETICS OF CANCER AND AGEING

Group Leader
Peter D. Adams

Research Scientists
Kathryn Gilroy
Maria Grazia Vizioli
Farah Jaber-Hijazi
Kristina Kirschner[1]
Francisco Marques
Arantxa Perez[2]
Neil Robertson

Scientific Officers
Claire Brock
Neil Fullarton[2]

Graduate Student
Ashley Newcombe[3]

[1]Wellcome Trust
[2]Medical Research Council
[3]BBSRC

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.

Research in our lab primarily focuses on the interface between ageing, epigenetics and cancer. We are particularly interested in age-associated epigenetic changes that lead to increased incidence of cancer with age (Fig. 1). In addition, we are planning systems biology–based approaches to comprehensively understand age-associated epigenetic changes and to exploit this knowledge to develop interventions to promote healthy ageing and suppression of age-associated diseases, including cancer. We are also exploiting epigenetics to develop novel drug combination therapies to combat cancer. We employ *in vitro* models, mouse models, human tissues and state-of-the-art analyses of large epigenomics datasets. We like to do collaborative, multidisciplinary research.

Cytoplasmic Chromatin Fragments (CCF) in senescent cells as drivers of inflammation via the cGAS/STING cytoplasmic DNA–sensing antiviral pathway

Cellular senescence is a potent tumour suppressor mechanism by virtue of proliferation arrest and the senescence-associated secretory phenotype (SASP), which promotes clearance of pre-malignant cells by the immune system. However, the mechanism responsible for initiation of SASP is unknown. In 2013, my lab first characterised and named CCF as fragments of chromatin expelled from the nucleus of senescent cells into the cytoplasm (Ivanov *et al.* J Cell Biol 2013; 202: 129–43). Then, in 2015, in collaboration with Shelley Berger's lab (Philadelphia), we showed that formation of CCF depends on the interaction between lamin B1 and autophagy adaptor LC3 in the nucleus, and that lamin B1 is a nuclear substrate of autophagy (Dou *et al.* Nature 2015; 527: 105–9). Most recently, again with Shelley Berger's lab, we have shown that CCF are sensed by the cytoplasmic DNA–sensing antiviral apparatus, cGAS and STING, and that this leads to activation of NF-κB and SASP in senescent cells.

A 'tug of war' between tumour suppressive, oncogene-induced senescence and oncogenic activated Wnt signalling in melanocytic neoplasia

The balance between these tumour suppressive and oncogenic activities determines the efficiency of senescence-mediated tumour suppression. For example, we showed that in oncogene-expressing melanocytes, a low level of activated Wnt signalling promotes benign nevus formation (Pawlikowski *et al.* Proc Natl Acad Sci U S A 2013; 110: 16009–14). However, a high level of activated Wnt signalling, caused by germline sequence variants, promotes giant congenital nevi in the form of congenital melanocytic nevus (CMN) syndrome (Pawlikowski *et al.* J Invest Dermatol 2015; 135: 2093–101). In a mouse model that closely recapitulates the human genetics, we showed that activated Wnt signalling and an activated *Ras* oncogene *(NRasQ61K)* cooperate to drive CMN syndrome, and that this is suppressed by acute postnatal treatment with MEK inhibitors (Pawlikowski *et al.* J Invest Dermatol 2015; 135: 2093–101). Based on these studies, our collaborator Veronica Kinsler has begun testing MEK inhibitors in babies afflicted by CMN syndrome (Kinsler *et al.* Br J Cancer 2017; 116: 990–3).

Ageing of the epigenome

Maintenance of cell phenotype and suppression of disease, including cancer, over the life course depends on a high level of epigenetic stability. However, since chromatin is inherently dynamic (Rai *et al.* Genes Dev 2014; 28: 2712–25), this steady-state stability likely reflects a challenge for the cell. Therefore, presumptive 'chromatin homeostasis' or 'chromostasis' mechanisms are predicted to actively maintain an epigenetic steady state over the life course, thereby suppressing age-associated disease (Rai *et al.* Genes Dev 2014; 28: 2712–25) (Fig. 2). We have shown that histone chaperone HIRA is one such factor that contributes to epigenetic stability in non-proliferating cells (Ye *et al.* Mol Cell 2007; 27: 183–96; Zhang *et al.* Dev Cell 2005; 8: 19–30). Recently, we reported the first DNA methylation clock in the mouse, and showed that diverse interventions – genetic, dietary and drug – that promote longevity of mice also suppress age-associated epigenetic changes and slow progression of this DNA methylation 'clock', i.e. enhance chromostasis.

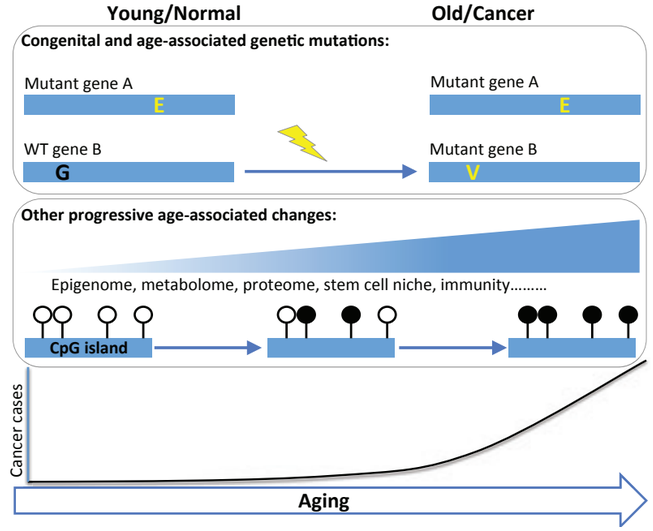


Figure 1
A model depicting the many factors proposed to contribute to the age-associated increase in cancer incidence. These include congenital genetic mutations and mutations acquired in early growth and development; genetic mutations acquired during ageing; and progressive age-associated changes to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

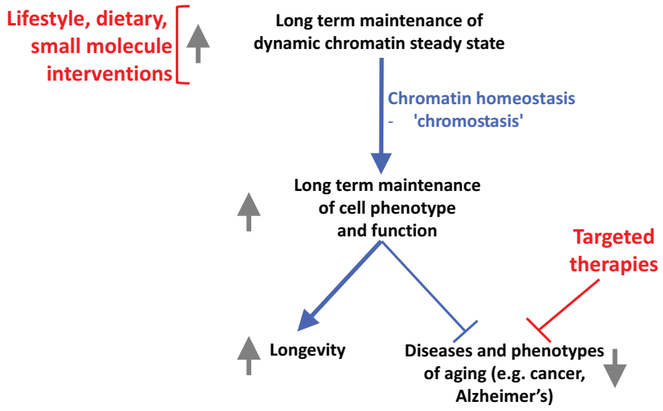


Figure 2
Presumptive 'chromostasis' confers epigenetic stability, healthy ageing and suppression of age-associated disease. Presumptive 'chromatin homeostasis' or 'chromostasis' mechanisms are predicted to actively maintain the dynamic epigenome at steady state over the life course, thereby suppressing age-associated disease. Diverse interventions – genetic, dietary, exercise and drug – that promote longevity, healthy ageing and suppression of disease are predicted to enhance chromostasis. Such preventative strategies can be an alternative to the current paradigm of targeted therapies for advanced disease. Strategies to enhance chromostasis – and other cellular homeostatic mechanisms – may prevent multiple diseases of ageing and be more successful than targeting advanced disease.

The epigenetic landscape of senescent cells

Cellular senescence is an irreversible proliferation arrest and pro-inflammatory phenotype triggered in primary cells by activated oncogenes and other molecular stresses. As a profound change in cell phenotype, the initiation and maintenance of senescence depends on reprogramming of chromatin, the epigenome and gene expression. Cellular senescence is a potent tumour suppressor mechanism. However, accumulation of senescent cells with age also causes tissue ageing, by blocking cell and tissue renewal and driving chronic inflammation. Indeed, in contrast to its acute tumour suppressive effects, chronic accumulation of inflammatory senescent cells is tumour promoting. In collaboration with Shelley Berger, we have mapped the distribution of several critical epigenetic regulators in proliferating and senescent cells, including DNA methylation, several histone modifications, histone variants and nuclear lamins. These collaborative studies have yielded critical insights into gene regulation in senescent cells (Rai *et al.* Genes Dev 2014; 28: 2712–25; Cruickshanks *et al.* Nat Cell Biol 2013; 15: 1495–506; Shah *et al.* Genes Dev 2013; 27: 1787–99), as well as the tumour suppressive and pro-ageing effects of senescent cells (Cruickshanks *et al.* Nat Cell Biol 2013; 15: 1495–506).

Structural and functional studies on the HIRA histone chaperone complex and its role in senescence-mediated tumour suppression

In collaboration with Ronen Marmorstein (Philadelphia), we have dissected the structure–function relationships between HIRA and its binding partners, UBN1, CABIN1 and ASF1a, and substrate histone H3.3 (Zhang *et al.* Dev Cell 2005; 8: 19–30). This included a crystal structure of the HIRA/ASF1a interaction surface and more recently the UBN1/histone H3.3 interaction surface (Tang *et al.* Nat Struct Mol Biol 2006; 13: 921–9). We were the first to describe the distribution of the HIRA complex across the mammalian epigenome (Pchelintsev *et al.* Cell Rep 2013; 3: 1012-9). In functional studies, we have demonstrated the role of this DNA replication–independent histone chaperone complex in the control of chromatin in non-proliferating senescent cells (Rai *et al.* Genes Dev 2014; 28: 2712–25). These studies have been facilitated by the mouse monoclonal and rabbit polyclonal antibodies that we have made to all subunits of the complex. More recently, we have generated the first conditional knockout mice of HIRA, UBN1 and CABIN1 and are using these to establish *in vivo* functions (Rai *et al.* Genes Dev 2014; 28: 2712–25). Of particular note, we have revealed a function for HIRA in promoting healthy ageing and suppression of cancer (Rai *et al.* Genes Dev 2014; 28: 2712–25).

Publications listed on page 92

TUMOUR METABOLISM

Group Leader
Eyal Gottlieb

Research Scientists
Simone Cardaci
Johan Vande Voorde

Scientific Officer
Elaine MacKenzie

Clinical Research Fellow
Henry Dabritz[1]

Graduate Students
Elodie Kuntz
Jiska van der Reest

[1]Beatson West of Scotland Cancer Centre Endowment Fund

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 showed 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 fulfil bioenergetic 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 neuroendrocrine 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 demethylases, thereby establishing, respectively, a pseudohypoxic and hypermethylator 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 *Sdhb*-ablated, 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 SDHB loss is 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 consume 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 (Cardaci *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

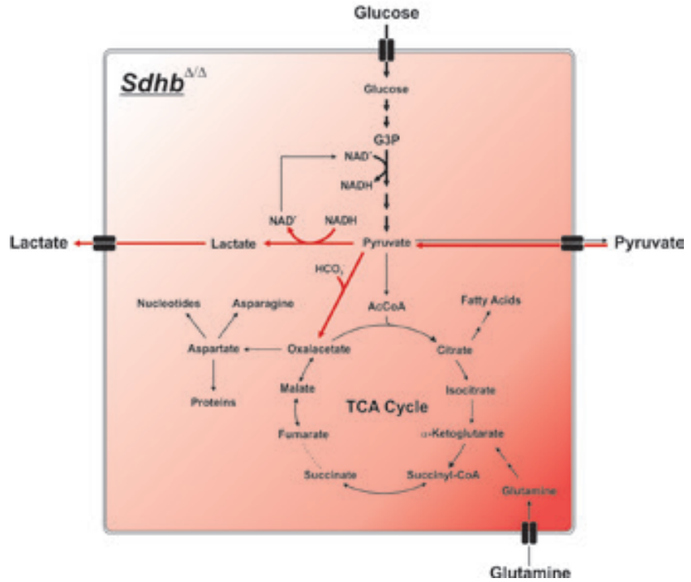


Figure 1
Schematic representation of metabolic rewiring observed in *Sdhb*-null cells
Lack of SDH activity commits cells to consume extracellular pyruvate, which sustains Warburg-like bioenergetic features. Moreover, pyruvate carboxylation diverts glucose-derived carbons into aspartate biosynthesis, thus sustaining cell growth of SDH-deficient cells. The size of arrows is proportional to the relative contribution/rate of the corresponding metabolic pathway. Red arrows indicate metabolic pathways required to support proliferation of *Sdhb*-null cells.

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 (^{13}C- and ^{15}N-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 carbons, 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-deprived cells. In both orthotopic glioblastoma mouse models and in patients, ^{13}C-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 (Tardito *et al.*, Nat Cell Biol. 2015; 17: 1556–68).

Publications listed on page 97

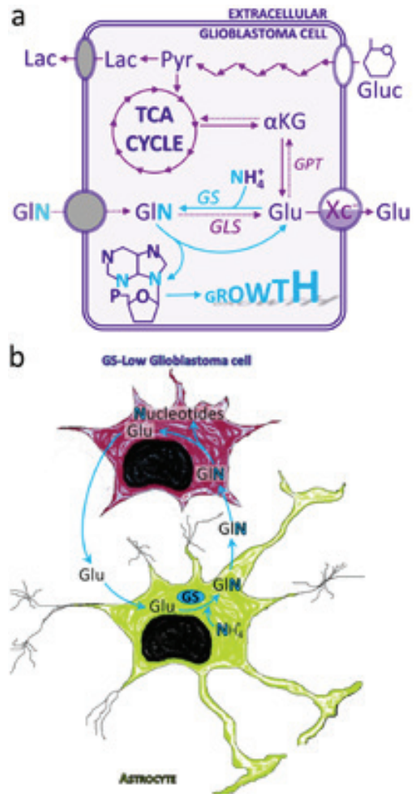


Figure 2
Glutamine metabolism in glioblastoma
(a) Glutamine-restricted glioblastoma cells utilise glucose-derived carbons to produce alpha-ketoglutarate (solid arrows). This keto acid is transaminated to glutamate, mostly by the glutamate pyruvate transaminases (GPT). Glutamate is then either secreted by the Xc^- antiporter or converted to glutamine by glutamine synthetase (GS). GS supplies glutamine to nitrogen-demanding catabolic pathways essential for growth, such as nucleotides biosynthesis. **(b)** A proposed model for a novel tumour–glia interaction. Glioblastoma cells expressing low levels of GS rely on the glutamine produced and released from GS-positive astrocytes to synthesise nucleotides for growth under glutamine-restricted conditions.

UBIQUITIN SIGNALLING

Group Leader
Danny Huang

Associate Scientist
Lori Buetow

Research Scientists
Mads Gabrielsen[1]
Karolina Majorek[1]
Mark Nakasone[1]
Feroj Syed

Scientific Officer
Gary Sibbet

Graduate Students
Dominika Kowalczyk
Helge Magnussen[1]
Chatrin Chatrin

[1]ERC

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 Mg^{2+} 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 intermediate (~ indicates the thioester bond). E3 plays a pivotal role in determining substrate fate. In general, E3 consists of an E2-binding module (HECT, RING or U-box domain) and a protein-protein interaction domain that can recruit the substrate directly or indirectly. With this configuration, E3 recruits E2~Ub and the substrate to promote Ub transfer from the E2 to a lysine side chain on the substrate. In humans, the Ub pathway consists of two E1s, ~30–40 E2s and ~600 E3s that collectively ubiquitinate thousands of different substrates. Our group is interested in understanding the regulation and mechanistic functions of RING E3s, with a particular focus on RING E3s that have been linked to cancer.

Ligase-independent function of MDM2 in limiting p53 activity

MDM2 is a RING E3 that plays a critical role in the regulation of the p53 tumour suppressor protein by inhibiting p53's transcriptional activity and targeting it for proteasomal degradation. MDM2 contains a C-terminal RING domain, which dimerises with itself or with an inactive RING domain from MDMX to form active MDM2 homodimer or MDM2-MDMX heterodimer, respectively. Mouse model studies have shown that both complexes have non-redundant roles in the inhibition of p53 activity, as loss of Mdm2 or MdmX leads to embryonic lethality at different developmental stages. However, it remains unclear how both complexes regulate p53, as it is difficult to separate their activities in cells. MDM2 inhibits p53 activity by binding to p53 via its N-terminal domain and other regions and recruits an E2~Ub conjugate via its RING domain to ubiquitinate p53. Small-molecule inhibitors targeting MDM2's N-terminal p53-binding domain have been developed, but these compounds exhibit high toxicity due to high levels of p53 activity, thereby limiting their efficacy. Here we investigated the effects of inhibition of the RING domain on p53 transcriptional activity.

We determined a 2.4 Å crystal structure of MDM2-MDMX RING dimer bound to an E2 UbcH5B covalently linked to Ub. The structure reveals the mechanism of E2~Ub activation by the MDM2-MDMX heterodimer and provides a rationale for how the MDM2 homodimer binds and activates the E2~Ub complex. Guided by the crystal structure, we designed MDM2 mutants that prevent E2~Ub binding without altering the RING domain structure. These mutants lost MDM2's E3 activity and were unable to ubiquitinate and degrade p53. However, they retained the ability to bind p53, thereby limiting p53's transcription activity. Cells expressing these mutants retained basal p53 levels and

Figure 1
Enzymatic cascade for Ub modifications

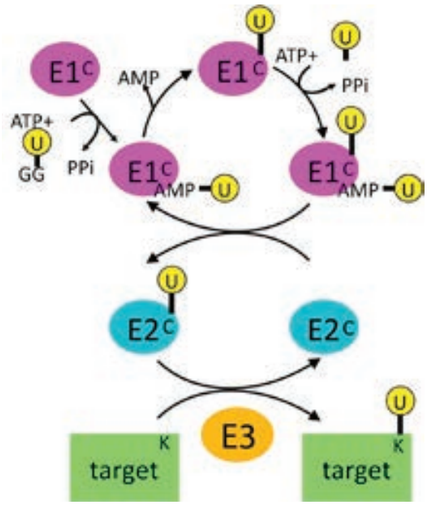
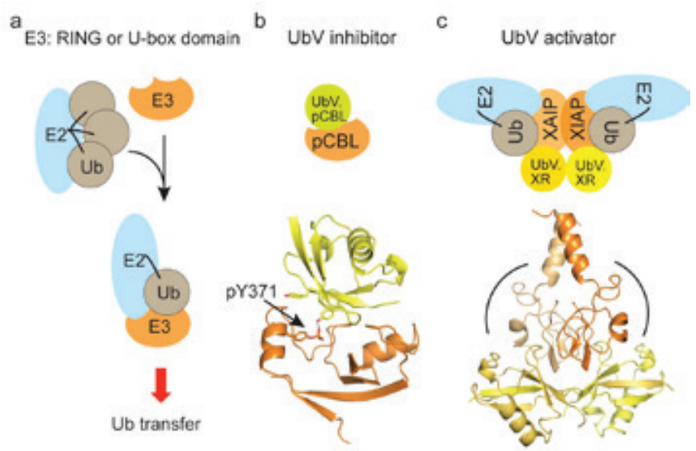


Figure 2
Targeting the RING domains
(a) Activation of the E2~Ub complex by the RING or U-box domain. The RING or U-box domain binds the E2~Ub complex and promotes the formation of the closed E2~Ub complex to facilitate Ub transfer. **(b)** UbV.pCBL functions as an inhibitor. It binds the E2~Ub binding surface of the pCBL RING domain and competes against E2~Ub binding. The crystal structure of the pCBL RING domain bound to UbV.pCBL reveals that phosphoTyr371 interacts with Tyr68 from UbV.pCBL. **(c)** UbV.XR functions as an activator. It dimerises and binds to a surface on the XIAP RING domain dimer that is remote from the E2~Ub binding site. UbV.XR contacts the closed E2~Ub conformation and further stabilises it 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 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 Zn^{2+} 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 modulation of the ubiquitin ligase activity could be a suitable approach for targeting RING E3s.

In collaboration with Professor Sachev Sidhu's lab at the University of Toronto, we have utilised a phage-displayed ubiquitin variant (UbV) library to screen for UbV(s) that bind selectively to the RING or U-box domain. The UbV library contains native Ub sequence that was randomised to generate billions of ubiquitin variant sequences. We identified three UbVs (UbV.E4B, UbV.pCBL and UbV.XR) that bind selectively to the RING or U-box domain of monomeric UBE4B, phosphorylated active CBL, and dimeric XIAP, respectively. We showed that UbV.E4B and UbV.pCBL function as inhibitors that bind selectively to the E2~Ub binding surface on the U-box domain of UBE4B and the RING domain of pCBL, respectively, thereby blocking E2~Ub binding. Interestingly, UbV.pCBL was selective only against Tyr371-phosphorylated CBL; the binding specificity was revealed in the crystal structure (Fig. 2b). Furthermore, cell-based analyses showed that UbV.E4B inhibits UBE4B-mediated p53 ubiquitination and UbV.pCBL inhibits CBL-catalysed EGFR ubiquitination in an EGF-dependent manner, a condition that leads to Tyr371 phosphorylation of CBL. Inhibition of EGFR ubiquitination resulted in EGFR stabilisation, decreased EGFR accumulation in early endosomes, and prolonged downstream signalling events.

In contrast to UbV.E4B and UbV.pCBL, UbV.XR binds XIAP dimeric RING domain and activates the ligase activity. The crystal structure of XIAP-UbV.XR showed that UbV.XR binds to a region in XIAP dimeric 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 UbV technology in the identification of inhibitors and activators of RING/U-box E3s.

Publications listed on page 97

CANCER METABOLOMICS

Group Leader
Jurre Kamphorst

CRUK Career
Development Fellow

Research Scientists
Francesca Romana Auciello
Vinay Bulusu
Sergey Tumanov

Scientific Officer
Jacqueline Tait-Mulder

Graduate Students
Grace McGregor[1]
Evdokia Michalopoulou

[1]joint with Owen Sansom,
supported by Rosetrees Trust

Lipids are a diverse class of biomolecules that are involved in tumour onset and progression. From a metabolic perspective, they are amongst 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 PIP_3 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 mainly 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. 2014; 2: 23). 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 cells actually consume 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 next investigated heavy (^{13}C) 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. 1). 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.

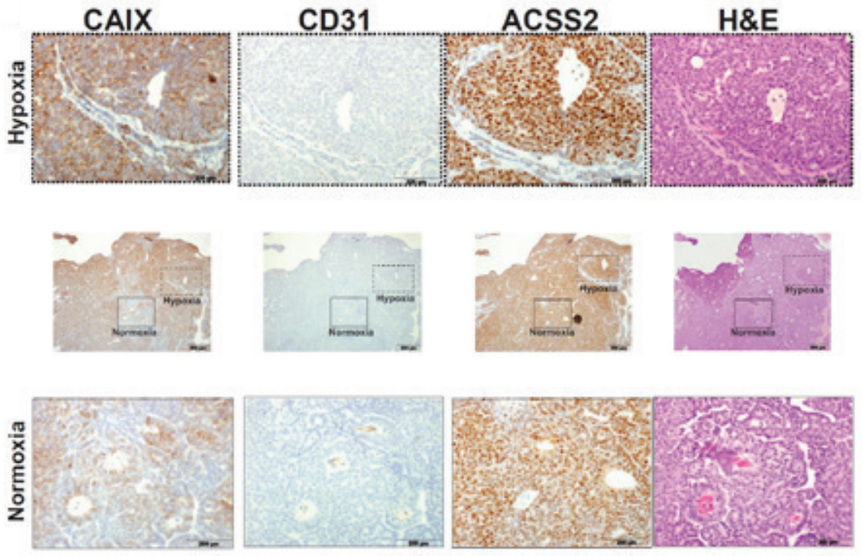


Figure 1
ACSS2 is prominently nuclear in hypoxic tumour regions
Immunohistochemical staining of serial sections from a representative tumour of the MMTV-PyMT mouse model for carbonic anhydrase 9 (CAIX, a hypoxic marker), CD31 (a marker for blood vessels), ACSS2 and H&E staining. ACSS2 is especially prominent in the nuclei of hypoxic and nutrient-deprived tumour cells, where it prevents loss of carbon and maintains histone acetylation to promote survival.

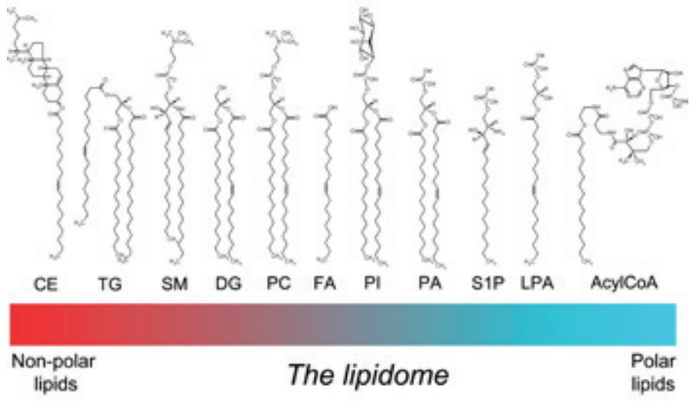


Figure 2
Lipids are structurally diverse
Examples of very apolar lipids (cholesterol esters, triglycerides) to relatively polar lipids (lysophosphatidic acid lipids, acyl-CoAs). We are developing a platform for their comprehensive analysis based on two extraction procedures and separation methods. Abbreviations: CE, cholesterol ester; TG, triglyceride; SM, sphingomyelin; DG, diglyceride; PC, phosphatidylcholine; FA, fatty acid; PI, phosphatidylinositol; S1P, sphingosine-1-phosphate; LPA, lysophosphatidic acid.

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 spectrometry–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 BIOLOGY

Group Leader
Hing Leung

Associate Scientist
Rachana Patel

Research Scientist
Arnaud Blomme

Scientific Officers
Ee Hong Tan
Catriona Ford

Research Associates
Vicky Harle[1,2]
Carolyn Loveridge[3]
Peter Repiscak[2]
Linda Rushworth[2]

Clinical Research Fellows
Chara Ntala
Mark Salji

Graduate Student
Rafael Sanchez Martinez[4]

Technician
Ernest Mui[2]

[1]CRUK Clinician Scientist Fellowship
[2]Prostate Cancer Foundation Challenge Award
[3]Prostate Cancer UK
[4]Horizon 2020 Innovative Training Network Early Stage Researcher

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 Zanivan, quantitative proteomic analysis was performed to study the proteome of the tumours. We identified two candidate proteins that were potently 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 orthograft 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

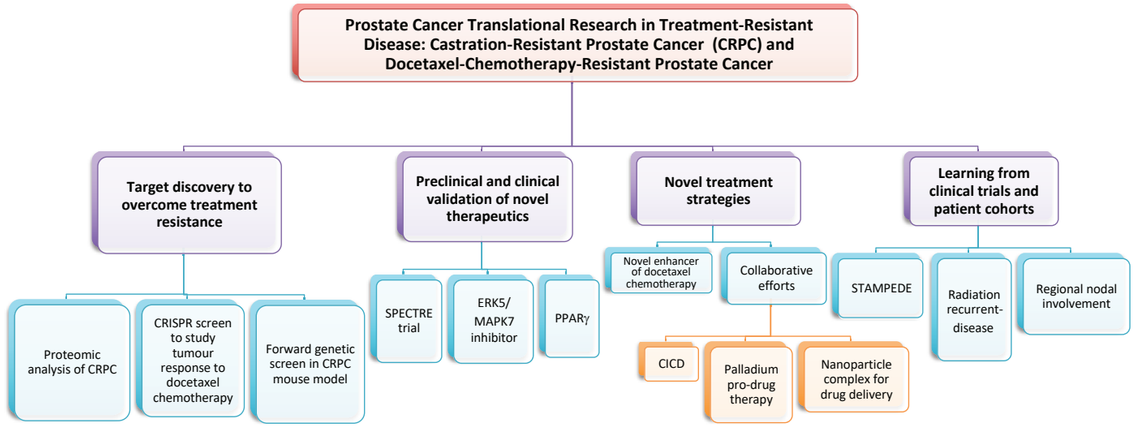
SPECTRE is a proof-of-concept efficacy clinical trial based on our recent publication (Patel *et al.*, 2017), in which we highlighted the importance of tumoural cholesterol uptake by castration-resistant prostate cancer in testosterone *de novo* synthesis.

We have previously proposed ERK5 as a potential target for therapy. Using a genetically modified mouse model of *Pten*-loss-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 II/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 Dufes, University of Strathclyde).

Learning from clinical trials and patient cohorts

We successfully applied archival diagnostic materials from the STAMPEDE clinical trial for next-generation RNA sequencing to interrogate the transcriptome of patients receiving chemotherapy within the STAMPEDE trial. Our timely study benefits from the recent publication of (modest) survival benefit combining hormone and chemotherapy upfront at the time of diagnosis (James *et al.*, Lancet 2016; 387: 1163–77). We are working hard to develop a molecular signature to identify patients who would respond unfavourably and therefore may benefit from additional or alternative treatment.

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

MOLECULAR IMAGING

Group Leader
David Lewis

Research Scientist
Piotr Dzien

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.

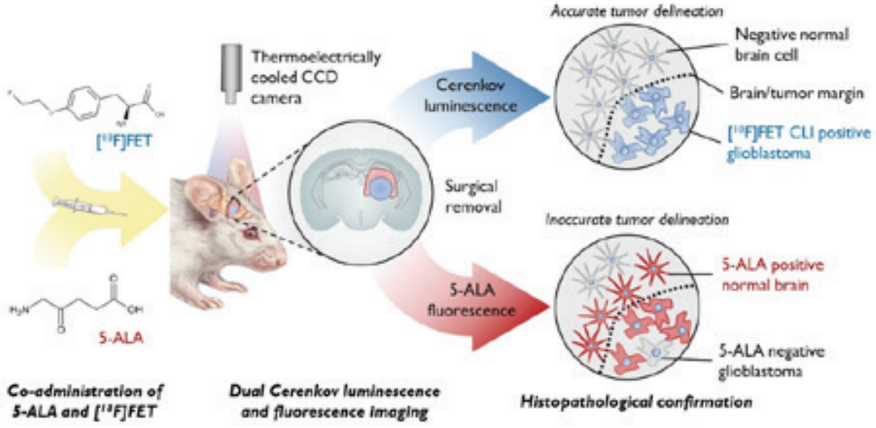


Figure 1
Tumour imaging with the radiolabelled amino acid [^{18}F] fluoro-ethyl-tyrosine (FET), which is taken up by glioma cells allowing accurate, sensitive and specific detection pre-operatively using PET/MRI and intra-operatively using Cerenkov luminescence imaging (CLI). CLI with FET performs better than guiding resections with fluorescence derived from 5-ALA, which is the current gold standard for visualising glioblastoma during surgery.

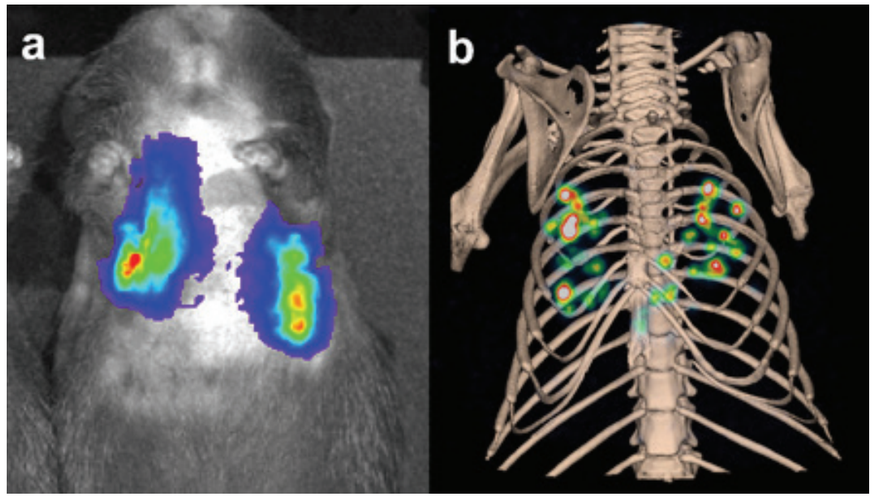


Figure 2
Oncogenesis can be imaged by using reporter genes, but this has so far been limited to optical reporters such as luciferase.
(a) Optical measurements are planar and surface weighted, and photon scatter and absorbance make quantitative tomographic measurements challenging.
(b) These limitations are removed by the use of radionuclide imaging reporters such as the sodium iodide symporter; higher-energy photons allow sensitive 3D imaging of tumour development. This figure shows the improved resolution for lung tumour imaging in the $KRAS^{G12D/+}$; $p53^{-/-}$ (KP) mouse with [^{99m}Tc] TcO_4^- SPECT/CT imaging after intranasal lentiviral LV-PGKCre-EF1NIS infection.

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 germline 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.

Publications listed on page 99

www.beatson.gla.ac.uk/daniel_murphy

ONCOGENE-INDUCED VULNERABILITIES

Group Leader

Daniel J. Murphy

Research Scientists
Katarina Gyuraszova
Bjorn Kruspig
Nathiya Muthalagu[1]

Graduate Students
Sarah Laing
Tiziana Monteverde
Jennifer Port
Declan Whyte[2]

[1]Worldwide Cancer Research, with Owen Sansom
[2]Prostate Cancer UK Future Leaders Academy

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

Our overarching hypothesis is that such oncogene-induced biological perturbations can be exploited for cancer therapy, even in the absence of direct suppression of driver oncogenes. We use deregulated MYC as our paradigm oncogene coupled with a mixture of candidate and RNAi-based screening approaches to identify induced vulnerabilities *in vivo* and *in vitro* and are actively exploring several strategies for selective elimination of cells that overexpress MYC.

MYC in cancer

Overexpression of the transcription factor MYC occurs in a huge number of human cancers arising from almost every tissue type. MYC overexpression may arise from focal or broad chromosomal amplification, gene translocation, enhanced mRNA and protein stability or indeed increased signalling through upstream regulatory factors such as Ras, Notch or 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 glucose breakdown, and in parallel induces expression of glutamine transporters and exploits this pathway to maintain the citric acid cycle. The energetic strain that MYC deregulation thus places upon the cell is evident in progressive activation of the AMP-activated protein kinase AMPK, which plays a key role in maintaining energetic homeostasis. AMPK in turn inhibits TORC1 to attenuate the rate of macromolecular synthesis, effectively allowing cells to balance the rate of ATP consumption with that of ATP production. Importantly, the AMPK-related kinase ARK5/NUAK1 is also required for maintenance of ATP homeostasis in cells wherein MYC is overexpressed. NUAK1 plays a specific role in MYC-dependent activation of AMPK and also maintains mitochondrial respiratory capacity. Suppression of NUAK1 thus impairs the ability of MYC-overexpressing cells to respond to declining ATP levels while simultaneously depriving cells of ATP-generating capacity, suggesting that suppression of NUAK1 may be an effective means to selectively kill cancer cells with high levels of MYC expression.

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

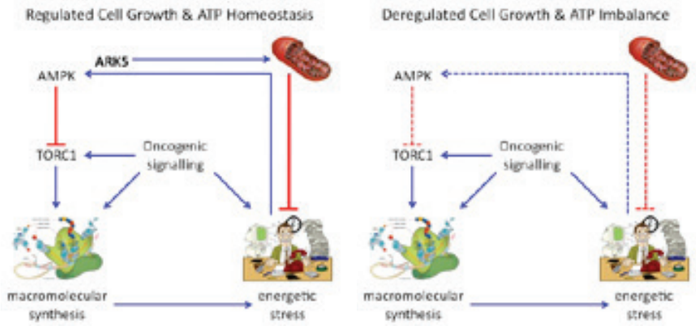


Figure 1
Induced dependencies need not reflect direct molecular interactions. Oncogene-induced cell growth, typically requiring signal transduction via the mechanistic target of rapamycin (mTOR) pathway, drives rampant ATP consumption, which must be compensated for through increased cellular intake of fuel (e.g. glucose, amino acids and fatty acids) combined with AMPK-mediated attenuation of macromolecular synthesis. Upon suppression of Ark5, this feedback mechanism is impaired, leading to ATP depletion and bioenergetic catastrophe. In principle, any intervention that similarly impairs bioenergetic homeostasis may selectively kill tumour cells.

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

Oncogene cooperation during lung cancer progression

Lung cancer remains one of the deadliest forms of cancer worldwide, accounting for some 18% of all cancer-related deaths, and the incidence of lung cancer is on the rise, especially in the increasingly industrialised and densely populated cities of emerging economies. Poor prognosis arises in large part from the combination of late disease detection and limited matching of patients with emerging targeted therapies. We have found that modestly elevating MYC levels in a KRAS-driven model of lung cancer is sufficient to drive progression to metastatic disease. This progression arises in part through increased transcription of promiscuous ERBB family ligands. We have identified an unexpected requirement for signal transduction through the ERBB receptor tyrosine kinase network for both establishment and maintenance of KRAS mutant lung cancer. Our data suggest that KRAS-driven tumours actively seek ways to amplify signalling through the RAS pathway in order to sustain the tumour phenotype. As there are presently no clinically proven small molecule inhibitors of KRAS, our observation raises the exciting possibility that simultaneously inhibiting signalling components upstream and downstream of KRAS with existing therapeutic agents may benefit the very large number of lung cancer patients whose disease is driven by mutant KRAS.

Oncogene cooperation in pancreatic cancer

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

Major developments in 2017

A major development was a successful application, spearheaded by our colleague Jennifer Morton, to establish a Pancreatic Cancer UK–funded 'future leaders in pancreatic cancer' postgraduate academy of five PhD students, resulting in Declan Whyte commencing his studies in my group. At the other end of the PhD journey, Jennifer Port and Tiziana Montevderde both completed their studies and submitted their respective theses in September. Tiziana subsequently moved to the CRUK Manchester Institute, joining the group of Michela Garofalo, while Jennifer secured a scientific writing position in the Netherlands. We embarked on an exciting new direction to develop new *in vivo* models for mesothelioma, buttressed by collaboration with the MRC Toxicology groups in Leicester, 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*. We additionally 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

www.beatson.gla.ac.uk/kevin_ryan

TUMOUR CELL DEATH

Group Leader

Kevin Ryan FRSE

Research Scientists
Florian Beaumatin[1]
Jaclyn Long[2]
Jun-ichi Sakamaki[2]
Barbara Zunino[3]

Scientific Officer
James O'Prey

Graduate Students
Valentin Barthet
Martina Brucoli
Christoph Nössing
Pablo Sierra Gonzalez[3]

[1]Worldwide Cancer Research
[2]Astellas Pharma Inc
[3]left during 2017

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 viability by preserving cellular integrity 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.

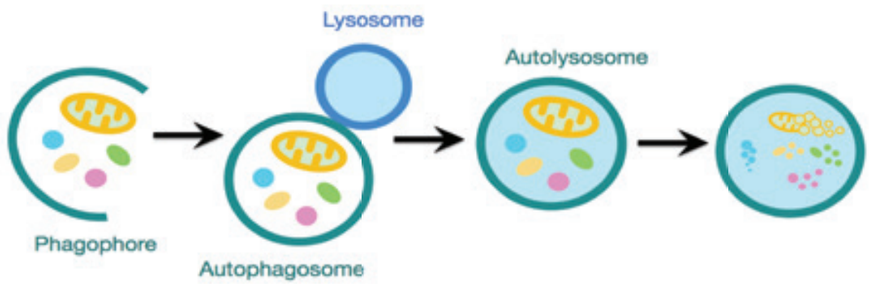


Figure 1
The process of macroautophagy
Within the cytoplasm of cells, membranes nucleate and grow to encapsulate cargoes in double-membraned structures called autophagosomes. Ultimately, autophagosomes fuse with lysosomes to form an autolysosome, within which the cargo is degraded by acidic hydrolases. The breakdown products are then recycled into the cytoplasm where they are either further catabolised or recycled into biosynthetic pathways.

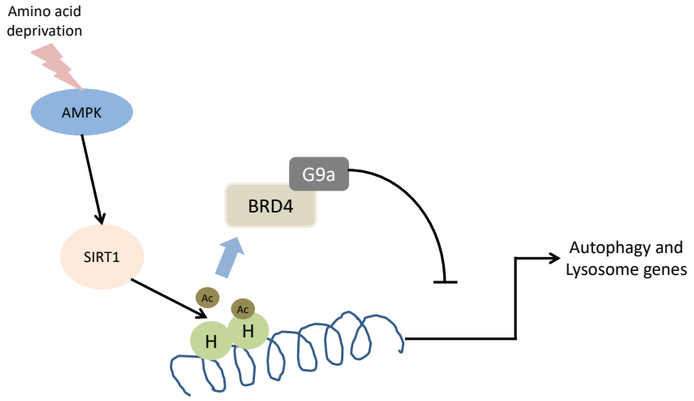


Figure 2
BRD4 represses expression of genes involved in autophagy and lysosome function. Amino acid deprivation signals histone deacetylation via AMPK and SIRT1. This causes displacement of the BRD4-G9a complex from chromatin, resulting in de-repression of genes involved in autophagy and lysosome function. H, histones; Ac, acetylation.

BRD4 is a chromatin reader protein which binds to acetylated histones. The protein then recruits proteins which can modulate gene expression, such 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 through the deacetylation of histones. This response involves the histone deacetylase 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 chromosome translocation involving *BRD4* and a gene called *NUT* produces a fusion protein called BRD4-NUT, which is considered the 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 cytoprotective, 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 Regulatory 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* (two genes essential for macroautophagy) 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 Verapamil – 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 Verapamil resulted in an enhanced cytotoxic effect, indicating that combinations of Verapamil with autophagy inhibitors is worthy of further investigation.

Publications listed on page 104

www.beatson.gla.ac.uk/stephen_tait

MITOCHONDRIA AND CANCER CELL DEATH

Group Leader
Stephen Tait

Research Scientists
Florian Bock
Kirsteen Campbell[1]
Kai Cao[2]
Joel Riley[3]

Scientific Officer
Cat Cloix[2]

Clinical Research Fellow
Anna Koessinger[4]

Graduate Students
Alba Roca[2]
Esmée Vringer[4,5]

[1]Breast Cancer Now project grant
[2]CRUK programme grant
[3]BBSRC project grant
[4]CRUK Glasgow Centre
[5]University of Glasgow

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 responses, 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.

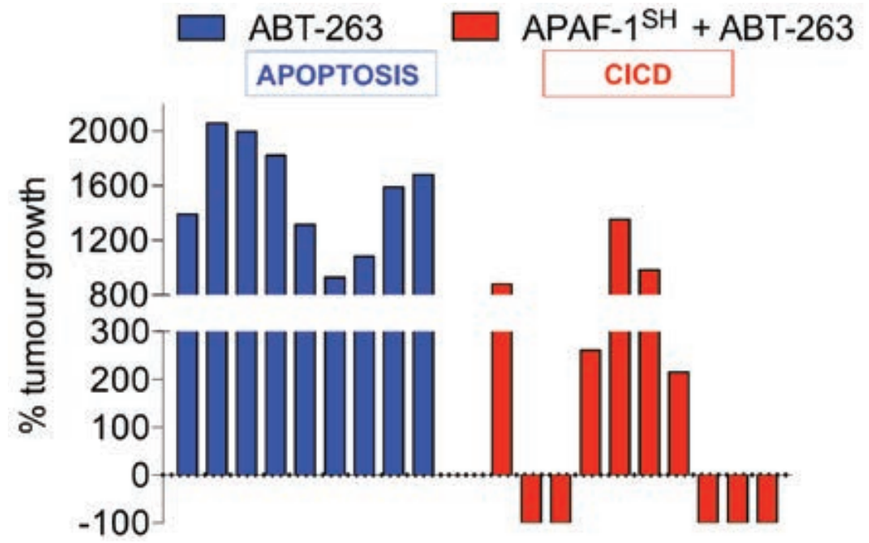
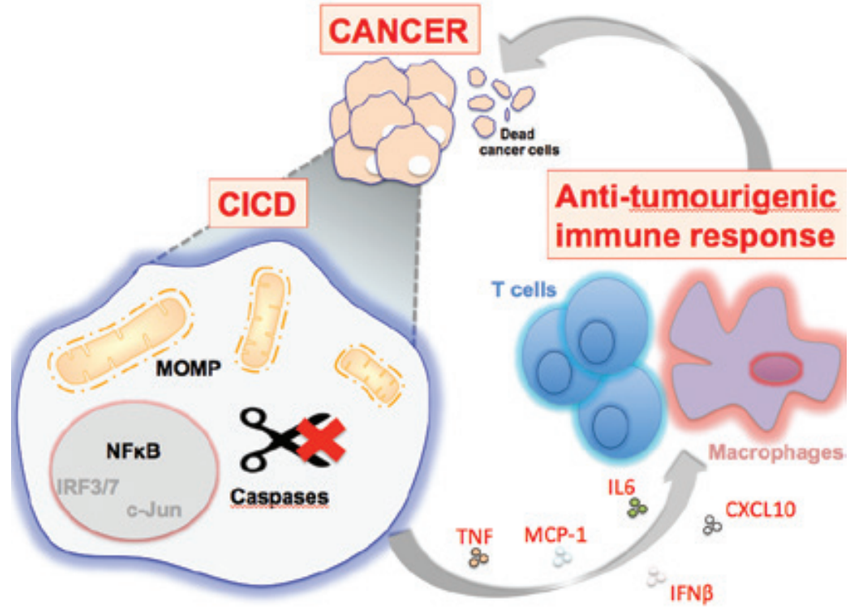


Figure 1
Caspase-independent cell death is more effective than apoptosis at inhibiting tumour growth
Control, apoptosis-proficient CT26 colorectal cancer cells were rendered CICD proficient through shRNA knockdown of APAF-1. Cells were implanted into syngeneic, immunocompetent BALB/c recipient mice. 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 (%) is shown. Inducing CICD inhibited tumour growth in all cases, where complete tumour regression was observed in 50% of mice.

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 inhibits caspase-associated toxicity. Second, cancer cells undergoing CICD are immunogenic – this requires NF-κB-dependent cytokine upregulation. By dying in this manner, CICD triggers host anti-tumour immunity that can kill remaining tumour cells.



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-κB transcription factor pathway. This, in turn, is required for inflammatory signaling during CICD. Mechanistically, mitochondria activate NF-κB by releasing proteins that downregulate cIAP1/2, resulting in NIK and NF-κB activation. Similar to others, we have found that permeabilised mitochondria, by releasing mtDNA, 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 in 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.

Publications listed on page 108

ONCOMETABOLISM

Group Leader
Saverio Tardito

Research Scientist
Victor Villar Cortes

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.

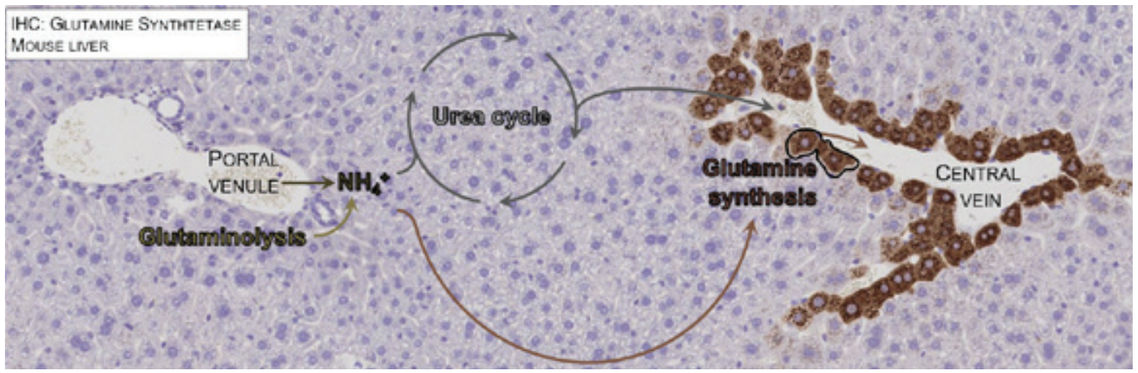


Figure 1
Liver zonation of glutamine metabolism
The liver is an ammonia-detoxifying organ and maintains homeostatic levels of circulating ammonia and glutamine. The functional unit of the liver constitutes an elegant example of metabolic zonation. In fact, the periportal region expresses a liver-type glutaminase which converts the excess of circulating glutamine into glutamate and ammonia. The latter flows downstream through hepatocytes competent in the urea cycle. These cells, despite having a low affinity for ammonia, constitute the bulk of the liver, and convert large amounts of ammonia into urea. The ammonia escaping this metabolic zone is captured by a few rows of hepatocytes surrounding the central vein, which express high levels of glutamine synthetase. This enzyme has a high affinity for ammonia, and fixes it into the non-toxic glutamine, which is finally returned into circulation.

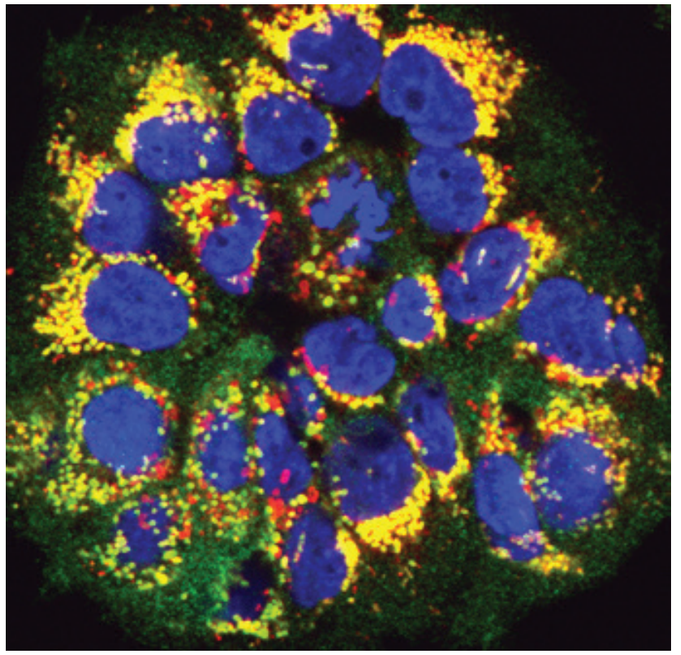


Figure 2
Human liver cancer cells (HuH-6) stained for glutamine synthetase (green), mitochondria (Tomm20, red) and nuclei (DAPI, blue). Glutamine synthetase activity is found in the cytoplasm, where it can localise in puncta in close proximity to the mitochondria. The relationship between the activity of glutamine synthetase and mitochondrial functions is yet to be elucidated.

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 glycaemia. A similar ratio applies to glutamine, the most abundant amino acid in circulation. Conversely, five non-essential amino acids normally circulating in blood are missing from DMEM.

On this basis, we 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.

Publications listed on page 109

www.beatson.gla.ac.uk/alexei_vazquez

MATHEMATICAL MODELS OF METABOLISM

Group Leader

Alexei Vazquez

Research Scientists

Alejandro Lage-Castellanos[1]
Johannes Meiser[2]
Kristell Oizel
Matthias Pietzke

Graduate Students

Jorge Fernandez-de-Cossio-Diaz[1]
Silvia Halim

[1]Infernet, European Union H2020
[2]Deutsche Forschungsgemeinschaft Fellowship

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 hypothesise 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 resynthesise serine via cytosolic one-carbon metabolism. In cells with defective mitochondrial one-carbon metabolism, the cytosolic pathway is reverted, compensating for the loss of mitochondrial formate production. When both cytosolic and mitochondrial pathways are compromised, cells can utilise exogenous formate or endogenous formaldehyde as alternative sources of one-carbon units.

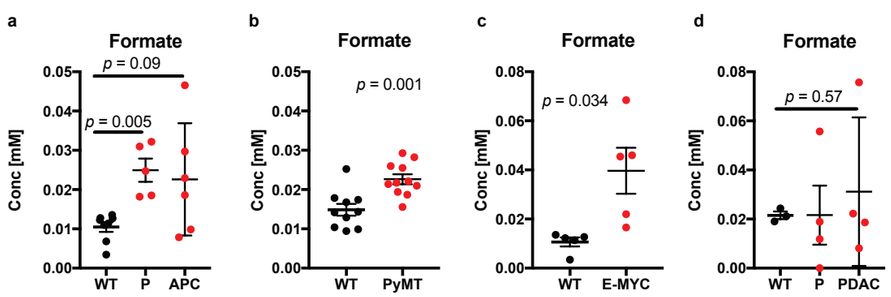


Figure 1
Increased serum formate levels in genetically engineered mouse models (GEMMs). (a–d) Serum formate in wild-type (WT), pre-neoplastic (P) and neoplastic tissues from different GEMMs for **(a)** intestinal cancer, **(b)** mammary carcinoma, **(c)** lymphoma, and **(d)** pancreatic ductal adenocarcinoma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

In 2011, while working at the Rutgers Cancer Institute of New Jersey, I predicted that serine catabolism, and subsequent formate production, should occur at rates exceeding the one-carbon demand of biosynthesis (Vazquez *et al*. PLoS One; 2011; 6: e25881). The excess formate would be released from the cells, a process referred to as formate overflow. In 2016, our laboratory experimentally verified that cancer cells release formate at high rates, comparable to or even exceeding the rate of incorporation of one-carbon units into purines (Meiser *et al*. Sci Adv 2016; 2: e1601273). Further *in vitro* and *in vivo* mechanistic studies revealed that formate overflow is dependent on the expression of mitochondrial one-carbon metabolism enzymes and competent oxidative phosphorylation (Meiser *et al*. Sci Adv 2016; 2: e1601273). *In vitro* cell cultures treated with complex I inhibitors manifest reduced formate release or even switch to formate uptake. Treatment of mice with the complex I inhibitor phenformin inhibits the whole-body rate of serine catabolism to plasma formate. However, whether formate overflow is observed in tumours *in vivo*, and if it is dependent on oxidative metabolism, remained to be elucidated.

In 2017 we developed the experimental methods to investigate formate overflow *in vivo*. We have established an experimental protocol based on ^{13}C-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 *APC*$^{Min/+}$ 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 *MTHFD1L*, 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 that 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.

Publications listed on page 109

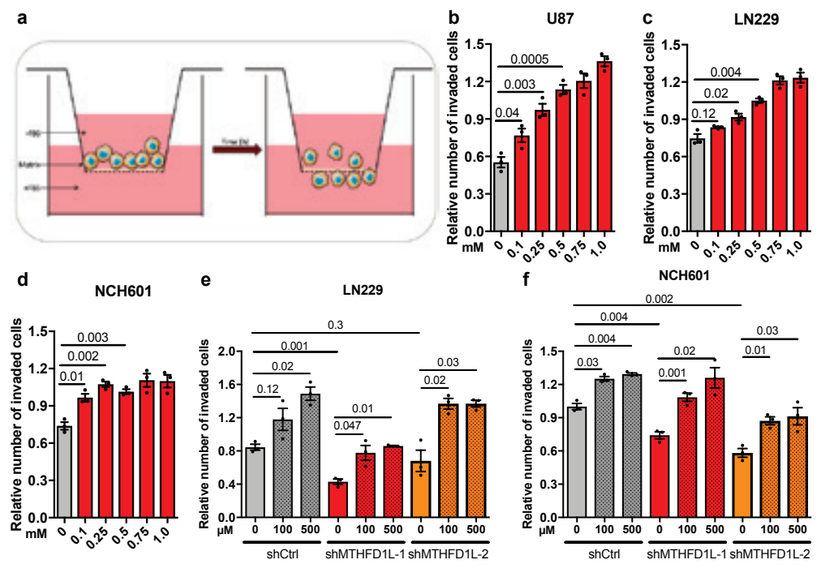


Figure 2
Formate overflow promotes cancer cell invasion in glioblastoma
(a) Cartoon illustrating the experimental setup to analyse cancer cell invasion using coated Boyden chambers. (b-d) Addition of extracellular sodium formate increases invasiveness in a concentration-dependent manner in **(b)** U87, **(c)** LN229 and **(d)** NCH601 cells. (e-f) Reduced invasiveness by *MTHFD1L* knockdown can be rescued with extracellular formate in **(e)** LN229 and **(f)** NCH601 cells.

CANCER METASTASIS AND RECURRENCE

www.beatson.gla.ac.uk/tom_bird

LIVER DISEASE AND REGENERATION

Group Leader
Tom Bird

Research Scientist
Miryam Mueller[1]

Graduate Student
Christos Kiourtis

Visitor
Joep Sprangers[2]

[1]Wellcome Trust
[2]Erasmus, Utrecht University

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.

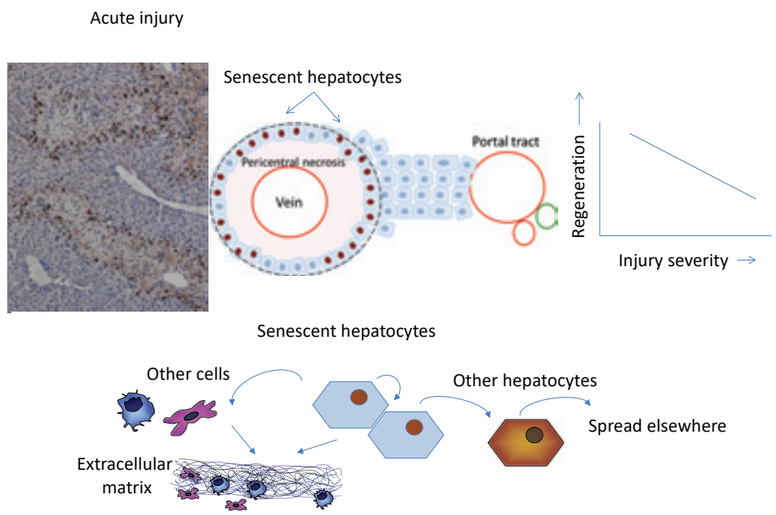


Figure 1
Damage-induced senescence in the liver
Following acute paracetamol toxicity, a rim of senescence develops around the area damaged by the toxic insult (pericentral). Instead of local regeneration, hepatocellular proliferation is activated elsewhere. However, when injury becomes increasingly severe,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

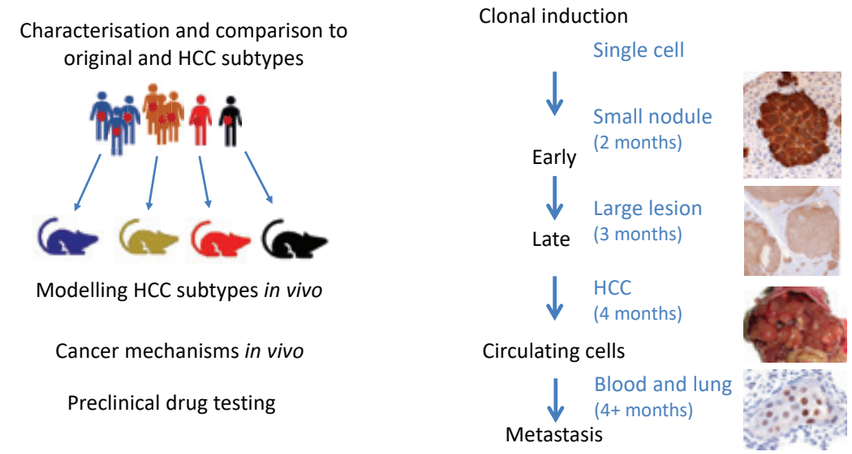


Figure 2
Human HCCs can be grouped into different functional and genetic subclasses. We are mimicking the genetic alterations in human HCC subclasses using *in vivo* models. Our strategy is to induce clonal hepatocytes with these targeted genetic alterations and then follow the clones' development into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

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 potentially at risk of liver disease, 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; and 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, prevent their disease and provide rescue therapies for those detected with late-stage disease.

Using large patient cohorts, we are studying how we can improve the use of serum biomarkers to identify patients who 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.

Publications listed on page 93

www.beatson.gla.ac.uk/david_bryant

MOLECULAR CONTROL OF EPITHELIAL POLARITY

Group Leader

David Bryant

Research Scientists
Álvaro Román-Fernández[1]
Mohammed Mansour[2]

Scientific Officer
Emma Sandilands[1]

Graduate Students
Marisa Nacke[3]
Konstantina Nikolatou[3]
Eva Freckmann[4]

[1]University of Glasgow
[2]Royal Society Newton International Fellowship
[3]CRUK Glasgow Centre
[4]University of Glasgow Industrial PhD Partnership

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.

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

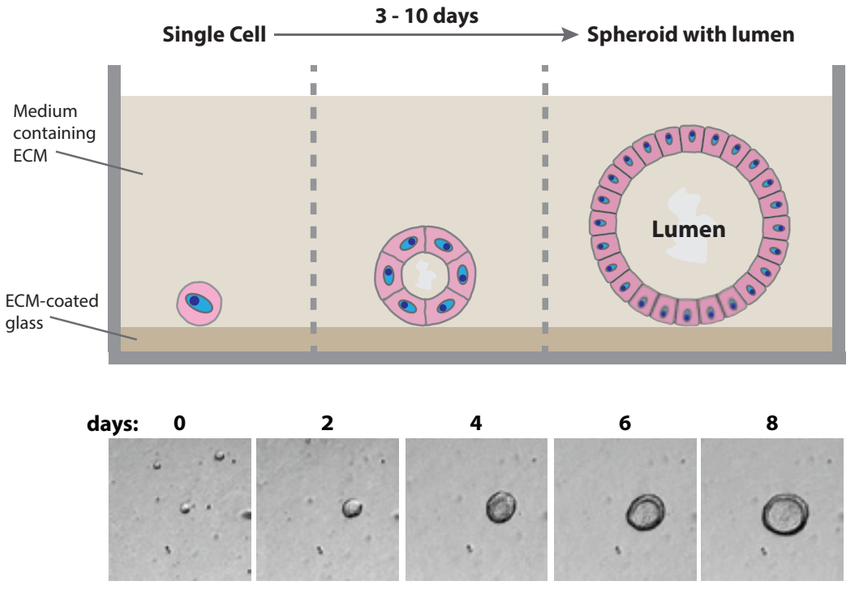


Figure 1
By culturing cells on glass-bottomed chambers coated with extracellular matrix (ECM), we direct the self-assembly of single cells into multicellular spheroid structures with a single, central lumen. This process occurs over 10 days, allowing us to study the dynamics of tissue formation.

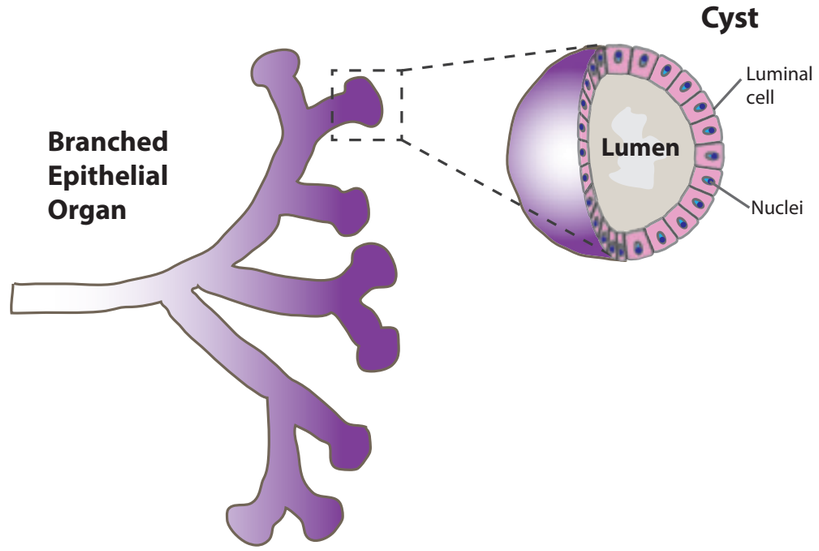


Figure 2
3D cultures of cells to form cysts (also called spheroids or organoids) allows us to model the basic structure of epithelial organs. This allows us to understand and manipulate factors that are altered in cancer patients and model their effect on tissue organisation.

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 preamble to identify which, and how selectively, ARF GTPases may be targets for future therapeutic inhibition studies.

Podocalyxin function in collective cancer cell invasion

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

In collaboration with the Zanivan group, we are using in-depth quantitative mass spectrometry to identify the interacting partners of Podocalyxin ('Podxl interactome') that control its pro-invasive function. Additionally, we are mapping the proteomic changes required during cancer progression to promote Podocalyxin function. Furthermore, we have collaborated with the Shanks lab / 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 vitro* modulators of invasion are consistently altered in prostate cancer patients, such that they may be potential therapeutic targets in the clinic in the future.

Understanding the effect of common genomic alterations on epithelial–stromal crosstalk in ovarian 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.

Publications listed on page 94

LEUKOCYTE DYNAMICS

Group Leader
Leo Carlin

Fellow of the Royal Microscopical Society (FRMS)

Research Scientists
Amanda McFarlane
Ximena L. Raffo Iraolagoitia

Graduate Students
John Mackey[1]
Judith Secklehner[2]

[1]National Heart & Lung Institute Foundation
[2]Imperial College London President's PhD Scholarship

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 come from a better understanding of how leukocytes are (dys) regulated. However, these strategies do not work for all cancers or all patients.

Specialised vasculature and leukocyte dynamics

Our group has a particular interest in the lung, 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 matrikines – chemoactive products of matrix degradation). Additionally, the accumulation of apoptotic neutrophils and their subsequent clearance is thought to directly contribute to anti-inflammatory programmes at the end of acute inflammatory responses. Taken together, these features mean neutrophils have the potential to contribute to both tumour antagonism and tumour-promoting inflammation, and recent work has demonstrated that neutrophils actually benefit cancer spread in the process of lung metastasis. Because of this diversity of actions and importance in the host defence, we need more mechanistic details in order to interact with neutrophils in a way that would inhibit cancer but not leave the patient at risk of serious infection. Neutrophils can be regulated by – and can regulate the function of – other immune cells, so an important goal is to look at a number of different cell types simultaneously to glean more information about the way that they interact and to uncover potential pathways to modify.

By looking across multiple, relevant cancer models we 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 neutrophil's 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).

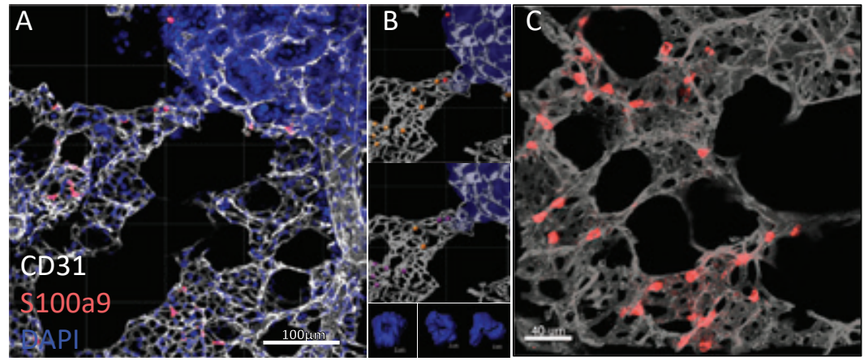


Figure 1
Imaging the immune microenvironment in cancer
(a) Confocal maximum intensity projection image showing lung tumour (area of high DAPI signal, top right; Blue), blood vessels (CD31; grey) and neutrophils (S100a9; red) in a precision-cut lung slice from a model lung adenocarcinoma. **(b)** These images are analysed using software to measure and colour-code the positions of individual cells in relation to the tumour and blood vessels (top, middle) and also to investigate the morphology of individual neutrophil nuclei (bottom). **(c)** A similar approach can be applied to a model of lung metastasis from breast cancer.

Images acquired in BAIR by Ximena Raffo and John Mackey in collaboration with Daniel Murphy and Seth Coffelt labs.

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' 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 Lo Celso's group at Imperial College in their work on acute myeloid leukaemia and with Sara Zanivan's group at the Institute studying cancer cell–blood vessel interactions.

Publications listed on page 95

www.beatson.gla.ac.uk/seth_coffelt

IMMUNE CELLS AND METASTASIS

Group Leader
Seth Coffelt

Research Scientist
Toshiyasu Suzuki[1]

Scientific Officer
Anna Kilbey[2]

Graduate Students
Sarah-Jane Remak[2,3]
Mark Lawrence[4]

Summer Interns
Kyla Foster[5]
Liam Hayman[6,7]

[1]Naito Foundation of Japan
[2]CRUK Glasgow Centre
[3]William Forrest Charitable Trust PhD Research Fund
[4]Pancreatic Cancer UK Future Leaders Fund
[5]University of Colorado
[6]Wellcome Trust Biomedical Vacation Scholarship
[7]Abertay University

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 Tenovus. 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 γδT cell biology.

γδ T cells in breast 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 cancer. 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 (*Tcrd*$^{-/-}$) 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 BTNL1, which is required for the maintenance of Vγ7-expressing γδ T cells. BTNL1 tethers Vγ7 cells specifically to the gut; therefore, Vγ7 cells are likely to be absent from these BTNL1-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.

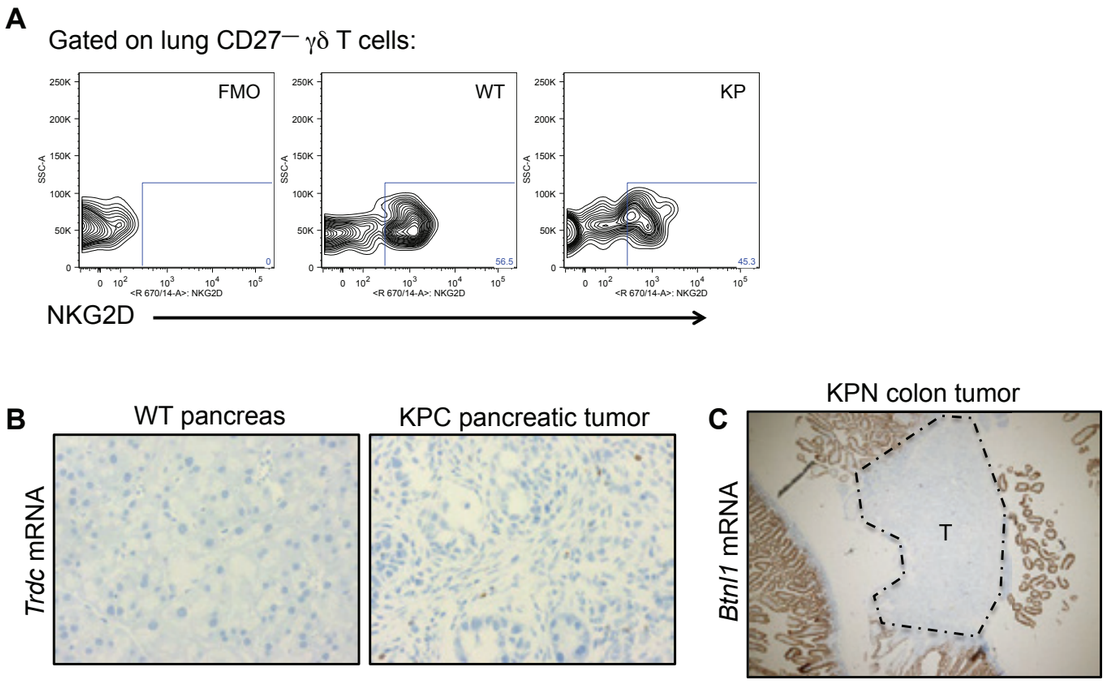


Figure 1
γδ T cells in mammary, pancreatic and colon cancers
(a) Flow cytometry analysis of NKG2D expression on CD27- γδ T cells isolated from the lungs of wild-type (WT) mice or mammary tumour–bearing *K14-Cre;Trp53F/F* (KP) mice. **(b)** γδ T cell abundance in pancreas of WT mice and pancreatic ductal adenocarcinoma of *KrasG12D;Trp53R125H;Pdx1-Cre* (KPC) mice as determined by RNAscope for the Trdc transcript. **(c)** Btnl1 mRNA expression in gut tissue from *Villin-CreER;KrasG12D;Trp53F/F;Nicd1LSL/+* (KPN) mice as determined by RNAscope. T = tumour

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.

Publications listed on page 95

CELL MIGRATION AND CHEMOTAXIS

Group Leader
Robert Insall FRSE

Research Scientists
Luke Tweedy[1]
Shashi Singh
Clelia Amato
Yvette Koh

Scientific Officer
Peter Thomason

Graduate Students
Sophie Claydon
Adam Dowdell

[1]CRUK Multidisciplinary Award

One of the most damaging aspects of cancer is metastasis, in which cells spread beyond the tumour in which they arose and colonise other organs. In normal organs, and most early solid tumours, cells do not migrate. However, when tumours become metastatic, suppression of cancer cell migration may be lost – cells spread into the blood and lymph systems to form secondary tumours. It is believed that cells cannot spread or move efficiently unless they are steered by something. However, it is not understood what provides the directional steer, or how cells manage to read and respond to it. Our group brings together multiple tools, from 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 structures that cells use to migrate, known as 'pseudopods'. Pseudopods are made by assembling fibres of a protein called actin; we try and understand what controls how actin is built, and how this leads to formation of pseudopods. The lab contains mathematicians, biochemists, microscopists and geneticists. 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 exquisitely chemotactically 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.

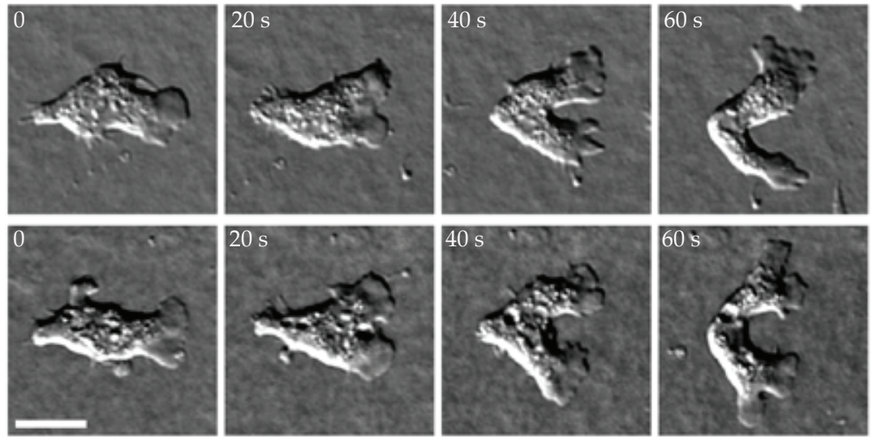


Figure 1
Loss of WASP makes no obvious difference to the actin protrusions of migrating *Dictyostelium* cells (top line: wild type cells; bottom line: WASP mutant cells)

Regulators of actin and the Arp2/3 complex

Most mammalian cells use pseudopods made of polymerised actin to power migration. Our current research focuses on the proteins and pathways that control these pseudopods. We use three approaches. For genetic studies we use *Dictyostelium*, taking advantage of its ease of manipulation, and prominent cell movement and chemotaxis. To apply our knowledge to cancer, we use melanoma cells cultured from tumours with different degrees of metastasis, and actual tumours from mouse models and, when possible, from fresh patient tissue.

Actin drives nearly all cell movement, and the principal driver of actin is an assembly called the Arp2/3 complex. We are particularly interested in the family of proteins that turns on the Arp2/3 complex. One such regulator is SCAR/WAVE, which is a fundamentally important regulator of cell movement. Mutants in a variety of species show that it is required whenever cells need to make large actin-based structures such as lamellipods; without SCAR/WAVE such structures are either small and malformed, or completely absent. It is found as part of a five-membered complex with the Rac-binding protein PIR121, Nap1, Abi and HSPC300. The prevailing view in the field is that all these proteins act simultaneously as a huge, homogenous complex that couples Rac and lipid signalling to actin polymerisation. However, this view seems very simplistic in view of the size of the complex and its dynamic behaviour.

Our experiments are currently focused on identifying the activators and other proteins that regulate each component of the complex. We are using the Institute's expertise in mass spectrometry to identify proteins that crosslink to SCAR in living cells at different migration rates. We have set up a system that allows us to purify biochemically useful amounts of SCAR complex from living cells. This is a unique facility. We have now found that SCAR is phosphorylated at 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 signalling molecules such as receptors and G-proteins. The behaviour of SCAR's relative WASP is slightly anomalous – there is a high degree of consensus among cell biologists about how it is controlled, but the standard view does a poor job of explaining the observed behaviour. We are therefore mutating and dissecting WASP to see how it works.

Publications listed on page 98

STRUCTURAL BIOLOGY OF CILIA

Group Leader
Shehab Ismail

Research Scientists
Louise Stephen
Tamas Yelland

Scientific Officer
Michael McIlwraith

Graduate Students
Yasmin Elmaghloob
Youhani Samarakoon

Our group investigates the cellular mechanisms that maintain the distinct composition of cilia and immunological synapses. In particular, we are interested in the trafficking of lipid-modified signalling proteins by a group of proteins called GDI-like solubilising factors. In our lab, we address our research questions using a combination of structural, biochemical and cellular biology approaches.

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 Arf (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 lipidated 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: e11859.

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

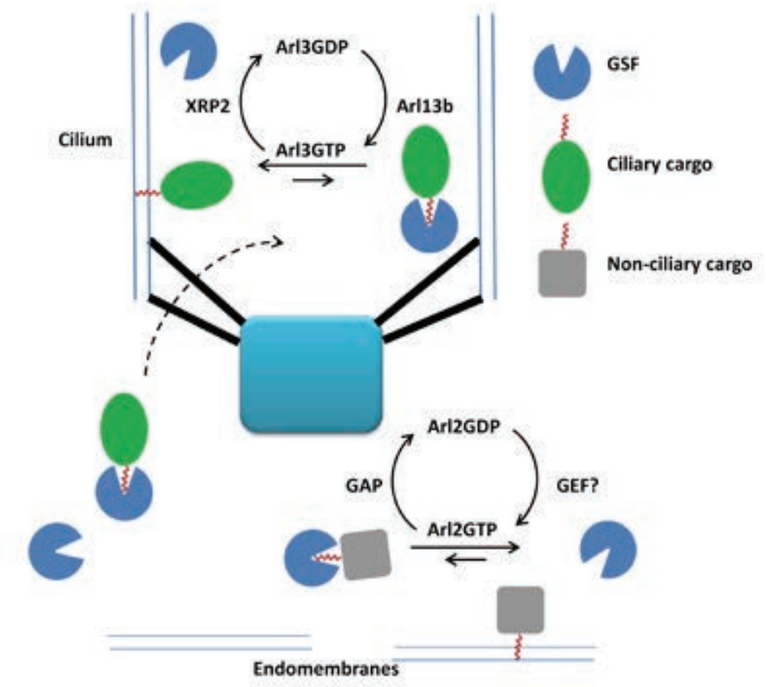


Figure 1
Sorting and shuttling of prenylated/myristoylated ciliary cargo into the cilium
The GSF (blue; e.g. Unc119b) binds to the lipid-modified tail of the ciliary cargo (green). The complex diffuses into the cilium, where Arl3, maintained in a GTP-bound state by Arl13b, binds the GSF, forcing a conformational shift that releases the ciliary cargo to the ciliary membrane. Binding of Arl3 to its GAP, XRP2, results in its inactivation. Non-ciliary cargo (grey) is solubilised by the GSF in the same manner before being released to endomembranes by Arl2GTP. The GEF required to activate Arl2, in its GTP-bound state, is not known.

www.beatson.gla.ac.uk/laura_machesky

MIGRATION, INVASION AND METASTASIS

Group Leader
Laura Machesky FRSE

Research Scientists
Amelie Juin
Kirsty Martin
Nikki Paul[1]
Karthic Swaminathan
Jamie Whitelaw

Scientific Officer
Heather Spence

Graduate Students
Loic Fort
Anh Hoang Le
Savvas Nikolaou
Vassilis Papalazarou[2]

[1]Pancreatic Cancer Research Fund
[2]CRUK Glasgow Centre, joint with Manuel Salmeron-Sanchez, University of Glasgow

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 Rho GTPase 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 protrusion: 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 most higher eukaryotes. Postdoc Jamie Whitelaw and students Anh Le and Savvas Nikolau 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 embryo melanoblasts. Student Emma Woodham and postdoc Nikki Paul, together with Prof. Cord Brakebusch (BRIC, 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 Burgess 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-bundling 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

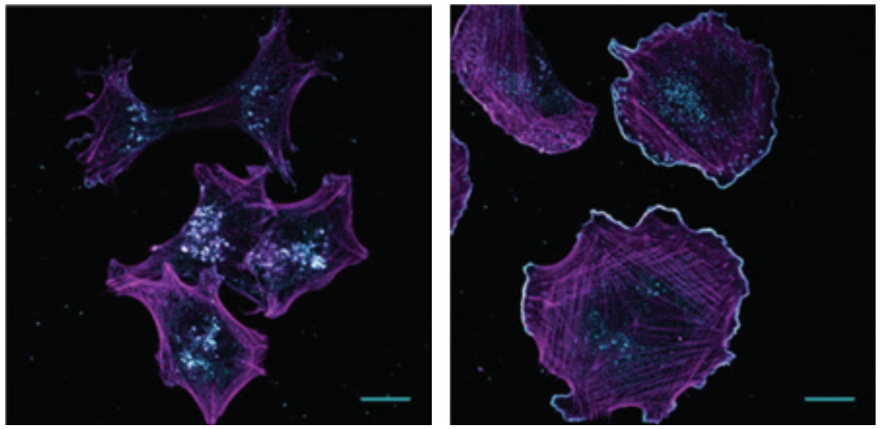


Figure 1
CHL-1 melanoma cells with control or CYRI CRISPR knockout. Purple = actin; blue = cortactin. Photo credit: Loic Fort

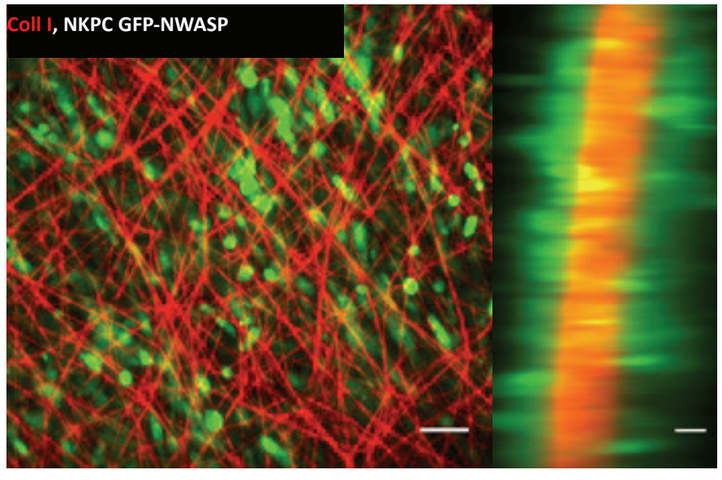


Figure 2
Pancreatic cancer cells invading native peritoneal basement membrane. Red = collagen-I; Green = GFP–N-WASP–expressing cancer cells. Photo credit: Amelie Juin and Ewan McGhee (BAIR).

INTEGRIN CELL BIOLOGY

Group Leader
Jim Norman

Research Scientists
Emmanuel Dornier
Sergi Marco-Martin
Sarah Palmer[1]
Madeleine Moore

Associate Clinical Scientist
Iain MacPherson

Scientific Officer
Louise Mitchell

Clinical Research Fellow
Dominik Kössinger

Graduate Students
Nicholas Rabas
David Novo

[1]West of Scotland Women's Bowling Association Fellow

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 p53s 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 foster certain ECM microenvironments in metastatic target organs, and how this can promote recruitment of circulating tumour cells and components of the innate and acquired immune systems to break dormancy and drive metastasis. 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 γ-amino butyric 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.

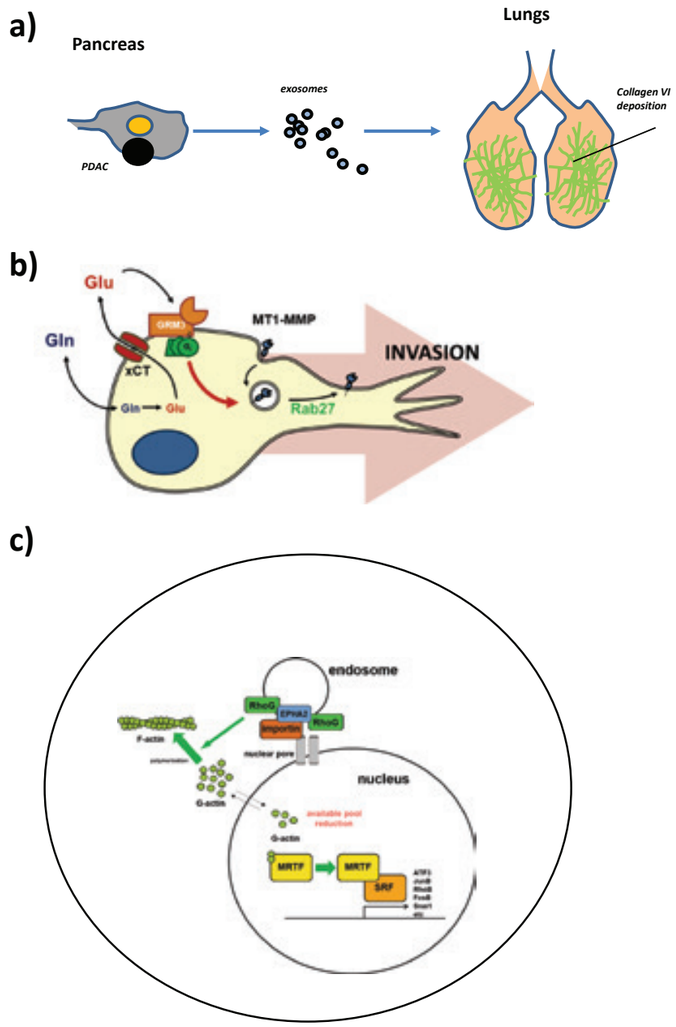


Figure 1
(a) Exosomes from mutant p53–expressing pancreatic adenocarcinoma tumours influence extracellular matrix deposition during lung remodelling.
(b) Glutamate secretion from mammary carcinoma promotes invasion by activation of the GRM3 metabotropic glutamate receptor.
(c) EphA2 mediates the nuclear capture of endosomes to promote local actin polymerisation to activate SRF-dependent transcription.

'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 these are now entering clinical trials. We are currently investigating whether the 'nuclear-capture' of endosomes mediates delivery of ASOs to the nucleus where they act to oppose proliferation of cancer cells. As nuclear-capture mechanisms are most active in cancer cells engaged in disseminations, we are investigating how this pathway may be exploited to target the delivery of ASOs to cancer cells that are actively metastasising.

Publications listed on page 103

www.beatson.gla.ac.uk/michael_olson

MOLECULAR CELL BIOLOGY

Group Leader
Michael Olson

Research Scientists
Steven Bryce
David McGarry[1]
Nicola Rath
Mathieu Unbekandt

Scientific Officer
June Munro

Clinical Research Fellow
Greg Naylor[2]

Graduate Students
Narisa Phinichkusolchit
Dominika Rudzka

Visiting Student
Fiona Donnan[3]

[1]Medical Research Council
[2]CRUK Glasgow Centre
[3]The Genetics Society

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-231 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-231 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 BDP8900 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, BDP8900 and BDP9066 displayed consistent anti-proliferative effects, with greatest activity in haematological cancer cells. Mass spectrometry identified MRCKα S1003 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 S1003 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 TKCC5 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

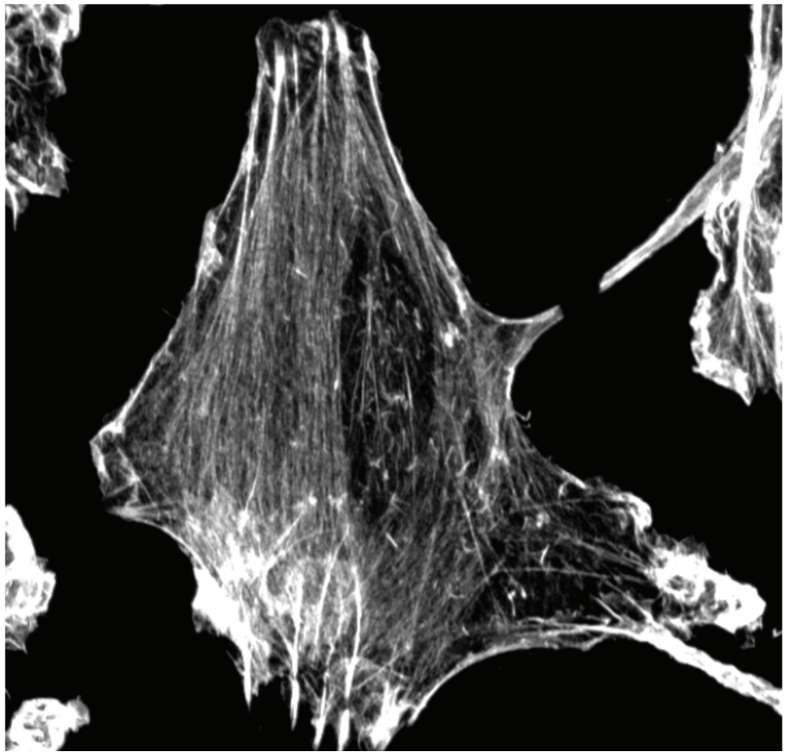


Figure 1
The filamentous actin (F-actin) cytoskeleton in an MDA-MB-231 human breast cancer cell

www.beatson.gla.ac.uk/owen_sansom

COLORECTAL CANCER AND WNT SIGNALLING

Group Leader

Owen Sansom FRSE

Research Scientists

Constantinos Alexandrou
Rene Jackstadt
Joel Johansson[1]
Georgios Kanellos[2]
John Knight
Dana Koludrovic[3]
Noha Mohamed[4]
Arafath Najumudeen
Madelon Paauwe[5]
Alexander Raven
Nikola Vlahov
David Vincent[6]

Scientific Officers

Andrew Campbell
Xavier Cortes Lavaud
Tam Jamieson
Rachel Ridgway

Graduate Students

Sigrid Fey[7]
David Gay[6]
Michael Hodder[8]
Joshua Leach[9]
Rachael Smith[7]
Lucas Zeiger[5]

[1]Novartis
[2]Celgene
[3]Marie Curie
[4]AstraZeneca
[5]CRUK Grand Challenge
[6]ERC
[7]Pancreatic Cancer UK
[8]MRC
[9]MRC Clinical Research Training Fellowship

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 modelled the likelihood of cancer comparing a single activating mutation of *β-catenin* versus bi-allelic *APC* mutation. We took into account the likelihood of the mutation, the requirement for two *APC* mutations and the fact that it took much longer for a *β-catenin* mutation to produce a phenotype. Using these parameters, the model predicted that if the stem cell was the cell-of-origin one would expect *β-catenin* mutations but if you include in addition transit amplifying (TA) cells then bi-allelic *APC* mutations were much more likely. Interestingly, the human colon has many more TA cells than the mouse, which may explain why an *Apc* mutation leads to small intestinal tumours in the mouse and colonic tumours in man.

Inhibiting Wnt signalling *in vivo*

Given the strong link between deregulated Wnt signalling and colon cancer, we have 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 monoclonal (i.e. with only one stem cell) rather than polyclonal (with 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 (Huels *et al.*, Nat Commun 2018; 9: 1132).

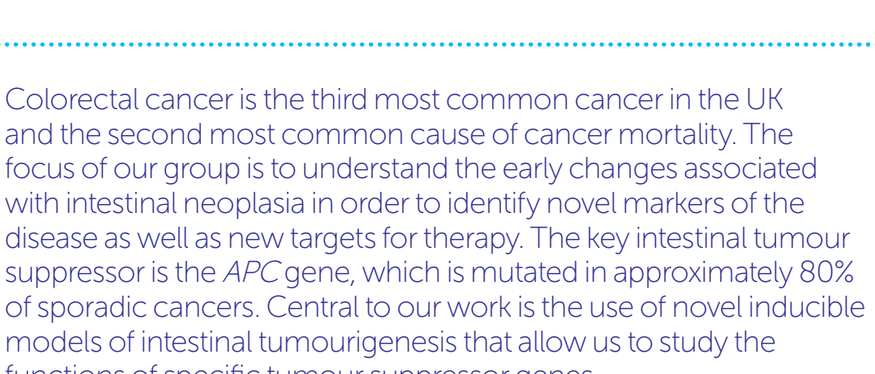
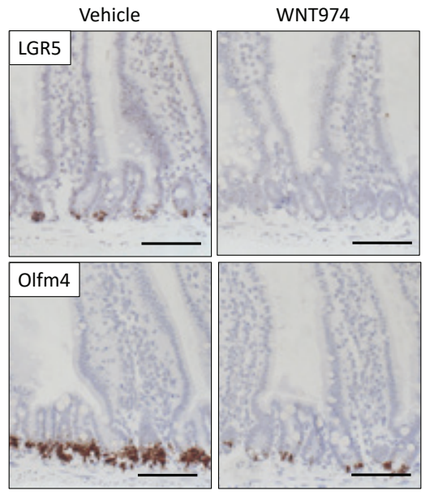


Figure 1
Simulation suggests that transit amplifying (TA) cells can act as the cell-of-origin in colon cancer. Left-hand panel: if just the stem cell is considered then *β-catenin* mutations are more likely to occur (blue line) rather than two *APC* mutations (red line). Right-hand panel: if the TA cells are included then two *APC* mutations accumulate.

Figure 2
WNT974 treatment reduces intestinal stem cell markers. Porcupine inhibition reduces the expression of the stem cell markers Lgr5 and Olfm4 using RNASCOPE *in situ* hybridisation, performed by Colin Nixon and his group (Huels *et al.*, Nat Commun 2018; 9: 1132).

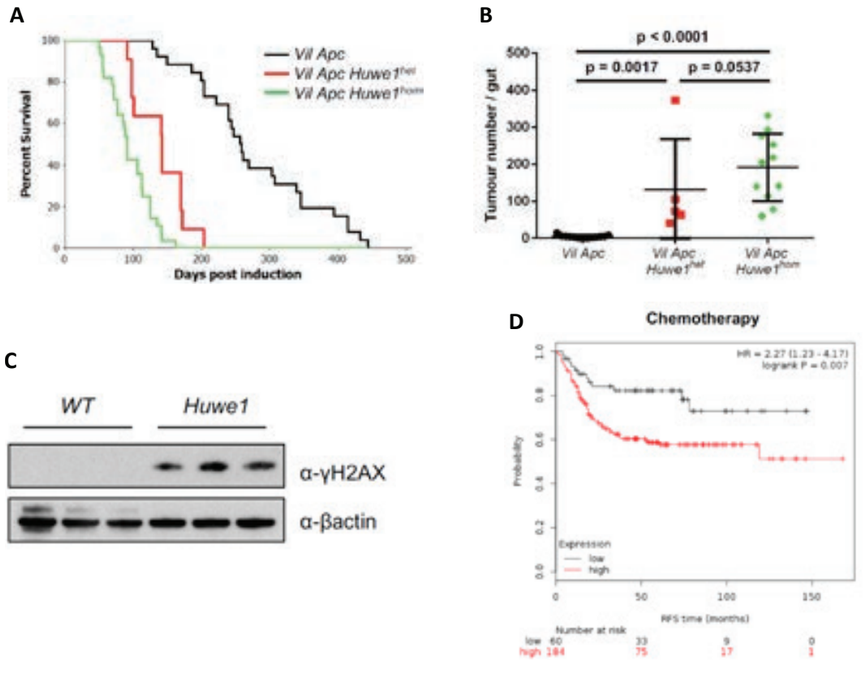


Demonstrating that HUWE1, which is mutated in up to 10% of colorectal cancer (CRC), is a *bona fide* tumour suppressor gene

In human cancers, many genes are mutated, often at relatively low frequencies. Assigning these as functional modifiers 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 deleted this 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 (Myant *et al.*, EMBO Mol Med 2017; 9: 181). 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

Figure 3
HUWE1 is a bona fide tumour suppressor gene. **(a)** Huwe1 loss accelerates time to intestinal tumourigenesis (green versus black line). **(b)** Huwe1 loss increases numbers of intestinal tumours. **(c)** HUWE1 loss increases levels of γ-h2aX **(d)** Patients that have tumours with low levels of HUWE1 (black line) have improved survival following chemotherapy compared to those with high levels of HUWE1 (red line).



TUMOUR MICROENVIRONMENT AND PROTEOMICS

Group Leader
Sara Zanivan

Research Scientists
Alice Santi
Sam Atkinson

Scientific Officer
Lisa Neilson

Graduate Students
Fernanda Kugeratski
Emily Kay
Ilaria Puoti

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 sizeable 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 we have previously shown to be a powerful technology to investigate cellular secretomes (Zanivan *et al.,* Mol Cell Proteomics 2013; 12: 3599–611) and molecular mechanisms underpinning EC functions (van den Biggelaar *et al.,* Blood 2014; 123: e22–e36 Patella *et al.,* Mol Cell Proteomics 2015; 14: 621–34; Patella *et al.,* J Proteome Res 2016; 15: 2187–97).

Pro-invasive functions of CAFs

Using methods that we have previously developed for in-depth quantitative MS–proteomic analysis of secreted proteins in cell culture (van den Biggelaar *et al.,* Blood 2014; 123: e22–e36) we have provided the first global portrait of proteins that are differentially secreted when patient-derived mammary fibroblasts are activated into CAFs by breast cancer cells. We have found that the chloride intracellular channel protein 3 (CLIC3) is one of the most upregulated and heavily secreted proteins in CAFs. Moreover, we have established an unprecedented role for this protein in the extracellular environment: CLIC3 is a pro-invasive oxidoreductase able to promote tumour invasion and vessel growth by increasing ECM stiffness though the reduction (activation) of the extracellular transglutaminase TGM2. Importantly, we have found that CLIC3 is highly abundant in the stroma of triple-negative breast cancer and high-grade serous ovarian cancer (HGSOC) patients. Indicating that CLIC3 may have a role in the progression of the disease, high levels of CLIC3 in the stroma correlate with poorer patient prognosis (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.

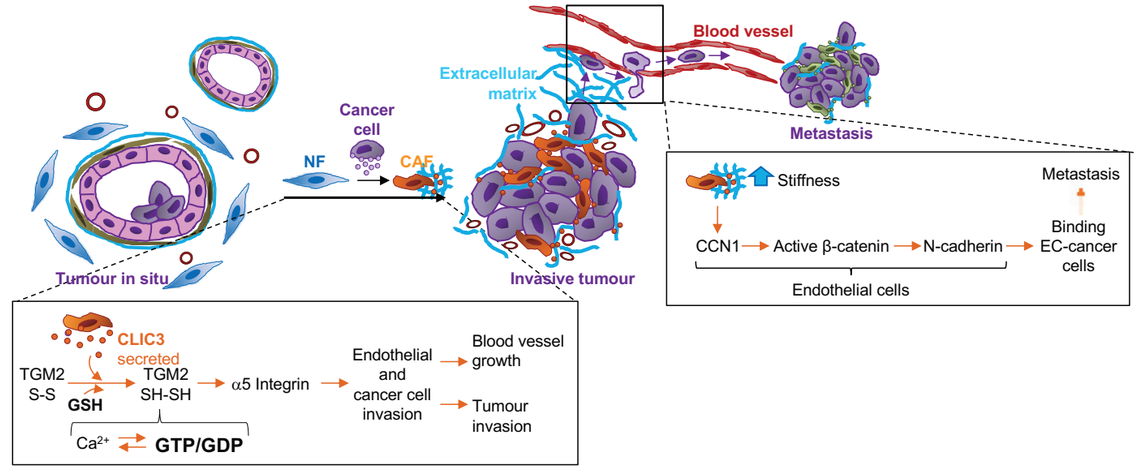


Figure 1
This cartoon summarises our recent findings in the context of tumour progression (via CLIC3, on the left) and metastasis (via CCN1, on the right). NF = normal fibroblasts; CAF = cancer associated fibroblasts (Adapted from Reid *et al.,* EMBO J 2017; 36: 2373–89)

Recently, we 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 intravasation 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 we are currently investigating further.

Tumour stiffness 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 adhesion receptors on the surface of the ECs. In particular, we have characterised an unprecedented mechanism through which high stiffness increases expression and exposure to the plasma membrane of N-cadherin via upregulation of CCN1. Importantly, we showed that the CCN1/N-cadherin pathway facilitates the binding of the cancer cells to blood vessels, which is the first step of cancer cell intravasation into the blood stream for the formation of distant metastasis (see Fig. 1). We have therefore identified 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

DRUG DISCOVERY

DRUG DISCOVERY UNIT

Co-Heads

Justin Bower

Heather McKinnon

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

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

Structural Biology
Kenneth Cameron
Andrea Gohlke
Gillian Goodwin
Christopher Gray
Marta Klejnot
Jennifer Konczal
Amy McCallum
Alexander Schuettelkopf

Lab Support
Michael Kilday

Infomatics Manager
Daniel James

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 (90%) 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 effector proteins 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 across multiple areas of the project, enabling us to progress our main chemical series more aggressively whilst also working on additional back-up series, presenting different opportunities and molecule profiles. Resource 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 RAS 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 GMPPnP, with very tight binding observed and 33nM in our KRAS G12D.GMPPnP 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 formats 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.

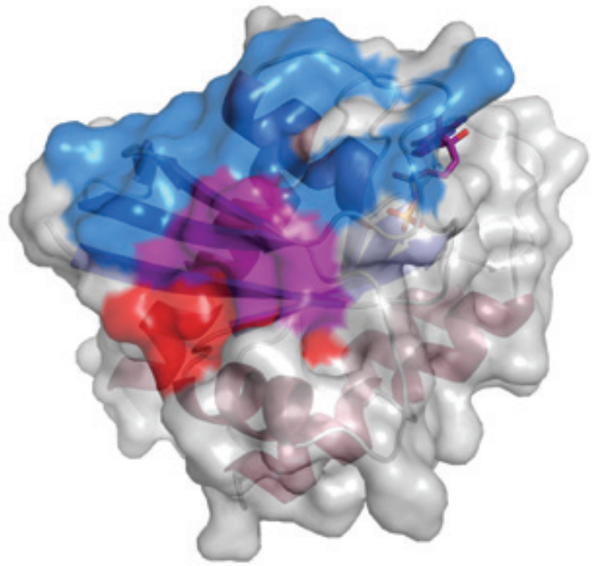


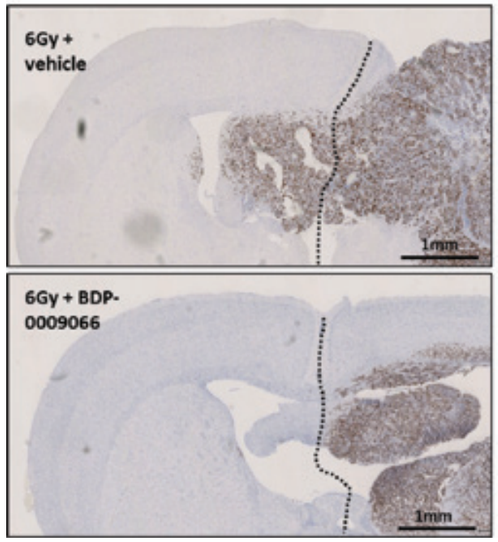
Figure 1
KRAS x-ray crystal structure, highlighting small molecule binding site (red) and areas of focus for small molecule optimisation (purple and blue).

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 RAS/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 EC_{50} 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 Professors 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 MLC and MYPT1 to facilitate the cytoskeletal changes which contribute to cancer cell motility and invasion. Previous 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 (MRCKβ K_i = 23pM) was designed starting from a ligand-efficient fragment BDP-00003246 (MRCKβ K_i = 4.49μM). Iterative rounds of medicinal chemistry and structure-based design using BDP-00003246 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.



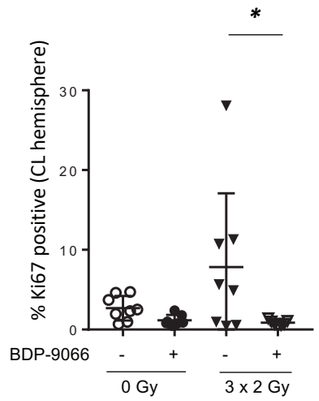


Figure 2
Inhibition of radiation-induced invasion in a mouse glioblastoma model with the MRCK inhibitor BDP-00009066. (J Birch, A Chalmers)

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

ADVANCED TECHNOLOGIES

BEATSON ADVANCED IMAGING RESOURCE (BAIR)

Head
Leo Carlin

Scientific Officers
Ewan McGhee
Margaret O'Prey
David Strachan

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 that model substrate or living organism.

Beatson Advanced Imaging Resource (BAIR) scientists work closely with the Institute's researchers to uncover and interrogate important molecular pathways in cancer. The BAIR is thus involved at some stage in nearly every paper from researchers at the Institute that contains a light micrograph. 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 imaging performed in the BAIR are showcased 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 orgnaniser, 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 woud 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 Machesky'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)

Amélie Juin, winner of the 2017 BAIR imaging competition. Her image shows a spiral of HUVECs stained with phalloidin and contactin.

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 with the existing BSU inverted multiphoton FLIM system. Second 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 chemoattractant; 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 (and, 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

Head
Sara Zanivan
(see page 62)

Scientific Officers
Sergio Lilla
Giovanny Rodriguez

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.

Publications listed on page 110

FUNCTIONAL SCREENING

Head
Emma Shanks

Research Scientist
Grant McGregor

Informatics Manager
Daniel James

Scientific Officers
Edward Kalkman[1]
Lynn McGarry

Graduate Student
Matthew Davidson

[1]CRUK Glasgow Centre

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

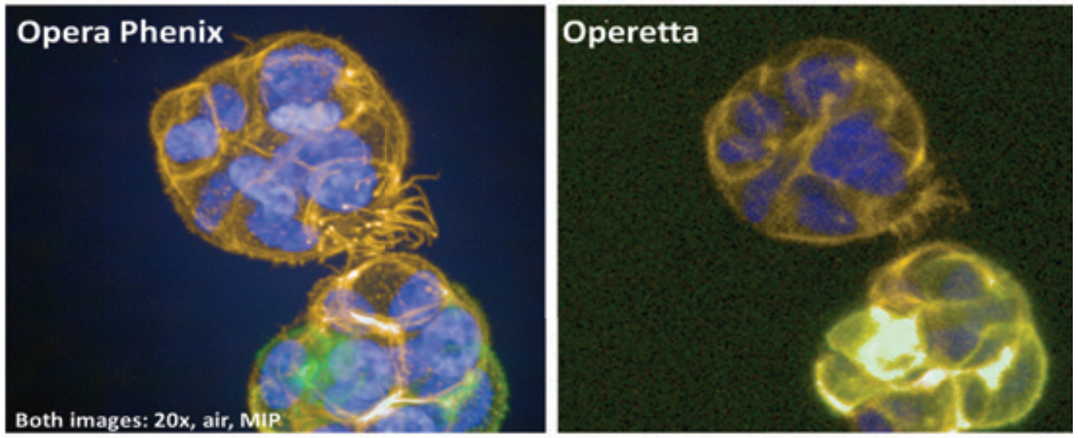


Figure 1
Technical comparison of a prostate cancer spheroid acquired on the Opera Phenix (left) and the Operetta (right). Clear enhancement of the spheroid physiology can be identified and subsequently quantified.

METABOLOMICS

Head
Gillian Mackay

Senior Scientific Officer
David Sumpton

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

PRECLINICAL PRECISION PANCREAS

Head
Jennifer Morton

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

Scientific Officers
Saadia Karim
Viola Paulus-Hock[1]
Curtis Rink[2]

Graduate Students
Laura Lapienyte[3]
Dale Watt[2]

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

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 **K**ras^{G12D} and **p**53^{R172H} targeted to the pancreas (using **C**re-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.

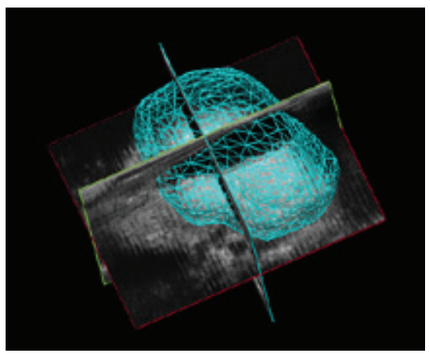


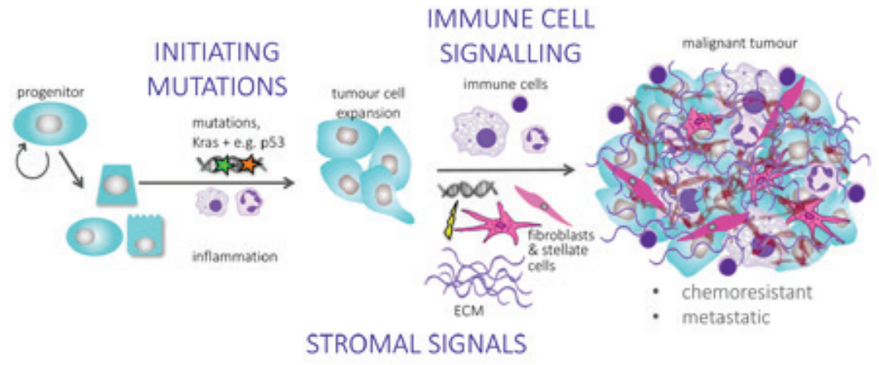
Figure 1
High-resolution ultrasound image showing 3D measurement of a pancreatic tumour in a KPC mouse

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 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.

Figure 2
Crosstalk between tumour cells and the microenvironment can affect cell proliferation, survival, metabolism, immune response and response to different therapeutic agents, while therapeutic agents in turn alter these signals and change the tumour subtype.

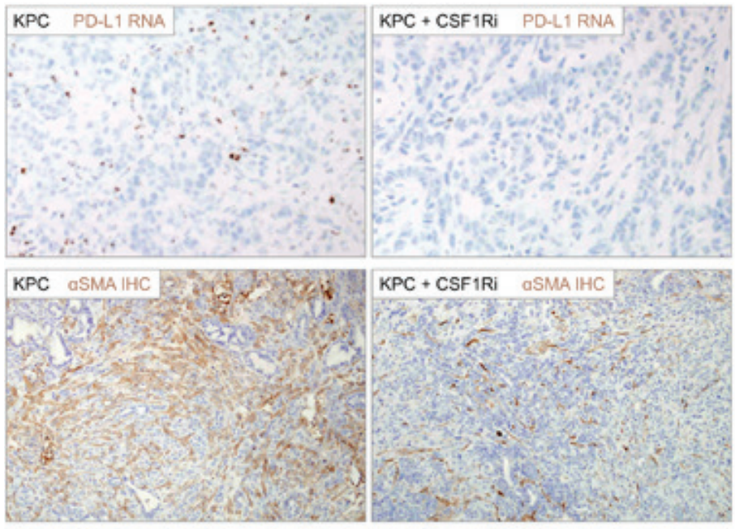


Genomics and immunotherapy

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 modelling 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 trialling novel concepts in the appropriate models.

Publications listed on page 101

Figure 3
IHC for αSMA and RNAscope ISH to detect PD-L1 demonstrate significant microenvironmental changes in CSF1R inhibitor–treated tumour-bearing KPC mice.



www.beatson.gla.ac.uk/karen_blyth

TRANSGENIC MODELS OF CANCER

Head
Karen Blyth

Research Scientist
Nicholas Rooney

Scientific Officers
Dimitris Athineos
Sandeep Dhayade
Susan Mason

Graduate Students
Alessandra Riggio
Kerri Sweeney[1]

[1]Breast Cancer Now

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.

Modelling cancer *in vivo*

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 2D 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 impressive tumour regression in preclinical models upon triggering of caspase-independent cell death (Giampazolias *et al.*, 2017), and with Sara Zanivan's lab showing the *in vivo* relevance of how the tumour microenvironment influences metastatic spread (Reid *et al.*, 2017).

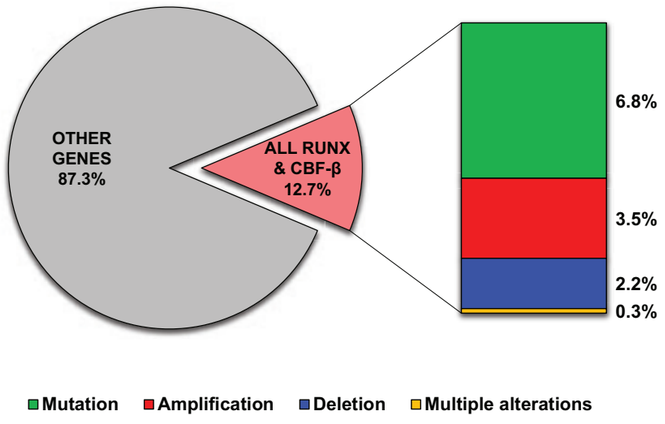


Figure 1
The RUNX/CBFβ co-factor complex is frequently altered in breast cancer
Using data generated by The Cancer Genome Atlas (TCGA, Cell 2015) and mined using cBioPortal (www.cbioportal.org/), we find that the *RUNX/CBFβ* genes are altered in over 12% of all breast cancers and occur as mutations, deletions and amplifications, demonstrating the context-dependent nature of these alterations. *RUNX1* is the most altered, in 6% of 974 breast cancers.

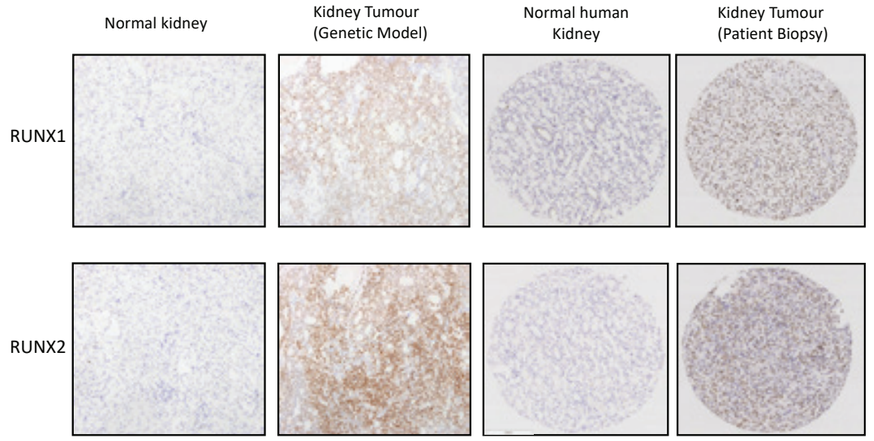


Figure 2
Tissue samples have been stained for expression of the RUNX1 (top) and RUNX2 (bottom) proteins. Normal kidney has low levels of expression of both proteins, whilst the tumours taken from genetic models (left) and patient biopsies (right) show elevated levels of expression of RUNX1 and RUNX2.

MCL1 as a prognostic indicator and drug target in breast cancer

In a study that was initiated in our lab and is an ongoing collaboration with Kirsteen Campbell and Stephen Tait (funded by Breast Cancer Now), we have identified that the pro-survival gene *MCL1* is frequently elevated in breast cancer, correlating with reduced survival and poor prognosis in patients. Kirsteen has elegantly shown that by deleting or pharmaceutically targeting this gene in xenograft and genetic models of breast cancer, tumour growth is severely compromised, highlighting the potential of this gene as a novel drug target for breast cancer (Campbell *et al.*, 2018). This is particularly exciting considering that inhibitors for this gene are in development and are undergoing clinical trials for certain leukaemias.

The *RUNX* genes in breast and other epithelial cancers

The *RUNX* genes, together with their binding partner CBFβ, 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 are 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 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, this tumour suppressor functionality is potentiated 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/CAS-9–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 identified 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

TRANSGENIC TECHNOLOGY

Head
Douglas Strathdee

Research Scientist
Eve Anderson

Scientific Officers
Cecilia Langhorne
Farah Naz Ghaffar

Graduate Student
Nicola Laprano

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 the 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-001 Exon 5; ENSE00003050674). 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 alternately 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 7.58kb in the mouse and 10.17kb 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 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 *FRT* site by Flp recombination in *E.coli*. The resulting vector was used as a targeting vector in embryonic stem cells. The frequency of targeted recombination was enhanced using CRISPR nucleases designed to cut the genomic DNA just inside the 3′ and 5′ homology arms (indicated by black arrows in Fig. 1). Following transfection in HM1 embryonic stem cells, the cells were selected on G418 and resistant colonies were picked and screened for the correct targeting by PCR. Correctly targeted ES cell clones identified will then be used in step 3.

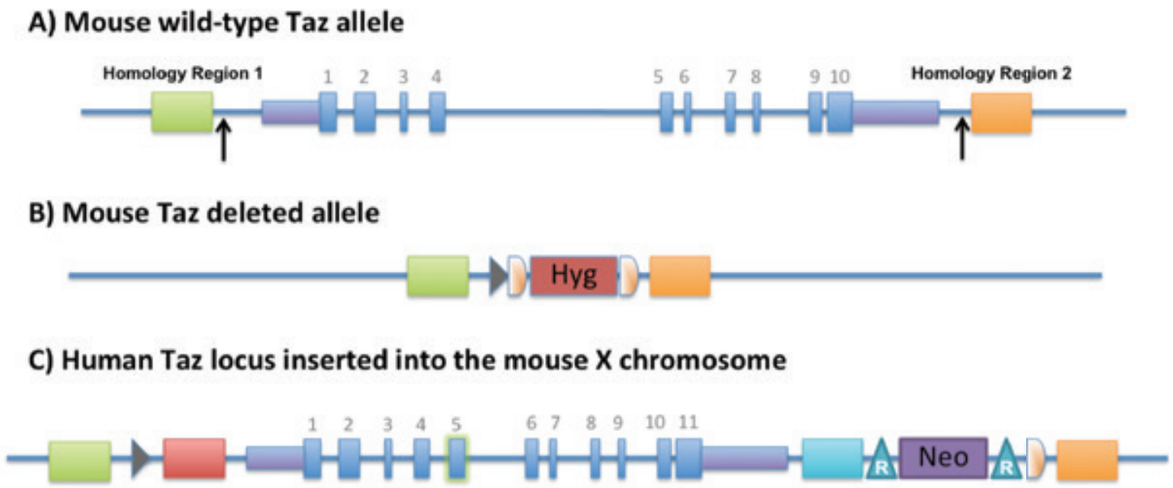


Figure 1
Replacing the mouse *Taz* gene with the human gene in stem cells
(a) The wildtype mouse *Taz* gene. Exons 1–10 are indicated by blue boxes. The 5′ and 3′ regions used to remove the gene are indicated by green and orange boxes, respectively. Black arrows indicate CRISPR cut sites. **(b)** The 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. A *loxP* site (grey triangle) and an *FRT* site (orange semicircle). **(c)** The human *Taz* gene inserted into the mouse X chromosome; this was achieved by dRMCE using the *loxP* and *FRT* sites. The red and light blue boxes indicate the homology regions used to recombineer the human sequences. The positions of the *Rox* sites (blue triangles) and the Neomycin cassette (purple box) are indicated.

Subsequently, a vector carrying the human genomic *Taz* sequences was cloned. This was created by recombineering from a human BAC. DNA comprising two short homology arms was synthesised in a short vector flanked by three site-specific recombination sites: a *loxP* site on the 5′ side and a *Rox* site and an *FRT* site on the 3′ side. A *Rox* site was used to recombine a neomycin-resistant selectable marker cassette by Dre recombination in expressing *E. coli*. Subsequently, recombination in DY380 *E. Coli* will all the retrieval of the human *Taz* locus into the vector by recombination at the small homology arms.

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 transfected into the ES cells generated in step 1, in which the mouse *Taz* gene has been deleted and which have *loxP* and *FRT* sites inserted at the site of the *Taz* locus. In order to achieve this, the retrieval vector was co-transfected with *pDIRE*, which simultaneously expresses Cre and Flp. The cells will then be selected on G418. This will select for cells in which the introduced vector has replaced the *Hyg* selectable marker at the *Taz* locus by recombination at the *loxP* and *FRT* sites. This will insert the human *Taz* gene in the same chromosomal location and the same orientation as 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. Futhermore we can now test the consequences of disease-relevant mutations in the context of the human gene..

Publications listed on page 108

TRANSLATIONAL MOLECULAR IMAGING (TMI)

Head
David Lewis

Senior PET Chemist
Dmitry Soloviev

Staff Scientist
Gaurav Malviya

Scientific Officers
Emma Johnson
Agata Mrowinska

The Translational Molecular Imaging facility has been renamed and relaunched to reflect its extended capabilities and broad new scope. The purpose of this new unit is to enable the clinical translation of novel molecular imaging approaches from preclinical cancer models at the Institute into innovative clinical trials. Investment from the Beatson Cancer Charity of £240k/year is allowing critical infrastructure investment in radiochemistry, including recruitment of a Senior PET Chemist, Dmitry Soloviev.

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 $^{11}C/^{18}F$ synthesisers (Synthra GmbH, Germany) in the R&D and GMP radiolabelling 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 – [^{11}C]acetate, [^{18}F]fluoro-ethyl-tyrosine (FET) and [^{11}C]methionine – will be

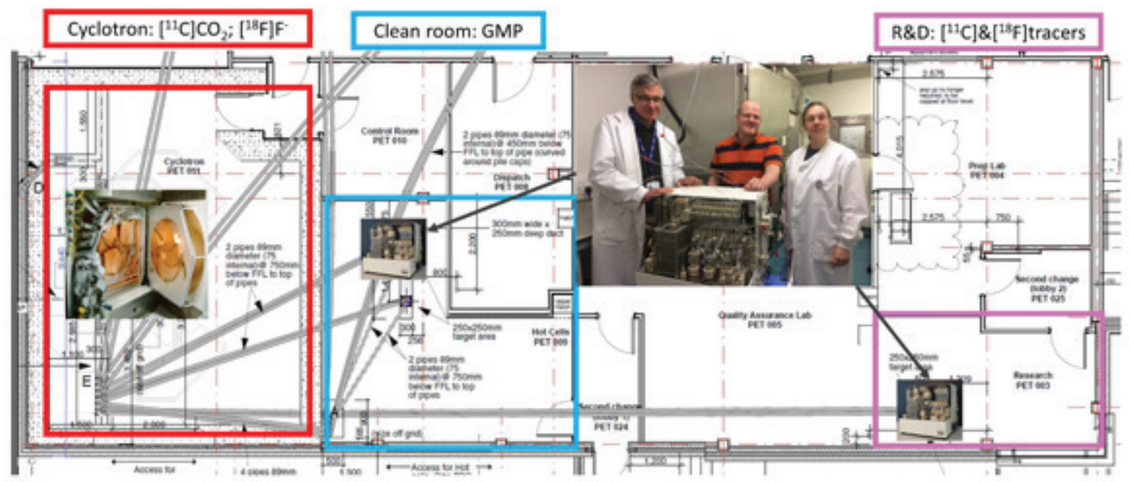


Figure 1
Blueprint for the installation of two identical and universal $^{11}C/^{18}F$ synthesisers (SYNTHRA GmbH, Germany) at the cyclotron facility of the Radiopharmaceutical Unit of the West of Scotland PET Centre, Gartnavel Hospital, allowing rapid clinical translation of new PET radiopharmaceuticals. R&D: development and production of radiotracers for preclinical research. GMP: production of the pharmaceutical-grade material for clinical studies.

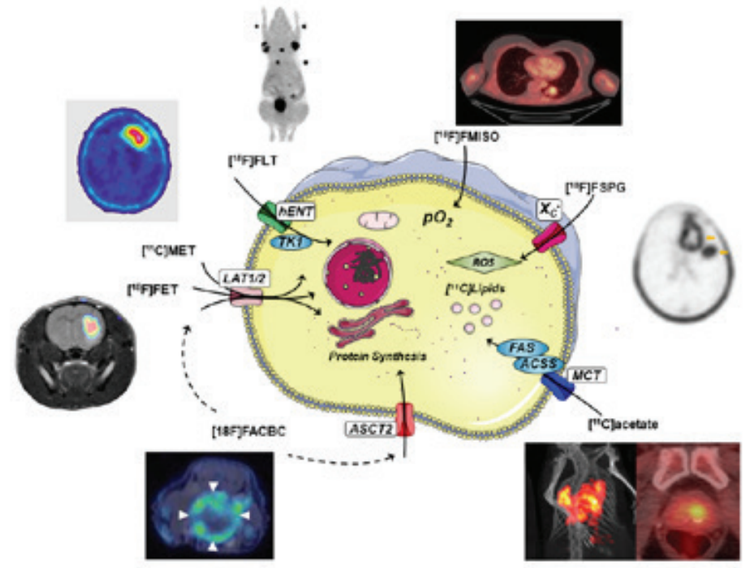


Figure 2
Positron emission tomography images, and cellular uptake mechanisms for several radiotracers for translational cancer imaging. ACE, [^{11}C]acetate; ACSS, acetyl-coenzyme A synthetase; ASCT2, neutral amino acid transporter, SLC1A5; FACBC, trans-1-amino-3-[^{18}F]-fluorocyclobutane-carboxylic acid; FAS, fatty acid synthase; FET, [^{18}F]fluoroethyltyrosine; FLT, 3′-deoxy-3′[^{18}F]-fluorothymidine; FMISO, [^{18}F] fluoromisonidazole; FSPG, (4S)-4-(3-[^{18}F]fluoropropyl)-l-glutamate; hENT, human equilibrative nucleoside transporter, SLC29A1; LAT1/2, large neutral amino acids transporters, SLC7A5 and SLC7A8; MCT, monocarboxylate transporter, SLC16A1; MET, [^{11}C]methionine; pO2, partial pressure of oxygen; ROS, reactive oxygen species; TK1, thymidine kinase 1; X_c^-, cystine/glutamate transporter, SLC7A11.

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.

This year, in collaboration with Hing Leung, we explored whether PET could monitor the effects of androgen deprivation therapy in prostate cancer. We observed reduced uptake of [^{18}F] FACBC, a novel SLC7A5 and SLC1A5 transporter tracer, in castrate-resistant compared to castrate-sensitive prostate cancer orthografts, suggesting that FACBC could be used for detecting the emergence of treatment resistance. This work was presented at the World Molecular Imaging Conference in Philadelphia. This year we will be exploring the role of PET/MRI in phenotyping subtypes of colon cancer and studying metabolic progression in breast and pancreatic tumourigenesis.

Publications listed on page 99

CRUK BEATSON INSTITUTE

RESEARCH FACILITIES PUBLICATIONS AND OPERATIONS

RESEARCH FACILITIES

Head of Research Facilities
Sue Fowler

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 Kernahan, John Trivett

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 additional office space.

Central Services

Margaret Laing (Supervisor), Elizabeth Cheetham, Dilhani 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.

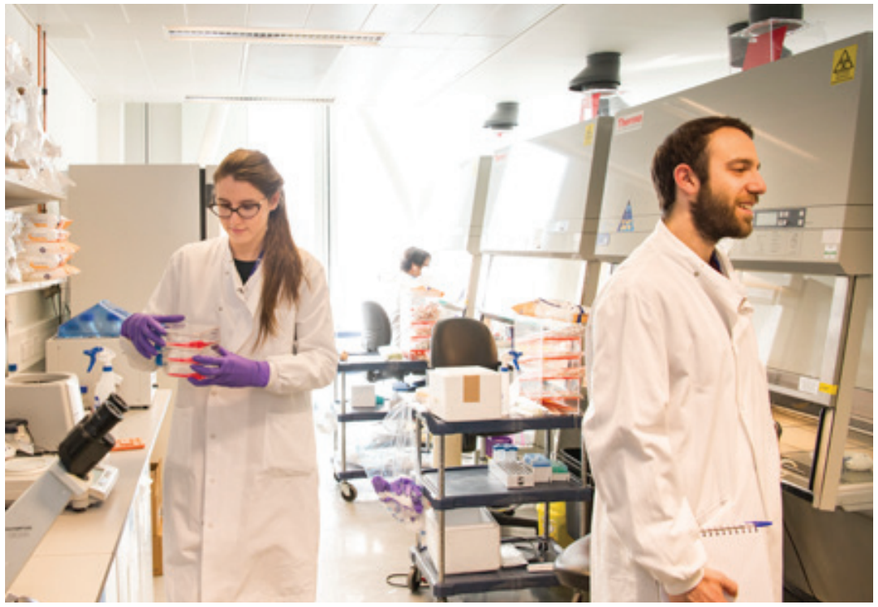
Flow Cytometry

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 Whitelaw, Fiona McGregor, Gemma Thomson, Mark Hughes, Saira Ghafoor, Shauna Currie Kerr, Vivienne 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. All sections are stained with haematoxylin and eosin for a general analysis of cell morphology and structure before more specialised histology stains are used to investigate specific tissue structures.

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 fluorescent images. This enables high-quality digital images to be scanned and stored and, if required, quantification to be performed. The image analysis software 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, Iain 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 test and production applications. This 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 analysis or other computationally intense applications. We currently provide virtual desktops for OS X users requiring access to Windows-based packages. We have rolled out vApps for specific imaging, proteomics and metabolomics applications. Significant investment has been put into creating documentation and procedures to allow us to run the service in a manner commensurate with ITIL. This is used as the foundation of our business continuity documentation and has led to the revision and ongoing improvement of many of our day-to-day working practices. Our intranet uses a content management system framework, allowing service managers and support departments the ability to easily upload forms and information for users.

A range of replacement hardware is stored on-site to allow fast repairs. A good selection of loan IT hardware, from USB drives to digital projectors, is held centrally. We provide video conferencing facilities, enabling conference calls between the Institute and other CRUK sites as well as many other locations. Audio-visual support services for large conferences have been provided at a number of international venues, as well as overseeing the in-house 178-seat theatre.

Laboratory Management

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

Laboratory Management 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 risk 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 starts 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 dealt with as efficiently and effectively as possible. Service contracts for core equipment are reviewed annually and procured centrally to ensure costs are kept as low as reasonably practical. We have effective procurement processes and liaise with Cancer Research UK purchasing to take advantage of any centralised agreements. We also have a good relationship with suppliers to ensure we achieve best prices and discounts for goods. 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 FX^{P} 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 Qiagen 8000 Biorobot. 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 mycoplasmal enzymes. Hoechst staining to detect the presence of mycoplasma DNA, enzyme immunoassay against the four most common species of mycoplasma or a colorimetric microplate assay to detect 16S ribosomal mycoplasma RNA can also be used.

The facility prepares cell-derived matrices from Tiff 5 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 10X TBST and bacterial growth reagents. Each product is tested for suitability of use and sterility where possible before being released for general stock. The preparation of antibiotic bacterial culture plates has been automated using a Mediaclave (Integra Biosciences AG) to sterilise and dispense into the plates.

PUBLICATIONS

Imran Ahmad (page 16)
Models of Advanced Prostate Cancer

Primary Research Papers

Loveridge, CJ, Mui EJ, Patel R, Tan EH, Ahmad I, Welsh M, Galbraith J, Hedley A, Nixon C, Blyth K, Sansom O, Leung HY.
Increased T cell infiltration elicited by Erk5 deletion in a Pten-deficient mouse model of prostate carcinogenesis. ***Canc Res*** 2017; 77: 3158–68

Loveridge, CJ, van't Hof RJ, Charlesworth G, King A, Tan EH, Rose L, Daroszewska A, Prior A, Ahmad I, Welsh M, Mui EJ, Ford C, Salji M, Sansom O, Blyth K, Leung HY.
Analysis of Nkx3.1:Cre-driven Erk5 deletion reveals a profound spinal deformity which is linked to increased osteoclast activity. ***Sci Rep*** 2017; 7: 13241

Salji, M, Hendry, J, Patel, A, Ahmad, I, Nixon, C and Leung, HY.
Peri-prostatic fat volume measurement as a predictive tool for castration resistance in advanced prostate cancer. ***Eur Urol Focus***. 2017 Mar 1. pii: S2405-4569(17)30028-7

Tan WS, Lamb BW, Tan MY, Ahmad I, Sridhar A, Nathan S, Hines J, Shaw G, Briggs TP, Kelly JD.
In-depth analysis of complications following Robot-assisted radical cystectomy with intracorporeal urinary diversion. ***Eur Urol Focus***. 2017; 3: 273–9.

Peter Adams (page 18)
Epigenetics of Cancer and Ageing

Primary Research Papers

Cole JJ, Robertson NA, Rather MI, Thomson JP, McBryan T, Sproul D, Wang T, Brock C, Clark W, Ideker T, Meehan RR, Miller RA, Brown–Borg HM, Adams PD.
Diverse interventions that extend mouse lifespan suppress shared age-associated epigenetic changes at critical gene regulatory regions. ***Genome Biol*** 2017; 18: 58

Dou Z, Ghosh K, Vizioli MG, Zhu J, Sen P, Wangensteen KJ, Simithy J, Lan Y, Lin Y, Zhou Z, Capell BC, Xu C, Xu M, Kieckhaefer JE, Jiang T, Shoshkes-Carmel M, Tanim K, Barber GN, Seykora JT, Millar SE, Kaestner KH, Garcia BA, Adams PD, Berger SL.
Cytoplasmic chromatin triggers inflammation in senescence and cancer. ***Nature*** 2017; 550: 402–6

Leung EY, McMahon JD, McLellan D, Syyed N, McCarthy CE, Nixon C, Orange C, Brock C, Hunter K, Adams PD.
DNA damage marker gammaH2AX is a potential predictive marker for progression of epithelial dysplasia of the oral cavity. ***Histopathology*** 2017; 71: 522–8

Piscitello D, Varshney D, Lilla S, Vizioli MG, Reid C, Gorbunova V, Seluanov A, Gillespie DA, Adams PD.
AKT overactivation can suppress DNA repair via p70S6 kinase-dependent downregulation of MRE11. ***Oncogene*** 2017; 37: 427–38.

Rai TS, Glass M, Cole JJ, Rather MI, Marsden M, Neilson M, Brock C, Humphreys IR, Everett RD, Adams PD.
Histone chaperone HIRA deposits histone H3.3 onto foreign viral DNA and contributes to anti-viral intrinsic immunity. ***Nucleic Acids Res*** 2017: 45: 11673–83.

van Tuyn J, Jaber-Hijazi F, MacKenzie D, Cole JJ, Mann E, Pawlikowski JS, Singh Rai T, Nelson DM, McBryan T, Ivanov A, Blyth K, Wu H, Milling S, Adams PD.
Oncogene-Expressing Senescent Melanocytes Upregulate Mhc Class Ii, A Candidate Melanoma Suppressor Function. ***J Invest Dermatol*** 2017; 137: 2197–207

Wang T, Tsui B, Kreisberg JF, Robertson NA, Gross AM, Yu MK, Carter H, Brown-Borg HM, Adams PD, Ideker T.
Epigenetic aging signatures in mice livers are slowed by dwarfism, calorie restriction and rapamycin treatment. ***Genome Biol*** 2017; 18: 57

Other Publications

Field AE, Adams PD.
Targeting chromatin aging - The epigenetic impact of longevity-associated interventions. ***Exp Gerontol*** 2017; 94: 29–33

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

Tom Bird (page 42)
Liver Disease and Regeneration

Primary Research Papers

Ogrodnik M, Miwa S, Tchkonia T, Tiniakos D, Wilson CL, Lahat A, Day CP, Burt A, Palmer A, Anstee QM, Grellscheid SN, Hoeijmakers JHJ, Barnhoorn S, Mann DA, Bird TG, Vermeij WP, Kirkland JL, Passos JF, von Zglinicki T, Jurk D.
Cellular senescence drives age-dependent hepatic steatosis. ***Nat Commun*** 2017; 8: 15691

Other Publications

Kelly S, Bird TG.
The Evolution of the Use of Serum Alpha-fetoprotein in Clinical Liver Cancer Surveillance. ***J Immunobiol*** 2016 Dec 31;1. pii: 1000116.

Karen Blyth (page 80)
Transgenic Models of Cancer

Primary Research Papers

Bulusu V, Tumanov S, Michalopoulou E, van den Broek NJ, MacKay G, Nixon C, Dhayade S, Schug ZT, Vande Voorde J, Blyth K, Gottlieb E, Vazquez A, Kamphorst JJ.
Acetate Recapturing by Nuclear Acetyl-CoA Synthetase 2 Prevents Loss of Histone Acetylation during Oxygen and Serum Limitation. ***Cell Rep*** 2017; 18: 647–58

Campbell KJ, Dhayade S, Ferrari N, Sims AH, Johnson E, Mason SM, Dickson A, Ryan KM, Kalna G, Edwards J, Tait SWG, Blyth K.
MCL-1 is a prognostic indicator and drug target in breast cancer. ***Cell Death Dis*** 2018; 9: 19. doi: 10.1038/s41419-017-0035-2. [Epub ahead of print]

Dornier E, Rabas N, Mitchell L, Novo D, Dhayade S, Marco S, Mackay G, Sumpton D, Pallares M, Nixon C, Blyth K, Macpherson I, Rainero E, Norman JC.
Glutaminolysis drives membrane trafficking to promote cancer invasion. ***Nat Commun*** 2017; 8: 2255.

Giampazolias E, Zunino B, Dhayade S, Bock F, Cloix C, Cao K, Roca A, Lopez J, Ichim G, Proics E, Rubio-Patino C, Fort L, Yatim N, Woodham E, Orozco S, Taraborrelli L, Peltzer N, Lecis D, Machesky L, Walczak H, Albert ML, Milling S, Oberst A, Ricci JE, Ryan KM, Blyth K, Tait SWG.
Mitochondrial permeabilization engages NF-kappaB-dependent anti-tumour activity under caspase deficiency. ***Nat Cell Biol*** 2017; 19: 1116–29

Hernandez-Fernaud JR, Ruengeler E, Casazza A, Neilson LJ, Pulleine E, Santi A, Ismail S, Lilla S, Dhayade S, MacPherson IR, McNeish I, Ennis D, Ali H, Kugeratski FG, Al Khamici H, van den Biggelaar M, van den Berghe PV, Cloix C, McDonald L, Millan D, Hoyle A, Kuchnio A, Carmeliet P, Valenzuela SM, Blyth K, Yin H, Mazzone M, Norman JC, Zanivan S.
Secreted CLIC3 drives cancer progression through its glutathione-dependent oxidoreductase activity. ***Nat Commun*** 2017; 8: 14206

Hock AK, Cheung EC, Humpton TJ, Monteverde T, Paulus-Hock V, Lee P, McGhee E, Scopelliti A, Murphy DJ, Strathdee D, Blyth K, Vousden KH.
Development of an inducible mouse model of iRFP713 to track recombinase activity and tumour development *in vivo*. ***Sci Rep*** 2017; 7: 1837

Loveridge CJ, Mui EJ, Patel R, Tan EH, Ahmad I, Welsh M, Galbraith J, Hedley A, Nixon C, Blyth K, Sansom O, Leung HY.
Increased T cell infiltration elicited by *Erk5* deletion in a *Pten*-deficient mouse model of prostate carcinogenesis. ***Cancer Res*** 2017; 77: 3158–68

Loveridge CJ, van 't Hof RJ, Charlesworth G, King A, Tan EH, Rose L, Daroszewska A, Prior A, Ahmad I, Welsh M, Mui EJ, Ford C, Salji M, Sansom O, Blyth K, Leung HY.
Analysis of Nkx3.1:Cre-driven Erk5 deletion reveals a profound spinal deformity which is linked to increased osteoclast activity. ***Sci Rep*** 2017; 7: 13241

Maddocks ODK, Athineos D, Cheung EC, Lee P, Zhang T, van den Broek NJF, Mackay GM, Labuschagne CF, Gay D, Kruiswijk F, Blagih J, Vincent DF, Campbell KJ, Ceteci F, Sansom

OJ, Blyth K, Vousden KH.
Modulating the therapeutic response of tumours to dietary serine and glycine starvation. ***Nature*** 2017; 544: 372–6

McCarroll CS, He W, Foote K, Bradley A, McGlynn K, Vidler F, Nixon C, Nather K, Fattah C, Riddell AH, Bowman P, Elliott EB, Bell M, Hawksby C, MacKenzie SM, Morrison LJ, Terry A, Blyth K, Smith GL, McBride MW, Kubin T, Braun T, Nicklin SA, Cameron ER, Loughrey CM.
Runx1 Deficiency Protects Against Adverse Cardiac Remodeling Following Myocardial Infarction. ***Circulation*** 2018; 137: 57–70. doi: 0.1161/CIRCULATIONAHA.117.028911. Epub 13 Oct 2017

Reid SE, Kay EJ, Neilson LJ, Henze AT, Serneels J, McGhee EJ, Dhayade S, Nixon C, Mackey JB, Santi A, Swaminathan K, Athineos D, Papalazarou V, Patella F, Roman-Fernandez A, ElMaghloob Y, Hernandez-Fernaud JR, Adams RH, Ismail S, Bryant DM , Salmeron-Sanchez M, Machesky LM, Carlin LM, Blyth K, Mazzone M,7, Zanivan S.
Tumor matrix stiffness promotes metastatic cancer cell interaction with the endothelium. ***EMBO J*** 2017; 36: 2373–89

Rumney RMH, Coffelt SB, Neale TA, Dhayade S, Tozer GM, Miller G.
PyMT-Maclow: A novel, inducible, murine model for determining the role of CD68 positive cells in breast tumor development. ***PLoS One*** 2017; 12: e0188591

van Tuyn J, Jaber-Hijazi F, MacKenzie D, Cole JJ, Mann E, Pawlikowski JS, Singh Rai T, Nelson DM, McBryan T, Ivanov A, Blyth K, Wu H, Milling S, Adams PD.
Oncogene-Expressing Senescent Melanocytes Upregulate Mhc Class Ii, A Candidate Melanoma Suppressor Function. J ***Invest Dermatol*** 2017; 137: 2197–207

Walton JB, Farquharson M, Mason S, Port J, Kruspig B, Dowson S, Stevenson D, Murphy D, Matzuk M, Kim J, Coffelt S, Blyth K, McNeish IA.
CRISPR/Cas9-derived models of ovarian high grade serous carcinoma targeting Brca1, Pten and Nf1, and correlation with platinum sensitivity. ***Sci Rep*** 2017; 7: 16827

Weigert M, Binks A, Dowson S, Leung EYL, Athineos D, Yu X, Mullin M, Walton JB, Orange C, Ennis D, Blyth K, Tait SWG, McNeish IA.
RIPK3 promotes adenovirus type 5 activity. ***Cell Death and Disease*** 2017; 8: 3206

Other Publications

Holen I, Speirs V, Morrissey B, Blyth K.
In vivo models in breast cancer research: progress, challenges and future directions. ***Dis Model Mech*** 2017; 10: 359–71

Morrissey B, Blyth K, Carter P, Chelala C, Jones L, Holen I, Speirs V.
The Sharing Experimental Animal Resources, Coordinating Holdings (SEARCH) Framework: Encouraging Reduction, Replacement, and Refinement in Animal Research. ***PLoS Biol*** 2017 15: e2000719

Riggio AI, Blyth K.
The Enigmatic Role of RUNX1 in Female-Related Cancers: Current Knowledge & Future Perspectives. ***FEBS J*** 2017; 284: 2345–62

Rooney N, Riggio AI, Mendoza-Villanueva D, Shore P, Cameron ER, Blyth K.
Runx Genes in Breast Cancer and the Mammary Lineage. ***Adv Exp Med Biol*** 2017; 962: 353–68

David Bryant (page 44)

Molecular Control of Epithelial Polarity

Primary Research Papers

Datta A, Sandilands E, Mostov KE, Bryant DM.
Fibroblast-derived HGF drives acinar lung cancer cell polarization through integrin-dependent RhoA-ROCK1 inhibition. ***Cell Signal*** 2017; 40: 91–8

Gao L, Yang Z, Hiremath C, Zimmerman SE, Long B, Brakeman PR, Mostov KE, Bryant DM, Luby-Phelps K, Marciano DK.
Afadin orients cell division to position the tubule lumen in developing renal tubules. ***Development*** 2017; 144: 3511–20

Reid SE, Kay EJ, Neilson LJ, Henze AT, Serneels J, McGhee EJ, Dhayade S, Nixon C, Mackey JB, Santi A, Swaminathan K, Athineos D, Papalazarou V, Patella F, Roman-Fernandez A, ElMaghloob Y, Hernandez-Fernaud JR, Adams RH, Ismail S, Bryant DM, Salmeron-Sanchez M, Machesky LM, Carlin LM, Blyth K, Mazzone M,7, Zanivan S.
Tumor matrix stiffness promotes metastatic cancer cell interaction with the endothelium. ***EMBO J*** 2017; 36: 2373–89

Ruch TR, Bryant DM, Mostov KE, Engel JN.
Par3 integrates Tiam1 and phosphatidylinositol 3-kinase signaling to change apical membrane identity. ***Mol Biol Cell*** 2017; 28: 252–60

Other Publications

Bryant D, Johnson A.
Meeting report - Intercellular interactions in context: towards a mechanistic understanding of cells in organs. ***J Cell Sci*** 2017; 130: 2083–5

Leo Carlin (pages 46 & 70)

Leukocyte Dynamics & Beatson Advanced Imaging Resource (BAIR)

Primary Research Papers

Duarte D, Hawkins ED, Akinduro O, Ang H, De Filippo K, Kong IY, Haltalli M, Ruivo N, Straszkowski L, Vervoort SJ, McLean C, Weber TS, Khorshed R, Pirillo C, Wei A, Ramasamy SK, Kusumbe AP, Duffy K, Adams RH, Purton LE, Carlin LM, Lo Celso C.
Inhibition of endosteal vascular niche remodeling rescues hematopoietic stem cell loss in AML. ***Cell Stem Cell*** 2018; 22: 64–77.e6. doi: 10.1016/j.stem.2017.11.006. Epub 21 Dec 2017.

Irshad S, Flores-Borja F, Lawler K, Monypenny J, Evans R, Male V, Gordon P, Cheung A, Gazinska P, Noor F, Wong F, Grigoriadis A, Fruhwirth GO, Barber PR, Woodman N, Patel D, Rodriguez-Justo M, Owen J, Martin SG, Pinder SE, Gillett CE, Poland SP, Ameer-Beg S, McCaughan F, Carlin LM, Hasan U, Withers DR, Lane P, Vojnovic B, Quezada SA, Ellis P, Tutt AN, Ng T.
RORgammat(+) Innate Lymphoid Cells Promote Lymph Node Metastasis of Breast Cancers. ***Cancer Res*** 2017; 77: 1083–96

Karadjian G, Fercoq F, Pionnier N, Vallarino-Lhermitte N, Lefoulon E, Nieguitsila A, Specht S, Carlin LM, Martin C.
Migratory phase of Litomosoides sigmodontis filarial infective larvae is associated with pathology and transient increase of S100A9 expressing neutrophils in the lung. ***PLoS Negl Trop Dis*** 2017; 11: e0005596

Reid SE, Kay EJ, Neilson LJ, Henze AT, Serneels J, McGhee EJ, Dhayade S, Nixon C, Mackey JB, Santi A, Swaminathan K, Athineos D, Papalazarou V, Patella F, Roman-Fernandez A, ElMaghloob Y, Hernandez-Fernaud JR, Adams RH, Ismail S, Bryant DM, Salmeron-Sanchez M, Machesky LM, Carlin LM, Blyth K, Mazzone M,7, Zanivan S.
Tumor matrix stiffness promotes metastatic cancer cell interaction with the endothelium. ***EMBO J*** 2017; 36: 2373–89

Other Publications

Secklehner J, Lo Celso C, Carlin LM.
Intravital microscopy in historic and contemporary immunology. ***Immunol Cell Biol*** 2017; 95: 506–13

Seth Coffelt (page 48)

Immune Cells and Metastasis

Primary Research Papers

Carron EC, Homra S, Rosenberg J, Coffelt SB, Kittrell F, Zhang Y, Creighton CJ, Fuqua SA, Medina D, Machado HL.
Macrophages promote the progression of premalignant mammary lesions to invasive cancer. ***Oncotarget*** 2017; 8: 50731–46

Kersten K, Coffelt SB, Hoogstraat M, Verstegen NJM, Vrijland K, Ciampricotti M, Doornebal CW, Hau CS, Wellenstein MD, Salvagno C, Doshi P, Lips EH, Wessels LFA, de Visser KE.
Mammary tumor-derived CCL2 enhances pro-metastatic systemic inflammation through upregulation of IL1β in tumor-associated macrophages. ***Oncoimmunology*** 2017 6: e1334744

Rumney RMH, Coffelt SB, Neale TA, Dhayade S, Tozer GM, Miller G.
PyMT-Maclow: A novel, inducible, murine model for determining the role of CD68 positive cells in breast tumor development. ***PLoS One*** 2017; 12: e0188591

van Baal J, Van de Vijver KK, Coffelt SB, van der Noort V, van Driel WJ, Kenter GG, Buist MR, Lok C.
Incidence of lymph node metastases in clinical early-stage mucinous and seromucinous ovarian carcinoma: a retrospective cohort study. ***BJOG*** 2017; 124: 486–94

Walton JB, Farquharson M, Mason S, Port J, Kruspig B, Dowson S, Stevenson D, Murphy D, Matzuk M, Kim J, Coffelt S, Blyth K, McNeish IA.
CRISPR/Cas9-derived models of ovarian high grade serous carcinoma targeting Brca1, Pten and Nf1, and correlation with platinum sensitivity. ***Sci Rep*** 2017; 7: 16827

Justin Bower & Heather McKinnon (page 66)

Drug Discovery Unit

Gray CH, Konczal J, Mezna 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 RalB. ***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; 133: 160–9

Other Publications

MRCK Inhibitors UK Patent Application Number 1713319.0 filed August 18th, 2017

MRCK Inhibitors UK Patent Application Number 1713318.2 filed 18th August, 2017

Jeff Evans (page 6)

Primary Research Papers

Carotenuto P, Fassan M, Pandolfo R, Lampis A, Vicentini C, Cascione L, Paulus-Hock V, Boulter L, Guest R, Quagliata L, Hahne JC, Ridgway R, Jamieson T, Athineos D, Veronese A, Visone R, Murgia C, Ferrari G, Guzzardo V, Evans TRJ, MacLeod M, Feng GJ, Dale T, Negrini M, Forbes SJ, Terracciano L, Scarpa A, Patel T, Valeri N, Workman P, Sansom O, Braconi C.
Wnt signalling modulates transcribed-ultraconserved regions in hepatobiliary cancers. ***Gut*** 2017; 66: 1268–77

Cook N, Basu B, Smith D-M, Gopinathan A, Evans TRJ, Steward WP, Palmer D, Propper D, Venugopal B, Hategan M, Anthoney DA, Hampson LV, Nebozhyn M, Tuveson D, Farmer-Hall H, Turner H, McLeod R, Halford S, Jodrell DA.
Phase I trial of the γ-secretase inhibitor MK-0752 in combination with gemcitabine in patients with pancreatic ductal adenocarcinoma. ***Br J Cancer*** 2018 Feb 13. doi: 10.1038/bjc.2017.495. [Epub ahead of print]

de la Cruz-Merino L, Di Guardo L, Grob J-J, Venosa A, Larkin J, McArthur GA, Ribas A, Ascierto PA, Evans TRJ, Gomez-Escobar A, Barteselli G, Eng S, Hsu JJ, Uyei A, Dréno B.
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

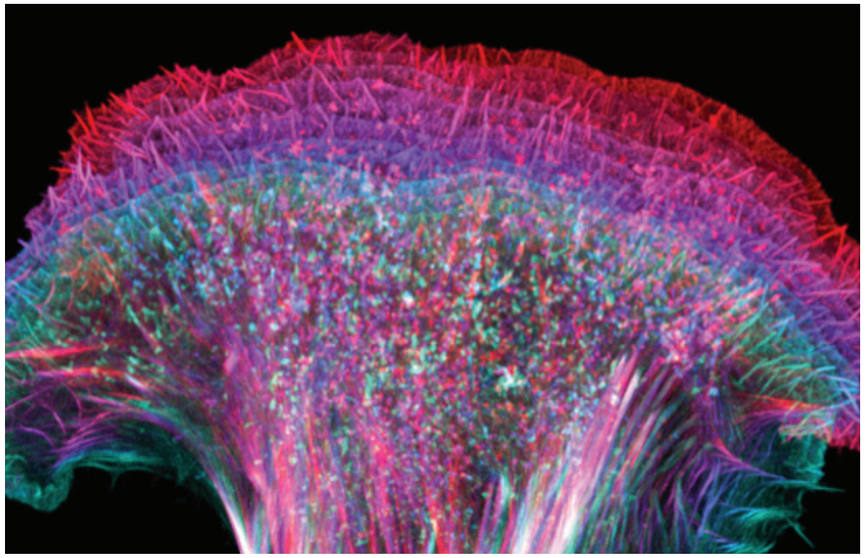
Evans TRJ, Van Cutsem E, Moore MJ, Bazin IS, Rosemurgy A, Bodoky G, Deplanque G, Harrison M, Melichar B, Pezet D, Elekes A, Rock E, Lin C, Strauss L, O'Dwyer PJ.
Phase 2 placebo-controlled, double-blind trial of dasatinib added to gemcitabine for patients with locally-advanced pancreatic cancer. ***Ann Oncol*** 2017; 28: 354–61

Kudo M, Finn R, Qin S, Han K-H, Ikeda K, Piscaglia F, Baron A, Park J-W, Han G, Jassem J, Blanc JF, Vogel A, Komov D, Evans, TRJ, Lopez C, Dutcus C, Guo M, Saito K, Kraljevic S, Tamai T, Ren M, Cheng, A-L.
A randomised phase 3 trial of lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma. ***Lancet*** 2018 Feb 9. pii: S0140-6736(18)30207-1. doi: 10.1016/S0140-6736(18)30207-1. [Epub ahead of print]

Meyer T, Fox R, Ma YT, Ross PJ, James MW, Sturgess R, Stubbs C, Stocken DD, Wall L, Watkinson A, Hacking N, Evans TRJ, Collins P, Hubner RA, Cunningham D, Primrose JN, Johnson PJ, Palmer DH.
Sorafenib in combination with transarterial chemoembolisation in patients with unresectable hepatocellular carcinoma (TACE 2): a randomised placebo-controlled, double-blind, phase 3 trial. ***Lancet Gastroenterol Hepatol*** 2017; 2: 565–75

Vennin C, Chin VT, Warren SC, Lucas MC, Herrmann D, Magenau A, Melenec P, Walters SN, Del Monte-Nieto G, Conway JR, Nobis M, Allam AH, McCloy RA, Currey N, Pinese M, Boulghourjian A, Zaratzian A, Adam AA, Heu C, Nagrial AM *et al*.
Transient tissue priming via ROCK inhibition uncouples pancreatic cancer progression, sensitivity to chemotherapy, and metastasis. ***Sci Transl Med*** 2017; 9: pii: eaai8504

Wilson RH, Evans TJ, Middleton MR, Molife LR, Spicer J, Dieras V, Roxburgh P, Giordano H, Jaw-Tsai S, Goble S, Plummer R.
A phase I study of intravenous and oral rucaparib in combination with chemotherapy in patients with advanced solid tumours. ***Br J Cancer*** 2017; 116: 88492



Other Publications

Wilson RAM, Evans TRJ, Fraser AR, Nibbs RJB.
Immune Checkpoint Inhibitors: New strategies to checkmate cancer. ***Clin Exp Immunol*** 2018; 191: 133–48. doi: 10.1111/cei.13081. Epub 27 Dec 2017.

Eyal Gottlieb (page 20)

Tumour Metabolism

Primary Research Papers

Bulusu V, Tumanov S, Michalopoulou E, van den Broek NJ, MacKay G, Nixon C, Dhayade S, Schug ZT, Vande Voorde J, Blyth K, Gottlieb E, Vazquez A, Kamphorst JJ.
Acetate Recapturing by Nuclear Acetyl-CoA Synthetase 2 Prevents Loss of Histone Acetylation during Oxygen and Serum Limitation. ***Cell Rep*** 2017; 18: 647–58

Fack F, Tardito S, Hochart G, Oudin A, Zheng L, Fritah S, Golebiewska A, Nazarov PV, Bernard A, Hau AC, Keunen O, Leenders W, Lund-Johansen M, Stauber J, Gottlieb E, Bjerkvig R, Niclou SP.
Altered metabolic landscape in IDH-mutant gliomas affects phospholipid, energy, and oxidative stress pathways. ***EMBO Mol Med*** 2017; 9: 1681–95

Kuntz EM, Baquero P, Michie AM, Dunn K, Tardito S, Holyoake TL, Helgason GV, Gottlieb E.
Targeting mitochondrial oxidative phosphorylation eradicates therapy-resistant chronic myeloid leukemia stem cells. ***Nat Med*** 2017 23: 1234–40

Monterisi S, Lobo MJ, Livie C, Castle JC, Weinberger M, Baillie GS, Surdo NC, Musheshe N, Stangherlin A, Gottlieb E, Maizels RJ, Bortolozzi M, Micaroni M, Zaccolo M.
PDE2A2 regulates mitochondria morphology and apoptotic cell death via local modulation of cAMP/PKA signalling. ***Elife*** 2017; 6: pii: e21374

Other Publications

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

Danny Huang (page 22)

Ubiquitin Signalling

Primary Research Papers

Gabrielsen M, Buetow L, Nakasone MA, Ahmed SF, Sibbet GJ, Smith BO2, Zhang W, Sidhu SS, Huang DT.
A General Strategy for Discovery of Inhibitors and Activators of RING and U-box E3 Ligases with Ubiquitin Variants. ***Mol Cell*** 2017; 68: 456–70

Nomura K, Klejnot M, Kowalczyk D, Hock AK, Sibbet GJ, Vousden KH, Huang DT.
Structural analysis of MDM2 RING separates degradation from regulation of p53 transcription activity. ***Nat Struct Mol Biol*** 2017; 24: 578–87

Robert Insall (page 50)
Cell Migration and Chemotaxis

Primary Research Papers

Davidson AJ, Amato C, Thomason PA, Insall RH.
WASP family proteins and formins compete in pseudopod- and bleb-based migration. ***J Cell Biol*** 2018; 217: 701–14. doi: 10.1083/jcb.201705160. Epub 30 Nov 2017.

Ferguson EA, Matthiopoulos J, Insall RH, Husmeier D.
Statistical inference of the mechanisms driving collective cell movement. ***Journal of the Royal Statistical Society: Series C (Applied Statistics)*** 2017; 66: 869–90

Periz J, Whitelaw J, Harding C, Gras S, Del Rosario Minina MI, Latorre-Barragan F, Lemgruber L, Reimer MA, Insall R, Heaslip A, Meissner M.
Toxoplasma gondii F-actin forms an extensive filamentous network required for material exchange and parasite maturation. ***Elife*** 2017; 6: pii: e24119.

Susanto O, Koh YWH, Morrice N, Tumanov S, Thomason PA, Nielson M, Tweedy L, Muinonen-Martin AJ, Kamphorst JJ, Mackay GM, Insall RH.
LPP3 mediates self-generation of chemotactic LPA gradients by melanoma cells. ***J Cell Sci*** 2017; 130: 3455–66

Thomason PA, King JS, Insall RH.
Mroh1, a lysosomal regulator localised by WASH-generated actin. ***J Cell Sci*** 2017; 130: 1785–95

Woodham EF, Paul NR, Tyrrell B, Spence HJ, Swaminathan K, Scribner MR, Giampazolias E, Hedley A, Clark W, Kage F, Marston DJ, Hahn KM, Tait SW, Larue L, Brakebusch CH, Insall RH, Machesky LM.
Coordination by Cdc42 of Actin, Contractility, and Adhesion for Melanoblast Movement in Mouse Skin. ***Curr Biol*** 2017; 27: 624–37

Other Publications

Susanto O, Insall RH.
LPP3, LPA and self-generated chemotactic gradients in biomedical science. ***Comm Integ Biol*** 2017 Dec 14: e1398870. https://doi.org/10.1080/19420889.2017.1398870

Shehab Ismail (page 54)
Structural Biology of Cilia

Primary Research Papers

Gray CH, Konczal J, Mezna 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 RalB. ***Protein Expr Purif*** 2017; 132: 75–84

Hernandez-Fernaud JR, Ruengeler E, Casazza A, Neilson LJ, Pulleine E, Santi A, Ismail S, Lilla S, Dhayade S, MacPherson IR, McNeish I, Ennis D, Ali H, Kugeratski FG, Al Khamici H, van den Biggelaar M, van den Berghe PV, Cloix C, McDonald L, Millan D, Hoyle A, Kuchnio A, Carmeliet P, Valenzuela SM, Blyth K, Yin H, Mazzone M, Norman JC, Zanivan S.
Secreted CLIC3 drives cancer progression through its glutathione-dependent oxidoreductase activity. ***Nat Commun*** 2017; 8: 14206

Martín-Gago P, Fansa EK, Klein CH, Murarka S, Janning P, Schürmann M, Metz M, Ismail S, Schultz-Fademrecht C, Baumann M, Bastiaens PIH, Wittinghofer A, Waldmann H.
A PDE6delta-KRas Inhibitor Chemotype with up to Seven H-Bonds and Picomolar Affinity that Prevents Efficient Inhibitor Release by Arl2. ***Angew Chem*** 2017; 129: 2463

Murarka S, Martin-Gago P, Schultz-Fademrecht C, Al Saabi A, Baumann M, Fansa EK, Ismail S, Nussbaumer P, Wittinghofer A, Waldmann H.
Development of Pyridazinone Chemotypes Targeting the PDEdelta Prenyl Binding Site. ***Chemistry*** 2017; 23: 6083–93

Reid SE, Kay EJ, Neilson LJ, Henze AT, Serneels J, McGhee EJ, Dhayade S, Nixon C, Mackey JB, Santi A, Swaminathan K, Athineos D, Papalazarou V, Patella F, Roman-Fernandez A, ElMaghloob Y, Hernandez-Fernaud JR, Adams RH, Ismail S, Bryant DM, Salmeron-Sanchez M, Machesky LM, Carlin LM, Blyth K, Mazzone M,7, Zanivan S.
Tumor matrix stiffness promotes metastatic cancer cell interaction with the endothelium. ***EMBO J*** 2017; 36: 2373–89

Other Publications

Ismail S.
A GDI/GDF-like system for sorting and shuttling ciliary proteins. ***Small GTPases*** 2017; 8: 208–11

Stephen L, Elmaghloob Y, Ismail S.
Maintaining protein composition in cilia. ***Biol Chem*** 2017; 399: 1–11

Jurre Kamphorst (page 24)
Cancer Metabolomics

Primary Research Papers

Bulusu V, Tumanov S, Michalopoulou E, van den Broek NJ, MacKay G, Nixon C, Dhayade S, Schug ZT, Vande Voorde J, Blyth K, Gottlieb E, Vazquez A, Kamphorst JJ.
Acetate Recapturing by Nuclear Acetyl-CoA Synthetase 2 Prevents Loss of Histone Acetylation during Oxygen and Serum Limitation. ***Cell Rep*** 2017; 18: 647–58

Rajeshkumar NV, Yabuuchi S, Pai SG, De Oliveira E, Kamphorst JJ, Rabinowitz JD, Tejero H, Al-Shahrour F, Hidalgo M, Maitra A, Dang CV.
Treatment of Pancreatic Cancer Patient-Derived Xenograft Panel with Metabolic Inhibitors Reveals Efficacy of Phenformin. ***Clin Cancer Res*** 2017; 23: 5639–47

Susanto O, Koh YWH, Morrice N, Tumanov S, Thomason PA, Nielson M, Tweedy L, Muinonen-Martin AJ, Kamphorst JJ, Mackay GM, Insall RH.
LPP3 mediates self-generation of chemotactic LPA gradients by melanoma cells. ***J Cell Sci*** 2017; 130: 3455–66

Other Publications

Kamphorst JJ, Lewis IA.
Editorial overview: Recent innovations in the metabolomics revolution. ***Curr Opin Biotechnol*** 2017; 43: iv-vii. doi: 10.1016/j.copbio.2017.01.005.

Tumanov S, Kamphorst JJ.
Recent advances in expanding the coverage of the lipidome. ***Curr Opin Biotechnol*** 2017; 43: 127–33

Hing Leung (page 26)
Prostate Cancer Biology

Primary Research Papers

Loveridge CJ, Mui EJ, Patel R, Tan EH, Ahmad I, Welsh M, Galbraith J, Hedley A, Nixon C, Blyth K, Sansom O, Leung HY.
Increased T-cell Infiltration Elicited by Erk5 Deletion in a Pten-Deficient Mouse Model of Prostate Carcinogenesis. ***Cancer Res*** 2017; 77: 3158–68

Loveridge CJ, van 't Hof RJ, Charlesworth G, King A, Tan EH, Rose L, Daroszewska A, Prior A, Ahmad I, Welsh M, Mui EJ, Ford C, Salji M, Sansom O, Blyth K, Leung HY.
Analysis of Nkx3.1:Cre-driven Erk5 deletion reveals a profound spinal deformity which is linked to increased osteoclast activity. ***Sci Rep*** 2017; 7: 13241

Munkley J, McClurg UL, Livermore KE, Ehrmann I, Knight B, McCullagh P, McGrath J, Crundwell M, Harries LW, Leung HY, Mills IG, Robson CN, Rajan P, Elliott DJ.
The cancer-associated cell migration protein TSPAN1 is under control of androgens and its upregulation increases prostate cancer cell migration. ***Sci Rep*** 2017; 7: 5249

Patek S, Willder J, Heng J, Taylor B, Horgan P, Leung H, Underwood M, Edwards J.
Androgen receptor phosphorylation status at serine 578 predicts poor outcome in prostate cancer patients. ***Oncotarget*** 2017; 8: 4875–87

Salji M, Hendry J, Patel A, Ahmad I, Nixon C, Leung HY.
Peri-prostatic Fat Volume Measurement as a Predictive Tool for Castration Resistance in Advanced Prostate Cancer. ***Eur Urol Focus*** 2017 Mar 1. pii: S2405-4569(17)30028-7. doi: 10.1016/j.euf.2017.01.019.

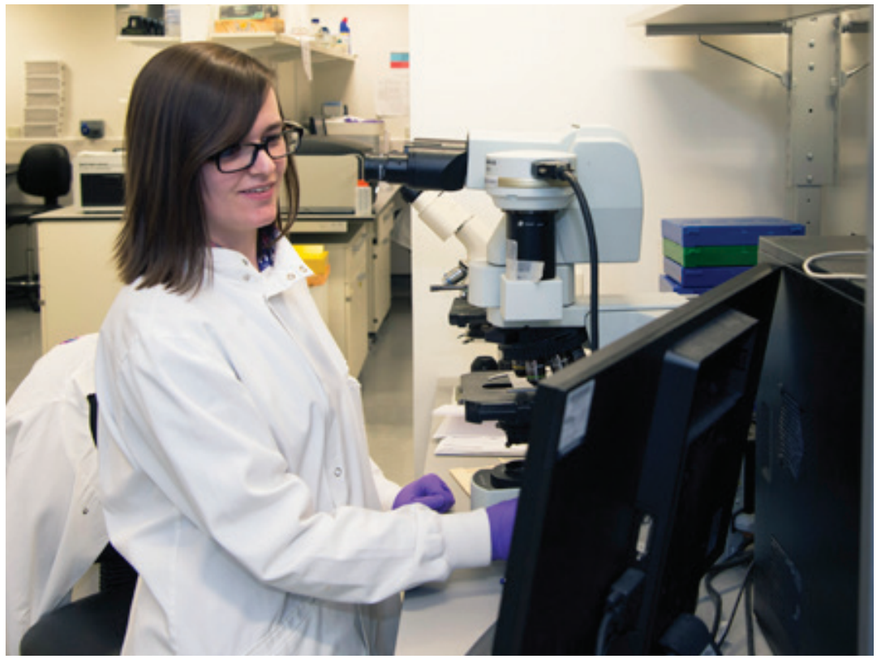
David Lewis (pages 28 & 84)
Molecular Imaging & Translational Molecular Imaging

Primary Research Papers

Neves AA, Xie B, Fawcett S, Alam IS, Witney TH, de Backer MM, Summers J, Hughes W, McGuire S, Soloviev D, Miller J, Howat WJ, Hu DE, Rodrigues TB, Lewis DY, Brindle KM.
Rapid Imaging of Tumor Cell Death *in vivo* using the C2A domain of Synaptotagmin-I. ***J Nucl Med*** 2017; 58: 881–7

Other Publications

Brindle KM, Izquierdo-Garcia JL, Lewis DY, Mair RJ, Wright AJ.
Brain Tumor Imaging. ***J Clin Oncol*** 2017; 35: 2432–8



Laura Machesky (page 54)
Migration, Invasion and Metastasis

Primary Research Papers

Giampazolias E, Zunino B, Dhayade S, Bock F, Cloix C, Cao K, Roca A, Lopez J, Ichim G, Proics E, Rubio-Patino C, Fort L, Yatim N, Woodham E, Orozco S, Taraborrelli L, Peltzer N, Lecis D, Machesky L, Walczak H, Albert ML, Milling S, Oberst A, Ricci JE, Ryan KM, Blyth K, Tait SWG.
Mitochondrial permeabilization engages NF-kappaB-dependent anti-tumour activity under caspase deficiency. ***Nat Cell Biol*** 2017; 19: 1116–29

Reid SE, Kay EJ, Neilson LJ, Henze AT, Serneels J, McGhee EJ, Dhayade S, Nixon C, Mackey JB, Santi A, Swaminathan K, Athineos D, Papalazarou V, Patella F, Roman-Fernandez A, ElMaghloob Y, Hernandez-Fernaud JR, Adams RH, Ismail S, Bryant DM, Salmeron-Sanchez M, Machesky LM, Carlin LM, Blyth K, Mazzone M,7, Zanivan S.
Tumor matrix stiffness promotes metastatic cancer cell interaction with the endothelium. ***EMBO J*** 2017; 36: 2373–89

Thomas SG, Poulter NS, Bem D, Finney B, Machesky LM, Watson SP.
The actin binding proteins cortactin and HS1 are dispensable for platelet actin nodule and megakaryocyte podosome formation. ***Platelets*** 2017; 28: 372–9

Woodham EF, Paul NR, Tyrrell B, Spence HJ, Swaminathan K, Scribner MR, Giampazolias E, Hedley A, Clark W, Kage F, Marston DJ, Hahn KM, Tait SW, Larue L, Brakebusch CH, Insall RH, Machesky LM.
Coordination by Cdc42 of Actin, Contractility, and Adhesion for Melanoblast Movement in Mouse Skin. ***Curr Biol*** 2017; 27: 624–37

Other Publications

Machesky L, Braga VMM.
So far, yet so close: alpha-Catenin dimers help migrating cells get together. ***J Cell Biol*** 2017; 216: 3437–9

Gillian Mackay (page 76)
Metabolomics

Primary Research Papers

Bulusu V, Tumanov S, Michalopoulou E, van den Broek NJ, MacKay G, Nixon C, Dhayade S, Schug ZT, Vande Voorde J, Blyth K, Gottlieb E, Vazquez A, Kamphorst JJ.
Acetate Recapturing by Nuclear Acetyl-CoA Synthetase 2 Prevents Loss of Histone Acetylation during Oxygen and Serum Limitation. ***Cell Rep*** 2017; 18: 647–58

Dolfi SC, Medina DJ, Kareddula A, Paratala B, Rose A, Dhami J, Chen S, Ganesan S, Mackay G, Vazquez A, Hirshfield KM.
Riluzole exerts distinct antitumor effects from a metabotropic glutamate receptor 1-specific inhibitor on breast cancer cells. ***Oncotarget*** 2017; 8: 44639–53

Dornier E, Rabas N, Mitchell L, Novo D, Dhayade S, Marco S, Mackay G, Sumpton D, Pallares M, Nixon C, Blyth K, MacPherson I, Rainero E, Norman JC.
Glutaminolysis drives membrane trafficking to promote cancer invasion. ***Nat Comms*** 2017; 8: 2255

Maddocks ODK, Athineos D, Cheung EC, Lee P, Zhang T, van den Broek NJF, Mackay GM, Labuschagne CF, Gay D, Kruiswijk F, Blagih J, Vincent DF, Campbell KJ, Ceteci F, Sansom OJ, Blyth K, Vousden KH.
Modulating the therapeutic response of tumours to dietary serine and glycine starvation. ***Nature*** 544: 372-376

Susanto O, Koh YWH, Morrice N, Tumanov S, Thomason PA, Nielson M, Tweedy L, Muinonen-Martin AJ, Kamphorst JJ, Mackay GM, Insall RH.
LPP3 mediates self-generation of chemotactic LPA gradients by melanoma cells. ***J Cell Sci*** 2017; 130: 3455–66

Jennifer Morton (page 78)
Preclinical Precision Pancreas

Primary Research Papers

Chou A, Froio D, Nagrial AM, Parkin A, Murphy KJ, Chin VT, Wohl D, Steinmann A, Stark R, Drury A, Walters SN, Vennin C, Burgess A, Pinese M, Chantrill LA, Cowley MJ, Molloy TJ, Australian Pancreatic Cancer Genome I, Waddell N, Johns A, Grimmond SM, Chang DK, Biankin AV, Sansom OJ, Morton JP, Grey ST, Cox TR, Turchini J, Samra J, Clarke SJ, Timpson P, Gill AJ, Pajic M.
Tailored first-line and second-line CDK4-targeting treatment combinations in mouse models of pancreatic cancer. ***Gut*** 2017 Oct 28. pii: gutjnl-2017-315144. doi: 10.1136/gutjnl-2017-315144. [Epub ahead of print]

Chuvin N, Vincent DF, Pommier RM, Alcaraz LB, Gout J, Caligaris C, Yacoub K, Cardot V, Roger E, Kaniewski B, Martel S, Cintas C, Goddard-Leon S, Colombe A, Valantin J, Gadot N, Servoz E, Morton J, Goddard I, Couvelard A, Rebours V, Guillermet J, Sansom OJ, Treilleux I, Valcourt U, Sentis S, Dubus P, Bartholin L.
Acinar-to-Ductal Metaplasia Induced by Transforming Growth Factor Beta Facilitates KRAS(G12D)-driven Pancreatic Tumorigenesis. ***Cell Mol Gastroenterol Hepatol*** 2017; 4: 263–82

Conway JRW, Vennin C, Cazet AS, Herrmann D, Murphy KJ, Warren SC, Wullkopf L, Boulghourjian A, Zaratzian A, Da Silva AM, Pajic M, Morton JP, Cox TR, Timpson P.
Three-dimensional organotypic matrices from alternative collagen sources as pre-clinical models for cell biology. ***Sci Rep*** 2017; 7: 16887

Farrell AS, Joly MM, Allen-Petersen BL, Worth PJ, Lanciault C, Sauer D, Link J, Pelz C, Heiser LM, Morton JP, Muthalagu N, Hoffman MT, Manning SL, Pratt ED, Kendsersky ND, Egbukichi N, Amery TS, Thoma MC, Jenny ZP, Rhim AD, Murphy DJ, Sansom OJ, Crawford HC, Sheppard BC, Sears RC.
MYC regulates ductal-neuroendocrine lineage plasticity in pancreatic ductal adenocarcinoma associated with poor outcome and chemoresistance. ***Nat Commun*** 2017; 8: 1728

Gundry C, Marco S, Rainero E, Miller B, Dornier E, Mitchell L, Caswell PT, Campbell AD, Hogeweg A, Sansom OJ, Morton JP, Norman JC.
Phosphorylation of Rab-coupling protein by LMTK3 controls Rab14-dependent EphA2 trafficking to promote cell:cell repulsion. ***Nat Commun*** 2017; 8: 14646

Harris NL, Vennin C, Conway JR, Vine KL, Pinese M, Cowley MJ, Shearer RF, Lucas MC, Herrmann D, Allam AH, Pajic M, Morton JP, Australian Pancreatic Cancer Genome I, Biankin AV, Ranson M, Timpson P, Saunders DN.
SerpinB2 regulates stromal remodelling and local invasion in pancreatic cancer. ***Oncogene*** 2017; 36: 4288–98

Nobis M, Herrmann D, Warren SC, Kadir S, Leung W, Killen M, Magenau A, Stevenson D, Lucas MC, Reischmann N, Vennin C, Conway JRW, Boulghourjian A, Zaratzian A, Law AM, Gallego-Ortega D, Ormandy CJ, Walters SN, Grey ST, Bailey J *et al.*
A RhoA-FRET Biosensor Mouse for Intravital Imaging in Normal Tissue Homeostasis and Disease Contexts. ***Cell Rep*** 2017; 21: 274–88

Rath N, Morton JP, Julian L, Helbig L, Kadir S, McGhee EJ, Anderson KI, Kalna G, Mullin M, Pinho AV, Rooman I, Samuel MS, Olson MF.
ROCK signaling promotes collagen remodeling to facilitate invasive pancreatic ductal adenocarcinoma tumor cell growth. ***EMBO Mol Med*** 2017; 9: 198–218

Rice AJ, Cortes E, Lachowski D, Cheung BCH, Karim SA, Morton JP, Del Rio Hernandez A.
Matrix stiffness induces epithelial-mesenchymal transition and promotes chemoresistance in pancreatic cancer cells. ***Oncogenesis*** 2017; 6: e352

Ritschka B, Storer M, Mas A, Heinzmann F, Ortells MC, Morton JP, Sansom OJ, Zender L, Keyes WM.
The senescence-associated secretory phenotype induces cellular plasticity and tissue regeneration. ***Genes Dev*** 2017; 31: 172–83

Rosenfeldt MT, O'Prey J, Flossbach L, Nixon C, Morton JP, Sansom OJ, Ryan KM.
PTEN deficiency permits the formation of pancreatic cancer in the absence of autophagy. ***Cell Death Differ*** 2017; 24: 1303–4

Vennin C, Chin VT, Warren SC, Lucas MC, Herrmann D, Magenau A, Melenec P, Walters SN, Del Monte-Nieto G, Conway JR, Nobis M, Allam AH, McCloy RA, Currey N, Pinese M, Boulghourjian A, Zaratzian A, Adam AA, Heu C, Nagrial AM *et al.*
Transient tissue priming via ROCK inhibition uncouples pancreatic cancer progression, sensitivity to chemotherapy, and metastasis. ***Sci Transl Med*** 2017; 9: pii: eaai8504

Other Publications

Leach JDG, Morton JP, Sansom OJ.
Neutrophils: homing in on the myeloid mechanisms of metastasis. ***Mol Immunol*** 2017; 9: 832–45

Morton JP, Sansom OJ.
CXCR2 inhibition in pancreatic cancer: opportunities for immunotherapy? ***Immunotherapy*** 2017; 9: 9–12

Vennin C, Murphy M, Morton JP, Cox TR, Pajic M, Timpson P.
Reshaping the Tumor Stroma as a New Approach for Treatment of Pancreatic Cancer. ***Gastroenterology*** 2017 Dec 26. pii: S0016-5085(17)36738-0. doi: http://dx.doi.org/10.1053/j.gastro.2017.11.280. [Epub ahead of print]

Daniel Murphy (page 30)

Oncogene-Induced Vulnerabilities

Primary Research Papers

Farrell AS, Joly MM, Allen-Petersen BL, Worth PJ, Lanciault C, Sauer D, Link J, Pelz C, Heiser LM, Morton JP, Muthalagu N, Hoffman MT, Manning SL, Pratt ED, Kendsersky ND, Egbukichi N, Amery TS, Thoma MC, Jenny ZP, Rhim AD , Murphy DJ, Sansom OJ, Crawford HC, Sheppard BC, Sears RC.
MYC regulates ductal-neuroendocrine lineage plasticity in pancreatic ductal adenocarcinoma associated with poor outcome and chemoresistance. ***Nat Commun*** 2017; 8: 1728

Hock AK, Cheung EC, Humpton TJ, Monteverde T, Paulus-Hock V, Lee P, McGhee E, Scopelliti A, Murphy DJ, Strathdee D, Blyth K, Vousden KH.
Development of an inducible mouse model of iRFP713 to track recombinase activity and tumour development *in vivo*. ***Sci Rep*** 2017; 7: 1837

Iltzsche F, Simon K, Stopp S, Pattschull G, Francke S, Wolter P, Hauser S, Murphy DJ, Garcia P, Rosenwald A, Gaubatz S.
An important role for Myb-MuvB and its target gene KIF23 in a mouse model of lung adenocarcinoma. ***Oncogene*** 2017; 36: 110–21

Monteverde T, Tait-Mulder J, Hedley A, Knight JR, Sansom OJ, Murphy DJ.
Calcium signalling links MYC to NUAK1. ***Oncogene*** 2017 Nov 6. doi: 10.1038/onc.2017.394. [Epub ahead of print]

Walton JB, Farquharson M, Mason S, Port J, Kruspig B, Dowson S, Stevenson D, Murphy D, Matzuk M, Kim J, Coffelt S, Blyth K, McNeish IA.
CRISPR/Cas9-derived models of ovarian high grade serous carcinoma targeting Brca1, Pten and Nf1, and correlation with platinum sensitivity. ***Sci Rep*** 2017; 7: 16827

Other Publications

Blyth KG, Murphy DJ.
Progress in Mesothelioma from Bench to Bedside. ***Resp Med*** 2018; 134: 31–41. Epub 2017 Nov 26.

Murphy DJ, Blyth KG.
Predicting lung cancer recurrence from circulating tumour DNA. Commentary on 'Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution'. ***Cell Death Differ*** 2017; 24: 1473–4

Port J, Murphy DJ.
Mesothelioma: Identical Routes to Malignancy from Asbestos and Carbon Nanotubes. ***Curr Biol*** 2017; 27: R1173-R1176

Jim Norman (page 56)

Integrin Cell Biology

Primary Research Papers

Diaz-Vera J, Palmer S, Hernandez-Fernaud JR, Dornier E, Mitchell LE, MacPherson I, Edwards J, Zanivan S, Norman JC.
A proteomic approach to identify endosomal cargoes controlling cancer invasiveness. ***J Cell Sci*** 2017; 130: 697–711

Dornier E, Rabas N, Mitchell L, Novo D, Dhayade S, Marco S, Mackay G, Sumpton D, Pallares M, Nixon C, Blyth K, MacPherson I, Rainero E, Norman JC.
Glutaminolysis drives membrane trafficking to promote cancer invasion. ***Nat Comms*** 2017; 8: 2255

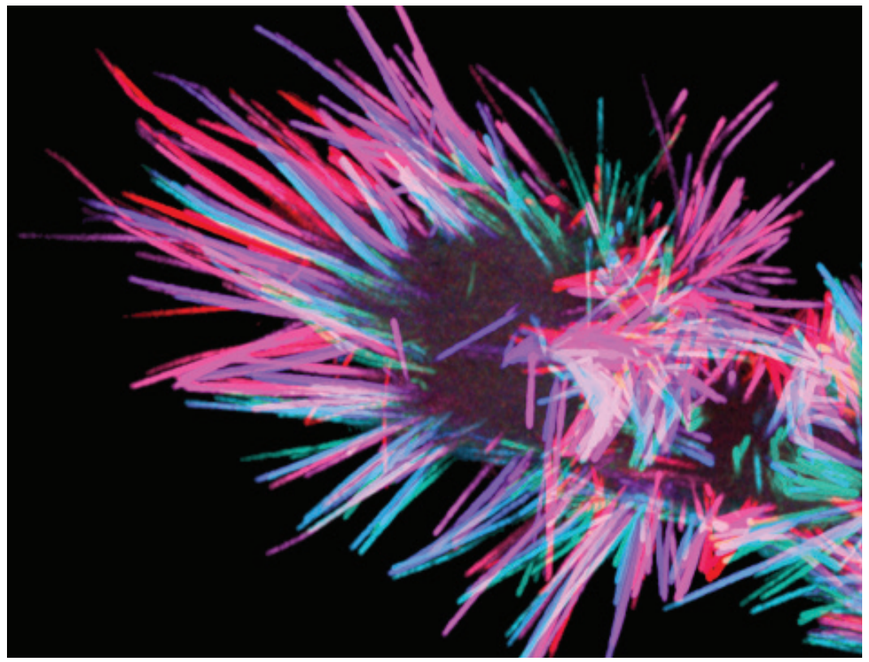
Gundry C, Marco S, Rainero E, Miller B, Dornier E, Mitchell L, Caswell PT, Campbell AD, Hogeweg A, Sansom OJ, Morton JP, Norman JC.
Phosphorylation of Rab-coupling protein by LMTK3 controls Rab14-dependent EphA2 trafficking to promote cell:cell repulsion. ***Nat Commun*** 2017; 8: 14646

Hall PS, Smith A, Hulme C, Vargas-Palacios A, Makris A, Hughes-Davies L, Dunn JA, Bartlett JMS, Cameron DA, Marshall A, Campbell A, Macpherson IR, Dan Rea, Francis A, Earl H, Morgan A, Stein RC, McCabe C; OPTIMA Trial Management Group.
Value of information analysis of multiparameter tests for chemotherapy in early breast cancer: The OPTIMA Prelim Trial. ***Value Health*** 2017; 20: 1311–8

Hernandez-Fernaud JR, Ruengeler E, Casazza A, Neilson LJ, Pulleine E, Santi A, Ismail S, Lilla S, Dhayade S, MacPherson IR, McNeish I, Ennis D, Ali H, Kugeratski FG, Al Khamici H, van den Biggelaar M, van den Berghe PV, Cloix C, McDonald L, Millan D, Hoyle A, Kuchnio A, Carmeliet P, Valenzuela SM, Blyth K, Yin H, Mazzone M, Norman JC, Zanivan S.
Secreted CLIC3 drives cancer progression through its glutathione-dependent oxidoreductase activity. ***Nat Commun*** 2017; 8: 14206

Other Publications

Cain H, Macpherson IR, Beresford M, Pinder SE, Pong J, Dixon JM.
Neoadjuvant therapy in early breast cancer: Treatment considerations and common debates in practice. ***Clin Oncol (R Coll Radiol)*** 2017; 29: 642–52



Dornier E, Norman JC.
Tensin links energy metabolism to extracellular matrix assembly. ***J Cell Biol*** 2017; 216: 867–9

Norman J, Zanivan S.
Chloride intracellular channel 3: A secreted pro-invasive oxidoreductase. ***Cell Cycle*** 2017; 16: 1993–4

Michael Olson (page 58)
Molecular Cell Biology

Primary Research Papers

Masre SF, Rath N, Olson MF, Greenhalgh DA.
ROCK2/ras(Ha) co-operation induces malignant conversion via p53 loss, elevated NF-kappaB and tenascin C-associated rigidity, but p21 inhibits ROCK2/NF-kappaB-mediated progression. ***Oncogene*** 2017; 36: 2529–42

Rath N, Morton JP, Julian L, Helbig L, Kadir S, McGhee EJ, Anderson KI, Kalna G, Mullin M, Pinho AV, Rooman I, Samuel MS, Olson MF.
ROCK signaling promotes collagen remodeling to facilitate invasive pancreatic ductal adenocarcinoma tumor cell growth. ***EMBO Mol Med*** 2017; 9: 198–218

Rudzka DA, Clark W, Hedley A, Kalna G, Olson MF.
Transcriptomic profiling of human breast and melanoma cells selected by migration through narrow constraints. ***Sci Data*** 2017; 4: 170172

Unbekandt M, Belshaw S, Bower J, Clarke M, Cordes J, Crighton D, Croft DR, Drysdale M, Garnett MJ, KGill K, Gray C, Greenhalgh D, Hall JAM, Konczal J, Lilla S, McArthur D, McConnell P, McDonald L, McGarry L, McKinnon H, McMenemy C, Mezna M, Morrice N, Munro J, Naylor G, Rath N, Schüttelkopf AW, Sime M, Olson MF.
Discovery of potent and selective MRCK inhibitors with therapeutic effect on skin cancer. ***Cancer Res***. 2018 Jan 30. pii: canres.2870.2017. doi: 10.1158/0008-5472. CAN-17-2870. [Epub ahead of print]

Zihni C, Vlassaks E, Terry S, Carlton J, Leung TKC, Olson M, Pichaud F, Balda MS, Matter K.
An apical MRCK-driven morphogenetic pathway controls epithelial polarity. ***Nat Cell Biol*** 2017; 19: 1049–60

Other Publications

Vennin C, Rath N, Pajic M, Olson MF, Timpson P.
Targeting ROCK activity to disrupt and prime pancreatic cancer for chemotherapy. ***Small GTPases*** 2017 Oct 3:1–8. doi: 10.1080/21541248.2017.1345712. [Epub ahead of print]

Kevin Ryan (page 32)
Tumour Cell Death

Primary Research Papers

Campbell KJ, Dhayade S, Ferrari N, Sims AH, Johnson E, Mason SM, Dickson A, Ryan KM, Kalna G, Edwards J, Tait SWG, Blyth K.
MCL-1 is a prognostic indicator and drug target in breast cancer. ***Cell Death Dis*** 2018; 9: 19. doi: 10.1038/s41419-017-0035-2. [Epub ahead of print]

Garcia-Mariscal A, Li H, Pedersen E, Peyrollier K, Ryan KM, Stanley A, Quondamatteo F, Brakebusch C.
Loss of RhoA promotes skin tumor formation and invasion by upregulation of RhoB. ***Oncogene*** 2018; 37:847-60. doi: 10.1038/onc.2017.333. Epub 2017 Oct 23.

Giampazolias E, Zunino B, Dhayade S, Bock F, Cloix C, Cao K, Roca A, Lopez J, Ichim G, Proics E, Rubio-Patino C, Fort L, Yatim N, Woodham E, Orozco S, Taraborrelli L, Peltzer N, Lecis D, Machesky L, Walczak H, Albert ML, Milling S, Oberst A, Ricci JE, Ryan KM, Blyth K, Tait SWG.
Mitochondrial permeabilization engages NF-kappaB-dependent anti-tumour activity under caspase deficiency. ***Nat Cell Biol*** 2017; 19: 1116–29

Kania E, Pajak B, O'Prey J, Sierra Gonzalez P, Litwiniuk A, Urbanska K, Ryan KM, Orzechowski A.
Verapamil treatment induces cytoprotective autophagy by modulating cellular metabolism. ***FEBS J*** 2017; 284: 1370–87

Lampada A, O'Prey J, Szabadkai G, Ryan KM, Hochhauser D, Salomoni P.
mTORC1-independent autophagy regulates receptor tyrosine kinase phosphorylation in colorectal cancer cells via an mTORC2-mediated mechanism. ***Cell Death Differ*** 2017; 24: 1045–62

Mitchell R, Hopcroft LEM, Baquero P, Allan EK, Hewit K, James D, Hamilton G, Mukhopadhyay A, O'Prey J, Hair A, Melo JV, Chan E, Ryan KM, Maguer-Satta V, Druker BJ, Clark RE, Mitra S, Herzyk P, Nicolini FE, Salomoni P, Shanks E, Calabretta B, Holyoake TL, Helgason GV.
Targeting BCR-ABL-Independent TKI Resistance in Chronic Myeloid Leukemia by mTOR and Autophagy Inhibition. ***J Natl Cancer Inst*** 2017 Nov 20. doi: 10.1093/jnci/djx236. [Epub ahead of print]

O'Prey J, Sakamaki J, Baudot AD, New M, Van Acker T, Tooze SA, Long JS, Ryan KM.
Application of CRISPR/Cas9 to Autophagy Research. ***Methods Enzymol*** 2017; 588: 79–108

Rosenfeldt MT, O'Prey J, Flossbach L, Nixon C, Morton JP, Sansom OJ, Ryan KM.
PTEN deficiency permits the formation of pancreatic cancer in the absence of autophagy. ***Cell Death Differ*** 2017; 24: 1303–4

Sakamaki JI, Wilkinson S, Hahn M, Tasdemir N, O'Prey J, Clark W, Hedley A, Nixon C, Long JS, New M, Van Acker T, Tooze SA, Lowe SW, Dikic I, Ryan KM.
Bromodomain Protein BRD4 Is a Transcriptional Repressor of Autophagy and Lysosomal Function. ***Mol Cell*** 2017; 66: 517–32.e9

Other Publications

Galluzzi L, Baehrecke EH, Ballabio A, Boya P, Bravo-San Pedro JM, Cecconi F, Choi AM, Chu CT, Codogno P, Colombo MI, Cuervo AM, Debnath J, Deretic V, Dikic I, Eskelinen EL, Fimia GM, Fulda S, Gewirtz DA, Green DR, Hansen M *et al.*
Molecular definitions of autophagy and related processes. ***EMBO J*** 2017; 36: 1811–36

New M, Van Acker T, Long JS, Sakamaki JI, Ryan KM, Tooze SA.
Molecular Pathways Controlling Autophagy in Pancreatic Cancer. ***Front Oncol*** 2017; 7: 28

Sakamaki JI, Long JS, New M, Van Acker T, Tooze SA, Ryan KM.
Emerging roles of transcriptional programs in autophagy regulation. ***Transcription*** 2018; 9:131-6. doi: 10.1080/21541264.2017.1372045. Epub 2017 Nov 3.

Sakamaki JI, Ryan KM.
Transcriptional regulation of autophagy and lysosomal function by bromodomain protein BRD4. ***Autophagy*** 2017; 13: 2006–7

Simon HU, Friis R, Tait SW, Ryan KM.
Retrograde signaling from autophagy modulates stress responses. ***Sci Signal*** 2017; 10: pii: eaag2791.

Owen Sansom (page 60)
Colorectal Cancer and Wnt Signalling

Primary Research Papers

Azad A, Yin Lim S, D'Costa Z, Jones K, Diana A, Sansom OJ, Kruger P, Liu S, McKenna WG, Dushek O, Muschel RJ, Fokas E.
PD-L1 blockade enhances response of pancreatic ductal adenocarcinoma to radiotherapy. ***EMBO Mol Med*** 2017; 9: 167–80

Cammareri P, Vincent DF, Hodder MC, Ridgway RA, Murgia C, Nobis M, Campbell AD, Varga J, Huels DJ, Subramani C, Prescott KLH, Nixon C, Hedley A, Barry ST, Greten FR, Inman GJ, Sansom OJ.
TGFbeta pathway limits dedifferentiation following WNT and MAPK pathway activation to suppress intestinal tumourigenesis. ***Cell Death Differ*** 2017; 24: 1681–93

Carotenuto P, Fassan M, Pandolfo R, Lampis A, Vicentini C, Cascione L, Paulus-Hock V, Boulter L, Guest R, Quagliata L, Hahne JC, Ridgway R, Jamieson T, Athineos D, Veronese A, Visone R, Murgia C, Ferrari G, Guzzardo V, Evans TRJ, MacLeod M, Feng GJ, Dale T, Negrini M, Forbes SJ, Terracciano L, Scarpa A, Patel T, Valeri N, Workman P, Sansom O, Braconi C..
Wnt signalling modulates transcribed-ultraconserved regions in hepatobiliary cancers. ***Gut*** 2017; 66: 1268–77

Chou A, Froio D, Nagrial AM, Parkin A, Murphy KJ, Chin VT, Wohl D, Steinmann A, Stark R, Drury A, Walters SN, Vennin C, Burgess A, Pinese M, Chantrill LA, Cowley MJ, Molloy TJ, Australian Pancreatic Cancer Genome I, Waddell N, Johns A, Grimmond SM, Chang DK, Biankin AV, Sansom OJ, Morton JP, Grey ST, Cox TR, Turchini J, Samra J, Clarke SJ, Timpson P, Gill AJ, Pajic M.
Tailored first-line and second-line CDK4-targeting treatment combinations in mouse models of pancreatic cancer. ***Gut*** 2017 Oct 28. pii: gutjnl-2017-315144. doi: 10.1136/gutjnl-2017-315144. [Epub ahead of print]

Chuvin N, Vincent DF, Pommier RM, Alcaraz LB, Gout J, Caligaris C, Yacoub K, Cardot V, Roger E, Kaniewski B, Martel S, Cintas C, Goddard-Leon S, Colombe A, Valantin J, Gadot N, Servoz E, Morton J, Goddard I,

Couvelard A, Rebours V, Guillermet J, Sansom OJ, Treilleux I, Valcourt U, Sentis S, Dubus P, Bartholin L.
Acinar-to-Ductal Metaplasia Induced by Transforming Growth Factor Beta Facilitates KRAS(G12D)-driven Pancreatic Tumorigenesis. ***Cell Mol Gastroenterol Hepatol*** 2017; 4: 263–82

D'Costa Z, Jones K, Azad A, van Stiphout R, Lim SY, Gomes AL, Kinchesh P, Smart SC, Gillies McKenna W, Buffa FM, Sansom OJ, Muschel RJ, O'Neill E, Fokas E.
Gemcitabine-Induced TIMP1 Attenuates Therapy Response and Promotes Tumor Growth and Liver Metastasis in Pancreatic Cancer. ***Cancer Res*** 2017; 77: 5952–62

Diamantopoulou Z, White G, Fadlullah MZH, Dreger M, Pickering K, Maltas J, Ashton G, MacLeod R, Baillie GS, Kouskoff V, Lacaud G, Murray GI, Sansom OJ, Hurlstone AFL, Malliri A.
TIAM1 Antagonizes TAZ/YAP Both in the Destruction Complex in the Cytoplasm and in the Nucleus to Inhibit Invasion of Intestinal Epithelial Cells. ***Cancer Cell*** 2017; 31: 621–34.e6

Farrell AS, Joly MM, Allen-Petersen BL, Worth PJ, Lanciault C, Sauer D, Link J, Pelz C, Heiser LM, Morton JP, Muthalagu N, Hoffman MT, Manning SL, Pratt ED, Kendsersky ND, Egbukichi N, Amery TS, Thoma MC, Jenny ZP, Rhim AD, Murphy DJ, Sansom OJ, Crawford HC, Sheppard BC, Sears RC.
MYC regulates ductal-neuroendocrine lineage plasticity in pancreatic ductal adenocarcinoma associated with poor outcome and chemoresistance. ***Nat Commun*** 2017; 8: 1728

Fumagalli A, Drost J, Suijkerbuijk SJ, van Boxtel R, de Ligt J, Offerhaus GJ, Begthel H, Beerling E, Tan EH, Sansom OJ, Cuppen E, Clevers H, van Rheenen J.
Genetic dissection of colorectal cancer progression by orthotopic transplantation of engineered cancer organoids. ***Proc Natl Acad Sci U S A*** 2017; 114: E2357–E2364

Gundry C, Marco S, Rainero E, Miller B, Dornier E, Mitchell L, Caswell PT, Campbell AD, Hogeweg A, Sansom OJ, Morton JP, Norman JC.
Phosphorylation of Rab-coupling protein by LMTK3 controls Rab14-dependent EphA2 trafficking to promote cell:cell repulsion. ***Nat Commun*** 2017; 8: 14646

Humphris JL, Patch AM, Nones K, Bailey PJ, Johns AL, McKay S, Chang DK, Miller DK, Pajic M, Kassahn KS, Quinn MC, Bruxner TJ, Christ AN, Harliwong I, Idrisoglu S, Manning S, Nourse C, Nourbakhsh E, Stone A, Wilson PJ *et al.*
Hypermutation In Pancreatic Cancer. ***Gastroenterology*** 2017; 152: 68–74.e2

Kersemans V, Beech JS, Gilchrist S, Kinchesh P, Allen PD, Thompson J, Gomes AL, D'Costa Z, Bird L, Tullis IDC, Newman RG, Corroyer-Dulmont A, Falzone N, Azad A, Vallis KA, Sansom OJ, Muschel RJ, Vojnovic B, Hill MA, Fokas E *et al.*
An efficient and robust MRI-guided radiotherapy planning approach for targeting abdominal organs and tumours in the mouse. ***PLoS One*** 2017; 12: e0176693

Loveridge CJ, Mui EJ, Patel R, Tan EH, Ahmad I, Welsh M, Galbraith J, Hedley A, Nixon C, Blyth K, Sansom O, Leung HY.
Increased T-cell Infiltration Elicited by Erk5 Deletion in a Pten-Deficient Mouse Model of Prostate Carcinogenesis. ***Cancer Res*** 2017; 77: 3158–68

Loveridge CJ, van 't Hof RJ, Charlesworth G, King A, Tan EH, Rose L, Daroszewska A, Prior A, Ahmad I, Welsh M, Mui EJ, Ford C, Salji M, Sansom O, Blyth K, Leung HY.
Analysis of Nkx3.1:Cre-driven Erk5 deletion reveals a profound spinal deformity which is linked to increased osteoclast activity. ***Sci Rep*** 2017; 7: 13241

Maddocks ODK, Athineos D, Cheung EC, Lee P, Zhang T, van den Broek NJF, Mackay GM, Labuschagne CF, Gay D, Kruiswijk F, Blagih J, Vincent DF, Campbell KJ, Ceteci F, Sansom OJ, Blyth K, Vousden KH.
Modulating the therapeutic response of tumours to dietary serine and glycine starvation. ***Nature*** 2017; 544: 372-6

Monteverde T, Tait-Mulder J, Hedley A, Knight JR, Sansom OJ, Murphy DJ.
Calcium signalling links MYC to NUAK1. ***Oncogene*** 2017 Nov 6. doi: 10.1038/onc.2017.394. [Epub ahead of print]

Myant KB, Cammareri P, Hodder MC, Wills J, Von Kriegsheim A, Gyorffy B, Rashid M, Polo S, Maspero E, Vaughan L, Gurung B, Barry E, Malliri A, Camargo F, Adams DJ, Iavarone A, Lasorella A, Sansom OJ.
HUWE1 is a critical colonic tumour suppressor gene that prevents MYC signalling, DNA damage accumulation and tumour initiation. ***EMBO Mol Med*** 2017; 9: 181–97

Nobis M, Herrmann D, Warren SC, Kadir S, Leung W, Killen M, Magenau A, Stevenson D, Lucas MC, Reischmann N, Vennin C, Conway JRW, Boulghourjian A, Zaratzian A, Law AM, Gallego-Ortega D, Ormandy CJ, Walters SN, Grey ST, Bailey J *et al.*
A RhoA-FRET Biosensor Mouse for Intravital Imaging in Normal Tissue Homeostasis and Disease Contexts. ***Cell Rep*** 2017; 21: 274–88

Ritschka B, Storer M, Mas A, Heinzmann F, Ortells MC, Morton JP, Sansom OJ, Zender L, Keyes WM.
The senescence-associated secretory phenotype induces cellular plasticity and tissue regeneration. Genes Dev 2017; 31: 172–83

Rose AM, Sansom OJ, Inman GJ.
Loss of TGF-beta signaling drives cSCC from skin stem cells - More evidence. ***Cell Cycle*** 2017; 16: 386–87

Rosenfeldt MT, O'Prey J, Flossbach L, Nixon C, Morton JP, Sansom OJ, Ryan KM.
PTEN deficiency permits the formation of pancreatic cancer in the absence of autophagy. ***Cell Death Differ*** 2017; 24: 1303–4

Tian A, Benchabane H, Wang Z, Zimmerman C, Xin N, Perochon J, Kalna G, Sansom OJ, Cheng C, Cordero JB, Ahmed Y.
Intestinal stem cell overproliferation resulting from inactivation of the APC tumor suppressor requires the transcription cofactors Earthbound and Erect wing. ***PLoS Genet*** 2017; 13: e1006870

van der Weyden L, Arends MJ, Campbell AD, Bald T, Wardle-Jones H, Griggs N, Velasco-Herrera MD, Tuting T, Sansom OJ, Karp NA, Clare S, Gleeson D, Ryder E, Galli A, Tuck E, Cambridge EL, Voet T, Macaulay IC, Wong K, Sanger Mouse Genetics Project, Spiegel S, Speak AO, Adams DJ.
Genome-wide in vivo screen identifies novel host regulators of metastatic colonization. ***Nature*** 2017; 541: 233–6

Vennin C, Chin VT, Warren SC, Lucas MC, Herrmann D, Magenau A, Melenec P, Walters SN, Del Monte-Nieto G, Conway JR, Nobis M, Allam AH, McCloy RA, Currey N, Pinese M, Boulghourjian A, Zaratzian A, Adam AA, Heu C, Nagrial AM *et al.*
Transient tissue priming via ROCK inhibition uncouples pancreatic cancer progression, sensitivity to chemotherapy, and metastasis. ***Sci Transl Med*** 2017; 9: pii: eaai8504.

Wildenberg ME, Koelink PJ, Diederen K, Te Velde AA, Wolfkamp SC, Nuij VJ, Peppelenbosch MP, Nobis M, Sansom OJ, Anderson KI, van der Woude CJ, D'Haens GR, van den Brink GR.
The ATG16L1 risk allele associated with Crohn's disease results in a Rac1-dependent defect in dendritic cell migration that is corrected by thiopurines. ***Mucosal Immunol*** 2017; 10: 352–60

Other Publications

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

Jackstadt R, Sansom OJ.
The Wae to repair: prostaglandin E2 (PGE2) triggers intestinal wound repair. ***EMBO J*** 2017; 36: 3–4

Leach JDG, Morton JP, Sansom OJ.
Neutrophils: homing in on the myeloid mechanisms of metastasis. ***Mol Immunol*** 2017 Dec 18. pii: S0161-5890(17)30615-6. doi: 10.1016/j.molimm.2017.12.013. [Epub ahead of print]

Morton JP, Sansom OJ.
CXCR2 inhibition in pancreatic cancer: opportunities for immunotherapy? ***Immunotherapy*** 2017; 9: 9–12

Phesse 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

Phesse TJ, Sansom OJ.
Lgr5 joins the club of gastric stem cell markers in the corpus. ***Nat Cell Biol*** 2017; 19: 752–4

Emma Shanks (page 74)

Functional Screening

Primary Research Papers

Cairney CJ, Godwin LS, Bilsland AE, Burns S, Stevenson KH, McGarry L, Revie J, Moore JD, Wiggins CM, Collinson RS, Mudd C, Tsonou E, Sadaie M, Bennett DC, Narita M, Torrance CJ, Keith WN.
A 'synthetic-sickness' screen for senescence re-engagement targets in mutant cancer backgrounds. ***PLoS Genet*** 2017; 13: e1006942

Other Publications

Davidson MA, Shanks EJ.
3q26-29 Amplification in head and neck squamous cell carcinoma: a review of established and prospective oncogenes. ***FEBS J*** 2017; 284: 2705–31

Douglas Strathdee (page 82)

Transgenic Technology

Primary Research Papers

Hock AK, Cheung EC, Humpton TJ, Monteverde T, Paulus-Hock V, Lee P, McGhee E, Scopelliti A, Murphy DJ, Strathdee D, Blyth K, Vousden KH.
Development of an inducible mouse model of iRFP713 to track recombinase activity and tumour development *in vivo*. ***Sci Rep*** 2017; 7: 1837

Nobis M, Herrmann D, Warren SC, Kadir S, Leung W, Killen M, Magenau A, Stevenson D, Lucas MC, Reischmann N, Vennin C, Conway JRW, Boulghourjian A, Zaratzian A, Law AM, Gallego-Ortega D, Ormandy CJ, Walters SN, Grey ST, Bailey J *et al.*
A RhoA-FRET Biosensor Mouse for Intravital Imaging in Normal Tissue Homeostasis and Disease Contexts. ***Cell Rep*** 2017; 21: 274–88

van de Lagemaat LN, Stanford LE, Pettit CM, Strathdee DJ, Strathdee 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

Correia-Melo C, Ichim G, Tait SW, Passos JF.
Depletion of mitochondria in mammalian cells through enforced mitophagy. ***Nat Protoc*** 2017; 12: 183–94

Daniels BP, Snyder AG, Olsen TM, Orozco S, Oguin TH, 3rd, Tait SW, Martinez J, Gale M, Jr., Loo YM, Oberst A.
RIPK3 Restricts Viral Pathogenesis via Cell Death-Independent Neuroinflammation. ***Cell*** 2017; 169: 301–13.e11

Giampazolias E, Zunino B, Dhayade S, Bock F, Cloix C, Cao K, Roca A, Lopez J, Ichim G, Proics E, Rubio-Patino C, Fort L, Yatim N, Woodham E, Orozco S, Taraborrelli L, Peltzer N, Lecis D, Machesky L, Walczak H, Albert ML, Milling S, Oberst A, Ricci JE, Ryan KM, Blyth K, Tait SWG.
Mitochondrial permeabilization engages NF-kappaB-dependent anti-tumour activity under caspase deficiency. ***Nat Cell Biol*** 2017; 19: 1116–29

Gutierrez KD, Davis MA, Daniels BP, Olsen TM, Ralli-Jain P, Tait SW, Gale M, Jr., Oberst A.
MLKL Activation Triggers NLRP3-Mediated Processing and Release of IL-1beta Independently of Gasdermin-D. ***J Immunol*** 2017; 195: 2156–64

Villa E, Proics E, Rubio-Patino C, Obba S, Zunino B, Bossowski JP, Rozier RM, Chiche J, Mondragon L, Riley JS, Marchetti S, Verhoeyen E, Tait SWG, Ricci JE.
Parkin-Independent Mitophagy Controls Chemotherapeutic Response in Cancer Cells. ***Cell Rep*** 2017; 20: 2846–59

Weigert M, Binks A, Dowson S, Leung EYL, Athineos D, Yu X, Mullin M, Walton JB, Orange C, Ennis D, Blyth K, Tait SWG, McNeish IA.
RIPK3 promotes adenovirus type 5 activity. ***Cell Death and Disease*** 2017; 8: 3206

Woodham EF, Paul NR, Tyrrell B, Spence HJ, Swaminathan K, Scribner MR, Giampazolias E, Hedley A, Clark W, Kage F, Marston DJ, Hahn KM, Tait SW, Larue L, Brakebusch CH, Insall RH, Machesky LM.
Coordination by Cdc42 of Actin, Contractility, and Adhesion for Melanoblast Movement in Mouse Skin. ***Curr Biol*** 2017; 27: 624–37

Other Publications

Giampazolias E, Tait SWG.
Caspase-independent cell death: an anti-cancer double-whammy. ***Cell Cycle*** 2017 Nov 23:1-5. doi: 10.1080/15384101.2017.1408229. [Epub ahead of print]

Ichim G, Tait SWG.
Cancer therapy-induced PAFR ligand expression: any role for caspase activity? ***Nat Rev Cancer*** 2017; 17: 253

Simon HU, Friis R, Tait SW, Ryan KM.
Retrograde signaling from autophagy modulates stress responses. ***Sci Signal*** 2017; 10: pii: eaag2791.

Saverio Tardito (page 36)

Oncometabolism

Primary Research Papers

Fack F, Tardito S, Hochart G, Oudin A, Zheng L, Fritah S, Golebiewska A, Nazarov PV, Bernard A, Hau AC, Keunen O, Leenders W, Lund-Johansen M, Stauber J, Gottlieb E, Bjerkvig R, Niclou SP.
Altered metabolic landscape in IDH-mutant gliomas affects phospholipid, energy, and oxidative stress pathways. ***EMBO Mol Med*** 2017; 9: 1681–95

Kuntz EM, Baquero P, Michie AM, Dunn K, Tardito S, Holyoake TL, Helgason GV, Gottlieb E.
Targeting mitochondrial oxidative phosphorylation eradicates therapy-resistant chronic myeloid leukemia stem cells. ***Nat Med*** 2017 23: 1234–40

Alexei Vazquez (page 38)

Mathematical Models of Metabolism

Primary Research Papers

Bulusu V, Tumanov S, Michalopoulou E, van den Broek NJ, MacKay G, Nixon C, Dhayade S, Schug ZT, Vande Voorde J, Blyth K, Gottlieb E, Vazquez A, Kamphorst JJ.
Acetate Recapturing by Nuclear Acetyl-CoA Synthetase 2 Prevents Loss of Histone Acetylation during Oxygen and Serum Limitation. ***Cell Rep*** 2017; 18: 647–58

Burgos-Barragan G, Wit N, Meiser J, Dingler FA, Pietzke M, Mulderrig L, Pontel LB, Rosado IV, Brewer TF, Cordell RL, Monks PS, Chang CJ, Vazquez A, Patel KJ.
Mammals divert endogenous genotoxic formaldehyde into one-carbon metabolism. ***Nature*** 2017; 548: 549–54

Dolfi SC, Medina DJ, Kareddula A, Paratala B, Rose A, Dhami J, Chen S, Ganesan S, Mackay G, Vazquez A, Hirshfield KM.
Riluzole exerts distinct antitumor effects from a metabotropic glutamate receptor 1-specific inhibitor on breast cancer cells. ***Oncotarget*** 2017; 8: 44639–53

Fernandez-de-Cossio-Diaz J, Vazquez A.
Limits of aerobic metabolism in cancer cells. ***Sci Rep*** 2017; 7: 13488

Other Publications

Meiser J, Vazquez A, Hiller K.
DJ1 at the interface between neuro-degeneration and cancer. ***Oncotarget*** 2017; 8: 9015–6

Vazquez A (2017)
Overflow Metabolism: From Yeast to Marathon Runners. 1st Edition. Academic Press

Sara Zanivan (pages 62 & 73)

Tumour Microenvironment and Proteomics

Primary Research Papers

Diaz-Vera J, Palmer S, Hernandez-Fernaud JR, Dornier E, Mitchell LE, Macpherson I, Edwards J, Zanivan S, Norman JC.
A proteomic approach to identify endosomal cargoes controlling cancer invasiveness. ***J Cell Sci*** 2017; 130: 697–711

Hernandez-Fernaud JR, Ruengeler E, Casazza A, Neilson LJ, Pulleine E, Santi A, Ismail S, Lilla S, Dhayade S, MacPherson IR, McNeish I, Ennis D, Ali H, Kugeratski FG, Al Khamici H, van den Biggelaar M, van den Berghe PV, Cloix C, McDonald L, Millan D, Hoyle A, Kuchnio A, Carmeliet P, Valenzuela SM, Blyth K, Yin H, Mazzone M, Norman JC, Zanivan S.
Secreted CLIC3 drives cancer progression through its glutathione-dependent oxidoreductase activity. ***Nat Commun*** 2017; 8: 14206

Kugeratski FG, Batista M, Lima CVP, Neilson LJ, da Cunha ES, de Godoy LM, Zanivan S, Krieger MA, Marchini FK.
Mitogen-Activated Protein Kinase Kinase 5 Regulates Proliferation and Biosynthetic Processes in Procyclic Forms of Trypanosoma brucei. ***J Proteome Res*** 2018; 17: 108–18. doi: 10.1021/acs.jproteome.7b00415. Epub 2017 Nov 7.

Reid SE, Kay EJ, Neilson LJ, Henze AT, Serneels J, McGhee EJ, Dhayade S, Nixon C, Mackey JB, Santi A, Swaminathan K, Athineos D, Papalazarou V, Patella F, Roman-Fernandez A, ElMaghloob Y, Hernandez-Fernaud JR, Adams RH, Ismail S, Bryant DM , Salmeron-Sanchez M, Machesky LM, Carlin LM, Blyth K, Mazzone M,7, Zanivan S.
Tumor matrix stiffness promotes metastatic cancer cell interaction with the endothelium. ***EMBO*** 2017; J 36: 2373–89

van den Eshof BL, Hoogendijk AJ, Simpson PJ, van Alphen FPJ, Zanivan S, Mertens K, Meijer AB, van den Biggelaar M.
Paradigm of Biased PAR1 (Protease-Activated Receptor-1) Activation and Inhibition in Endothelial Cells Dissected by Phosphoproteomics. ***Arterioscler Thromb Vasc Biol*** 2017; 37: 1891–1902

Other Publications

Norman J, Zanivan S.
Chloride intracellular channel 3: A secreted pro-invasive oxidoreductase. Cell Cycle 2017; 16; 1993–4

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

Santi A, Kugeratski FG, Zanivan S.
Cancer Associated Fibroblasts: The Architects of Stroma Remodeling. ***Proteomics*** 2017; Dec 27. doi: 10.1002/pmic.201700167. [Epub ahead of print]

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

Fort, Loïc (2017) FAM49B: first negative regulator of the Scar/WAVE complex. From evolution to an *in vivo* analysis [PhD thesis, University of Glasgow, Beatson Institute]

Sierra Gonzalez, Pablo (2017) Understanding a new role for D-mannose in modulating tumour growth and cancer therapy [PhD thesis, University of Glasgow, Beatson Institute]

Koh, Yvette Wui Hui (2017) Deciphering the role of LPA and pseudopod machinery during melanoma chemotaxis [PhD thesis, University of Glasgow, Beatson Institute]

Kugeratski, Fernanda Grande (2017) The secretome of hypoxic mammary cancer-associated fibroblasts unveils pro-angiogenic factors [PhD thesis, University of Glasgow, Beatson Institute]

Kuntz, Elodie Marie (2017) An investigation of metabolic vulnerabilities in chronic myeloid leukaemic stem cells [PhD thesis, University of Glasgow, Beatson Institute]

Monteverde, Tiziana (2017) Investigating the function and regulation of NUAK1 and its role in non-small cell lung cancer [PhD thesis, University of Glasgow, Beatson Institute]

Rodgers, Lisa (2017) The phosphagen system in prostate cancer [PhD thesis, University of Glasgow, Beatson Institute]

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 Dornier, Ramon Klein Geltink, Constantinos Alexandrou, David Lewis, Celia Berkers, Naama Kanarek, Andres Mendez-Lucas, Liron Bar-Pelled, Mattia Falcone (sponsored by WCR) and Arafath Najamudeen (sponsored by Advanced Cell Diagnostics). Transnetyx 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 Geltin who won The Biochemist short talk prize for his presentation describing mitochondrial priming by CD28.

Talk to 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 (NKI).

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, Milyenyi 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 OMERO (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

Distinguished Seminar Speaker:
Ultan McDermott, Wellcome Trust Sanger Institute, Cambridge

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

Thomi Brunner, University of Konstanz, Germany

September

Christopher Lord, The Breast Cancer Now Toby Robins Breast Cancer Research Centre, London

Chris Madsen, 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

Karin de Visser (NKI) speaking at this year's Beatson workshop

October

Richard Houlston, Institute for Cancer Research, London

Rob Snelgrove, National Heart & Lung Institute, Imperial College London

Ernst Lengyel, University of Chicago Medicine, USA

November

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

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

December

Eric O'Neill, CRUK/MRC Oxford Institute for Radiation Oncology

STUDENTSHIPS AND POSTDOCTORAL FELLOWSHIPS

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.

www.glasgow.gov.uk and www.peoplemakeglasgow.com give general information about Glasgow and other useful links.

The graduate students above are members of the Prostate Cancer UK Future Leaders Academy. From left to right: Mark Lawrence, Laura Lapienyte, 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 Kolliatsas, 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 Laing, 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.

THANKS FOR SUPPORTING US

The work of our various research groups would barely proceed without the substantial grant funding provided by Cancer Research UK to the Beatson Institute and the University of Glasgow, now amounting to £20 million per annum combined. We are also indebted to a number of other organisations that provide funding to our scientists, usually supporting projects in a particular sphere of special interest, or supporting the careers of talented junior scientists, enabling them to pursue their research interests within our laboratories. These organisations, whose funding we appreciate greatly, are listed below. The additional funding provided by these organisations makes possible much work that we otherwise could not be undertaking and has become integral and indispensable to our operations.

Cancer Research UK Beatson Institute

Tom Bird
Wellcome Trust

Karen Blyth
Breast Cancer Now

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
AstraZeneca, Celgene, European Community, Gilead, Janssen, NHS Greater Glasgow & Clyde Health Board Endowment Fund, Novartis, Pancreatic Cancer UK & Pancreatic Cancer Scotland, Wellcome Trust, West of Scotland Women's Bowling Association, Worldwide Cancer Research

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 Coffelt
European Community, William Forrest Charitable Trust, Naito Foundation

Jurre Kamphorst
Rosetrees Trust

Daniel Murphy
Worldwide Cancer Research

Stephen Tait
BBSRC, Breast Cancer Now

Dr David Lewis receives cheque from Mosshead Primary School pupils

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 Bohemen, in memory of R Neale
Margaret G Brown
Clyde Travel
Trina Corbett
Thomas Donaldson
Electa Chapter No.27 O.E.S
James 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
Annie Murray Hogarth and Alexander J Paton
Inner Wheel Club of Auchterarder and District
John Beattie & Sons
Irene Kennedy and Janet Lyke
Alan MacDonald, in memory of Walter Soutar
Craig McGuire Trust
The McLauchlan family
Fiona McNeill
The McQuarrie family, in memory of their daughter and sister Rhoda
Mentholatum Company Ltd and staff
Mosshead Primary School
Matthew Munro, in memory of John Waddell
Overton Park Bowling Club
Alexander J Paton and Annie Murray Hogarth
Sarah Percy
PMV Pharmaceuticals
James Port, on behalf of Mr Port's wife, Elizabeth
Brian Potter and Mrs Potter
Patricia Rooney (Darrows)
Jacqueline Thomson, in memory of her father
Thornhill Gardening Society
Trinity Mirror plc
West of Scotland Women's Bowling Association
Mrs Jean Whiteford and Miss A Paterson
Elizabeth Wiggins, in memory of her father

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

CONTACT DETAILS

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

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

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

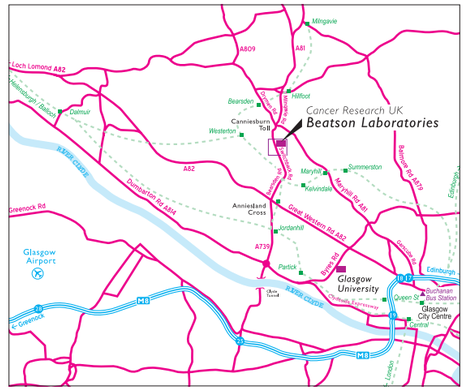
The Beatson Institute for Cancer Research is a registered charity in Scotland (SC006106) and registered as a company limited by guarantee in Scotland (84170).
Registered address: Cancer Research UK Beatson Institute,
Garscube Estate,
Switchback Road, Bearsden,
Glasgow G61 1BD

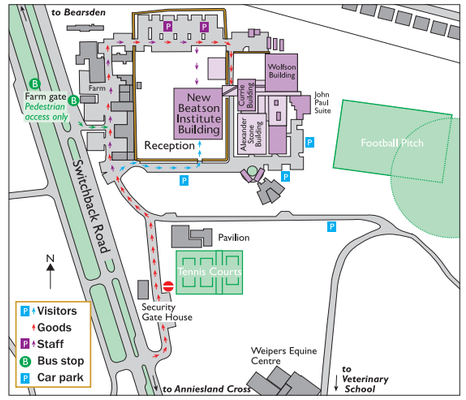
Tel +44(0) 141 330 3953
www.beatson.gla.ac.uk

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

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

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





NOTES

ISSN 1479-0378

CANCER RESEARCH UK
BEATSON INSTITUTE

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

www.beatson.gla.ac.uk