SCIENTIFIC REPORT 2016

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COVER IMAGE

Live small cell lung cancer cells, stained for actin, invading into a layer of matrigel. Colours represent depth at which the cells were imaged. *Image supplied by Andrew Porter & Sophie Adlard (Cell Signalling)*

SCIENTIFIC REPORT 2016

MANCHESTER INSTITUTE

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The University of Manchester

CANCER RESEARCH UK | MANCHESTER INSTITUTE

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The Cancer Research UK Manchester Institute is located across The Paterson Building (shown here) and The Manchester Cancer Research Centre Building

The Manchester Cancer Research Centre Building

DIRECTOR'S INTRODUCTION

Director of the Cancer Research UK Manchester Institute

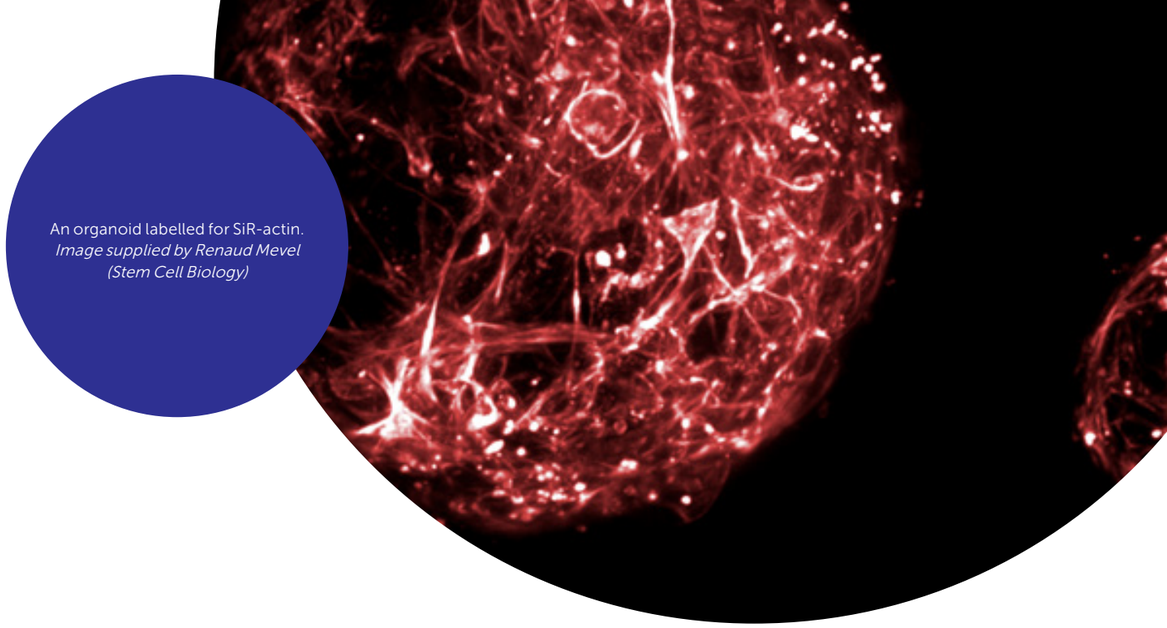
Professor Richard Marais

It has been a busy and successful year for the Institute. Highlights from 2016 include the demonstration by our Clinical and Experimental Pharmacology (CEP) group, in collaboration with AstraZeneca, of impressive pre-clinical activity of a Wee1 inhibitor in combination with olaparib in small cell lung cancer. This has led to a clinical trial in this disease that will begin early this year, at The Christie NHS Foundation Trust and several US sites.

We are also pleased that, building on the impressive work from our Leukaemia Biology group, the first Phase 1 clinical trial data with ORY-1001 – a first in class inhibitor of the histone demethylase LSD1 – was presented at the American Society of Hematology meeting in San Diego in December 2016. This revealed that ORY-1001 promotes differentiation of acute myeloid leukemia blast cells in patients, an exciting outcome that is now being taken forward in collaboration with Oryzon Genomics and Roche. We congratulate the Drug Discovery Unit (DDU) on declaring its first pre-clinical candidate drug, which targets the tyrosine kinase RET. This important milestone was the result of a collaboration with the venture capital company 6th Element Capital who partially funded this work. The drug candidate will now progress through to clinical trials in non-small cell lung cancer patients whose tumours harbour RET-fusion oncogenes. Finally, during the summer, my own group, Molecular Oncology, underwent a very successful quinquennial review of our research programme.

Highlights from our publications include Caroline Dive's study in *Nature Medicine* showing how to distinguish chemosensitive from chemorefractory small cell lung cancer patients using the genetic features of circulating tumour cells. Another notable publication was a study published in *Cell* from one of our Junior Group Leaders, Claus Jørgensen. Claus' group reported that in pancreatic ductal adenocarcinoma, in addition to its conventional role in directly driving tumour cell proliferation, oncogenic KRAS also regulates tumour cell signalling through the stromal cells. Also, my group reported the development of a comprehensive precision medicine platform for melanoma in *Cancer Discovery* that makes use of liquid biopsies and circulating-free DNA to monitor responses to therapy and identify mechanisms of resistance.

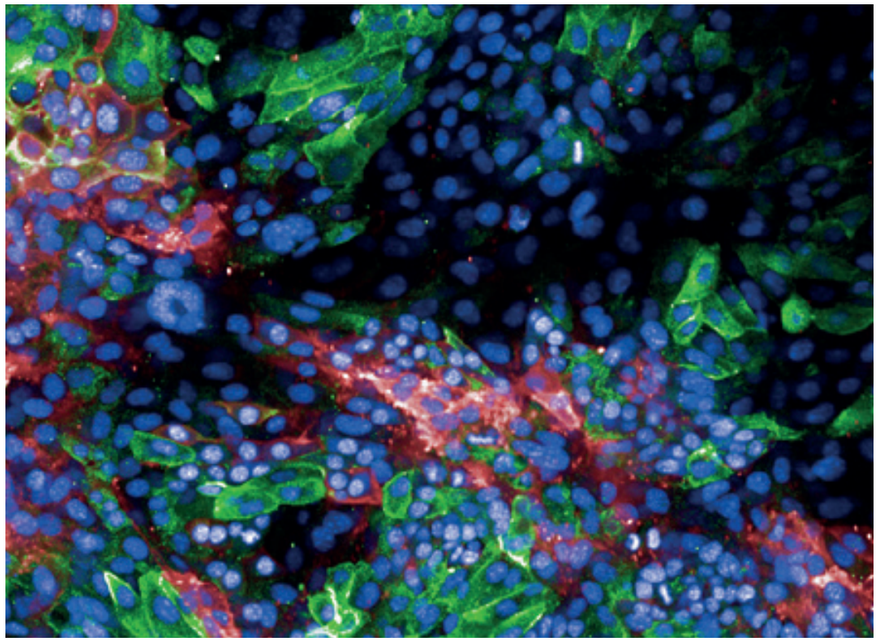
Extending the breadth and depth of our research portfolio is critical to achieving our aims and to this end, it is important that we attract additional funding to support our work. To increase our success in winning external grants, we therefore established a Grants Committee in 2016 to oversee the preparation of applications and ensure a robust system of internal peer review prior to submission. I am delighted that Iain Hagan agreed to chair this committee, because he brings considerable experience, having been a member of several grant funding committees and review panels. This committee is already having an impact, and I am also delighted at the success we enjoyed in our grant applications in 2016. Notably successes were Iain Hagan's Investigator Award from the Wellcome Trust, which will enable him to expand his work on the spatial and temporal control of mitotic commitment. Similarly, Amaya Viros, a former post-doctoral fellow in the Molecular Oncology group, secured a Wellcome Trust-funded Clinical Scientist Fellowship to enable her to establish an independent group at CRUK MI. Her Skin Cancer and Ageing group will investigate how the ageing microenvironment of the skin contributes to the more aggressive disease that occurs in older patients. Also impressive was Caroline Dive's successful bid, together with Professor Peter Kuhn at the University of Southern California, for funding from former US Vice President Joe Biden's Cancer Moonshot initiative. The aim of this programme is to develop and refine technologies to analyse tiny numbers or residual circulating cancer cells in the blood to identify the patients who are likely to relapse. We are also participating in several exciting collaborations with our local partners. During 2016, CRUK MI played major roles in Manchester's successful bid for CRUK Major Centre status and Manchester's successful bid for a NIHR Biomedical Research Centre (BRC). Both of these initiatives bring significant additional support to our research and will allow us to make progress on our major objective of delivering personalised medicine for cancer patients in the North-West. Our Deputy Director, Caroline Dive, is working closely with clinical colleagues and leading the cancer precision medicine theme in the BRC.



An organoid labelled for SiR-actin. *Image supplied by Renaud Mevel (Stem Cell Biology)*

It is always pleasing to see the success of our scientists recognised with prestigious prizes and awards. Caroline Dive was the recipient of the AstraZeneca British Pharmaceutical Society Prize for Women in Pharmacology, while Amaya Viros received the Leo Pharma Research Foundation Award, and I was delighted to receive the European Society for Pigment Cell Research Fritz Anders Medal. At The University of Manchester annual awards ceremony, Tim Somerville was named one of four Postgraduate Students of the Year and I was honoured to receive one of four Researcher of the Year Awards. Emma Williams was judged to have given the best oral presentation at the International PhD Student Cancer Conference in Cambridge, and Romina Girotti won the inaugural Christopher J. Marshall award for studies on signal transduction and melanoma, which was presented by the Society for Melanoma Research at their annual meeting in Boston in November. Such external recognition is highly rewarding and in total members of the Institute received 26 prizes and awards during the year. Each year, the Institute also bestows its own award, the Dexter Prize for Young Scientists. The Prize is named after Mike Dexter, a former Director of the Paterson Institute for Cancer Research (the former name of the Institute) and in 2016 it was awarded to Dan Wiseman. Dan is a Clinical Fellow who completed his PhD in the Leukaemia Biology group in 2016. His project focused on IDH mutations in Acute Myeloid Leukaemia and resulted in a significant body of work encompassing both the basic biology of this disease as well as more translational aspects. I am delighted that he is continuing his interest in IDH in his post-doctoral studies in the Leukaemia Biology group having secured a highly competitive Bloodwise Clinician Scientist Fellowship.

It was immensely rewarding to showcase Manchester and its success to the world's cancer research community by hosting the European Association for Cancer Research (EACR) meeting in the city during the summer. It was an honour to chair this meeting in my capacity as EACR President (2014-16). Moreover, Caroline Dive chaired a vibrant and stimulating National Cancer Research Institute meeting in Liverpool in November, which included an excellent plenary lecture from Iain Hagan and a



An in vitro co-culture of pancreatic cancer cells and cancer-associated fibroblasts. *Image supplied by Colin Hutton (Systems Oncology)*

Women in Science workshop that was organised and chaired by Caroline.

Engaging with the supporters who fund our research is incredibly important and the past year offered some unique opportunities to engage with the general public and to communicate the progress that we are making. In particular, 2016 marked Manchester's tenure as European City of Science. Our staff and students took part in a "science lates" evening at Manchester Museum and joined in with a soapbox science event at Piccadilly Gardens in the centre of Manchester. We held two open days where we invited our supporters into the Institute as well as hosting a regular series of laboratory tours. During these events, we explained to the general public how we use animals in our research, because although most of our work does not involve animals, some animal research is essential if we are to understand, prevent and cure cancer. As part of our drive to be open about how, when and why we use animals, we put on displays at various events including one at the Manchester Science Festival, and I am delighted that the efforts of the team that took part were acknowledged by a national award in public engagement from the organisation Understanding Animal Research.

During the summer, we said goodbye to two of our Group Leaders. John Brognard returned to the US to establish a group at the National Cancer Institute in Maryland while Valerie Kouskoff is continuing her research at the Division of Musculoskeletal and Dermatological Sciences at The University of Manchester. We thank both them and their research teams for their hard work and contributions to the Institute over the last few years and wish them all every success in the future.

Finally, looking forward, during 2017 we shall continue with further Group Leader recruitment in our areas of priority while continuing to develop our other activities. This includes continuing to work with our partners at University College London and in Belfast to develop our Lung and Prostate Centres of Excellence respectively. Similarly, we shall continue to collaborate with colleagues in the CRUK Manchester Centre and the MCRC over plans to develop the MCRC Phase 2 Building which will culminate in a new research facility that will house the Manchester Centre for Cancer Biomarker Sciences. This initiative is led by Caroline Dive and will support a range of translational and clinical studies to accelerate biomarker research into patient benefit. During the summer, the Institute will undergo a Quinquennial Review by an international panel of experts to assess our achievements over the last five years and consider our strategy for the years ahead. We look forward to sharing our success with the panel and receiving their input to help us progress our scientific agenda over the next five years.

RESEARCH HIGHLIGHTS

In this section we highlight some research publications from 2016 which report significant advances in specific areas. The selected papers demonstrate the breadth and the quality of the research being undertaken by the groups at the Cancer Research UK Manchester Institute.

Tape CJ, Ling S, Dimitriadi M, McMahon KM, Worboys JD, Leong HS, Norrie IC, Miller CJ, Poulogiannis G, Lauffenburger DA, Jørgensen C.
Oncogenic KRAS Regulates Tumor Cell Signaling via Stromal Reciprocation.
Cell, 2016; 165(4):910-20.

Pancreatic ductal adenocarcinoma (PDAC) is a dismal disease with five-year survival levels at less than 5%. This is due to late diagnosis, aggressive disease progression and limited treatment options. PDAC is characterised by activating mutations in the oncogene KRAS, with co-occurring inactivation of CDKN2A, TP53 and SMAD4. A hallmark of PDAC is an excessive expansion of genetically normal stromal cells, such as fibroblasts, which generate a pro-tumorigenic microenvironment that decreases the sensitivity of tumour cells to treatments. However, the mechanisms whereby tumour cells recruit and co-opt 'normal' cells, and how these cells in turn influence tumour cells, are poorly understood. Interrogating how tumour cells communicate with resident fibroblasts, we observed that tumour cells co-opt neighbouring fibroblasts to produce a reciprocal signal, ultimately altering tumour cell function. Specifically, the tumour cell response to expression of oncogenic KRAS was greatly influenced by the presence of fibroblasts. As such, we observed that specific kinases, for example AKT, were only activated in tumour cells when these were allowed to communicate with fibroblasts. Importantly, tumour cells were not sensitive to inhibitors of AKT when cultivated alone, but only when co-cultured with fibroblasts. This suggests that future efforts to evaluate drug targets should include stromal components.

Memon D, Dawson K, Smowton CSF, Xing W, Dive C, Miller CJ.
Hypoxia-driven splicing into noncoding isoforms regulates the DNA damage response.
npj Genomic Medicine, 2016; 1, Article number: 16020.

Blood vessels within a tumour are disorganised, leading to oxygen starvation (hypoxia). High levels of hypoxia are associated with poor patient outcome and resistance to therapy, making it a crucial aspect of tumour biology. When a gene is expressed to make a protein, the cell makes an RNA copy of the gene's DNA. This is then translated into a protein sequence that folds up to make the final protein. For most genes, some additional processing called 'alternative splicing' is performed, in which parts of the RNA are chopped out and the remaining bits spliced together. This lets the cell make a set of different, but related, proteins from the same gene.

By using RNA-sequencing to monitor changes to the RNA within cell lines starved of oxygen, the group identified specific genes that changed their splicing patterns to make RNA molecules that could not be translated into a valid protein. They then saw the same pattern occurring in patient samples from The Cancer Genome Atlas (TCGA), and showed that it affected a significant number of genes associated with repairing DNA damage. This is exciting because it reveals a new way that cells can switch on and off specific genes important in cancer.

Draper JE, Sroczynska P, Tsoulaki O, Leong HS, Fadlullah MZ, Miller C, Kouskoff V, Lacaud G.
RUNX1B expression is highly heterogeneous and distinguishes megakaryocytic and erythroid lineage fate in adult mouse hematopoiesis.
PLoS Genetics, 2016; 12(1):e1005814.

The transcription factor RUNX1 is a master regulator of blood cell production. It controls the accurate production of the various types of blood cells throughout adult life. Mutations in

RUNX1 cause defects in diverse blood lineages in human patients, including different types of leukaemia as well as blood clotting defects due to a shortage of platelet-producing megakaryocytes. RUNX1 is expressed from two promoters, which produce several distinct RNA transcripts and protein isoforms. To investigate the timing and localisation of the expression of these two promoters (termed distal and proximal), the Stem Cell Biology group created a mouse model with reporter genes expressed under the control of the Runx1 promoters. In a previous study, they determined the activities and requirements for the Runx1 promoters at the initiation of blood production in the developing embryo. In this study, the group investigated the output from the two promoters in adult organs, including bone marrow, spleen and thymus. They demonstrated that the distal Runx1 promoter is highly expressed but the proximal promoter activity is more restricted and in particular marks the point in adult blood production where the red blood cell and megakaryocyte pathways separate. The different proteins produced by these two Runx1 promoters may therefore have different roles in driving the production of these two cell types. They also determined that expression of the leukaemic fusion protein AML1-ETO9a resulted in preferential proximal upregulation suggesting that its expression may be important to establish a pre-leukaemic environment.

Girotti MR, Gremel G, Lee R, Galvani E, Rothwell D, Viros A, Mandal AK, Lim KH, Saturno G, Furney SJ, Baenke F, Pedersen M, Rogan J, Swan J, Smith M, Fusi A, Oudit D, Dhomen N, Brady G, Lorigan P, Dive C, Marais R.
Application of sequencing, liquid biopsies, and patient-derived xenografts for personalized medicine in melanoma.
Cancer Discovery, 2016; 6(3):286-99.

Melanoma is the most aggressive form of skin cancer. Recent breakthroughs in the development of immune checkpoint inhibitors and targeted therapies have transformed the management of melanoma and extended survival. Nonetheless, most patients still succumb to metastatic disease. Biomarkers to determine which patients will benefit from a specific treatment, to detect disease progression early and to select effective second-line therapies are needed. We have developed a personalised medicine platform utilising tumour tissue and liquid biopsies in combination with next generation sequencing (NGS) and xenograft models. Analysis of circulating tumour DNA (ctDNA) revealed that levels of mutant DNA predicted clinical responses and allowed detection of relapse due to common resistance mechanisms weeks ahead of clinical imaging. Patient-derived xenografts (PDXs) were used to test hypothesis-driven, personalised therapies and to validate combination therapies. As an alternative, in cases where tumours were not accessible, we generated circulating tumour cell derived xenografts (CDXs) from blood biopsies. CDXs recapitulated the genetic and histologic features of patient tumours and could predict response to therapy. Our findings demonstrate the power of a personalised medicine approach in which alterations identified by NGS can be used to monitor treatment responses, reveal resistance mechanisms, and may represent actionable targets that can be tested using xenograft models.

Marei H, Carpy A, Woroniuk A, Vennin C, White G, Timpson P, Macek B, Malliri A.
Differential Rac1 signalling by guanine nucleotide exchange factors implicates FLII in regulating Rac1-driven cell migration.
Nature Communications, 2016; 7:10664.

The protein Rac1 has been implicated in the formation of many tumour types and the dissemination of metastatic cells. Upon activation by guanine nucleotide exchange factors (GEFs), Rac1 associates with a variety of proteins in the cell thereby regulating various functions, including cell migration and invasion. However, activation of Rac1 is also required for epithelial cells to adhere strongly one to the other, raising the possibility of exacerbating tumour progression when targeting Rac1. Avoiding this complication calls for the identification of factors that control the selection of Rac1-driven cellular responses. Interestingly, there are at least 20 GEFs involved in Rac1 activation, suggesting a more complex role of GEFs in regulating Rac1 signalling besides promoting guanine nucleotide exchange. In this study, we highlighted the role of two Rac-specific GEFs, Tiam1 and P-Rex1, in dictating contrasting biological outcomes downstream of Rac1: Tiam1 inhibits, whereas P-Rex1 promotes migration. Importantly, proteomic analysis uncovered a role for both GEFs in modulating the Rac1 interactome, which results in the stimulation of GEF-specific signalling cascades. In particular, we demonstrate that P-Rex1 stimulates migration through enhancing the interaction between Rac1 and the actin-remodelling protein flightless-1 homologue, to modulate cell contraction.

Carter L, Rothwell DG, Mesquita B, Smowton C, Leong HS, Fernandez-Gutierrez F, Li Y, Burt DJ, Antonello J, Morrow CJ, Hodgkinson CL, Morris K, Priest L, Carter M, Miller C, Hughes A, Blackhall F, Dive C, Brady G.
Molecular analysis of circulating tumor cells identifies distinct copy-number profiles in patients with chemosensitive and chemorefractory small-cell lung cancer.
Nature Medicine, 2016 Epub 21 November

Small cell lung cancer (SCLC), the most aggressive form of lung cancer, kills around 220, 000 patients per year globally, with no significant change in survival or treatment options beyond platinum-based chemotherapy in 30 years. Most patients respond to chemotherapy well initially, but relapse rapidly with acquired drug resistance. Approximately 20% of patients present with chemorefractory disease progressing within 90 days of treatment. We generated the first DNA-based biomarker for SCLC in a 31 patient study which was based on copy number alterations (CNA) measured pre-treatment in single circulating tumour cells (CTCs). This CTC CNA classifier anticipated whether patients would be chemosensitive or chemorefractory. When examined in CTCs from initially chemosensitive patients who then progressed, the classifier did not correlate with that of chemorefractory patients' CTCs at baseline, implying distinct mechanisms of inherent and acquired chemotherapy resistance. Larger studies are now required to qualify this classifier. Currently patients receive chemotherapy upon a SCLC diagnosis. Until improved targeted treatments are forthcoming, it is very unlikely that the classifier will be used to alter treatment. However, the classifier could be helpful in designing early clinical trials of emerging therapies where anticipation of the duration of first line chemotherapy response could be beneficial.

Williamson SC, Metcalf RL, Trapani F, Mohan S, Antonello J, Abbott B, Leong HS, Chester CPE, Simms N, Polanski R, Nonaka D, Priest L, Fusi A, Carlsson F, Carlsson A, Hendrix MJC, Seftor REB, Seftor EA, Rothwell DG, Hughes A, Hicks J, Miller C, Kuhn P, Brady G, Simpson KL, Blackhall FH, Dive C.
Vasculogenic mimicry in small cell lung cancer.
Nature Communications, 2016; 7:13322.

The majority of small cell lung cancer patients present with widespread metastases and consistently circulating tumour cells (CTCs) are relatively prevalent in this tumour type. We discovered a rare CTC subset which co-expressed the endothelial marker vascular-endothelial cadherin (VE-Cad) with epithelial cytokeratins, a phenotype consistent with vasculogenic mimicry (VM). VM is a hypoxia driven process whereby tumour cells form 'endothelial-like' vessels. We hypothesise that VM may support tumour dissemination. Using a microarray from SCLC patient tissues we showed that high VM levels correlated with worse prognosis. Importantly, using copy number alterations, we used our CTC-derived patient explant models to confirm that VM vessels were tumour derived and that VE-Cad expressing CTCs were of SCLC origin. By knocking down VE-Cad in a VM proficient SCLC cell line, we showed in the xenograft model that VE-Cad was required for VM which increased initial tumour growth dynamics. Despite increased intratumoural cisplatin in VM proficient versus deficient tumours, VM proficient tumours were less cisplatin sensitive. The functional significance of VM in SCLC suggests that understanding its regulation may present opportunity for therapeutic interventions. The impact of VM on metastases is now under investigation.

James DI, Smith KM, Jordan AM, Fairweather EE, Griffiths LA, Hamilton NS, Hitchin JR, Hutton CP, Jones S, Kelly P, McGonagle AE, Small H, Stowell AI, Tucker J, Waddell ID, Waszkowycz B, Ogilvie DJ.
First-in-Class Chemical Probes against Poly(ADP-ribose) Glycohydrolase (PARG) Inhibit DNA Repair with Differential Pharmacology to Olaparib.
ACS Chemical Biology, 2016; 11(11):3179-3190.

DNA repair pathways are often compromised in cancer cells and this presents a double-edged sword for tumours. Whilst the compromised repair mechanisms allow cells to rapidly gain mutations conferring benefits such as drug resistance, these cells become highly dependent on the remaining pathways for survival. Blockade of these remaining processes with a small molecule can lead to therapeutic benefit. This approach is exemplified by inhibitors of the DNA repair protein poly(ADP-ribose) polymerase (PARP). However, many components of these pathways are poorly understood, partly because of a lack of chemical tools to probe their role in disease. In this study, the Drug Discovery Unit (DDU) reveal a unique compound which specifically targets poly(ADP-ribose) glycohydrolase (PARG), a vital component in the repair of DNA single strand breaks. PARG was previously considered by some to be undruggable, given the nature of the active site but this study (arising from a collaboration with AstraZeneca) revealed a novel start point that induced its own unique binding site. The DDU used computational, medicinal chemistry and assay development expertise to discover the first cell-permeable, selective inhibitors of PARG. The team anticipate that this compound will help unravel the role of PARG in DNA repair and other biological processes, eventually leading to the discovery of novel therapeutics.

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RESEARCH GROUPS

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CANCER INFLAMMATION AND IMMUNITY

Group Leader
Santiago Zelenay

Scientific Officer
Chikkanna-Gowda CP

Postdoctoral Fellows
Eduardo Bonavita[1]
Victoria Pelly[1]

Graduate Student
Eimear Flanagan[1]

[1]joined in 2016

The clinical success of therapies that harness the ability of the immune system to recognise and eliminate cancer cells is currently extending to numerous malignancies, including cancer types previously thought to be refractory to immunotherapy. However, complete and durable tumour regressions are observed only in a reduced minority of patients highlighting the need for mechanistic studies to uncover the basis for absent or partial responses. The Cancer Inflammation and Immunity group investigates the underlying mechanisms that allow cancer cells to evade natural and therapy-induced tumour immunity, combining the use of genetically engineered pre-clinical cancer models with the analysis of samples from cancer patients. Our ultimate goal is to develop novel targeted interventions to disrupt immune suppression, promote tumour immunity and enhance the efficacy of cancer therapy.

The remarkable success of immunotherapy, mainly from strategies based on immune checkpoint blockade, has fully reinvigorated interest in its application to the cancer immunology field. These therapies have shown great promise for the treatment of advanced cancers and also, more recently, as an adjuvant therapy following tumour resection. Arguably the most important open question in the field is why only some patients benefit from treatment and particularly why full and long-lasting clinical outcomes are observed only in a much reduced subset of patients. Intense recent research on this topic points to the presence of pre-existing tumour-infiltrating T cells, particularly $CD8^+$ T cells, as one of the best predictive markers for clinical benefit following immune checkpoint blockade. This is consistent with the notion that these drugs work, at least in part by blocking inhibitor receptors upregulated in activated endogenous T cells.

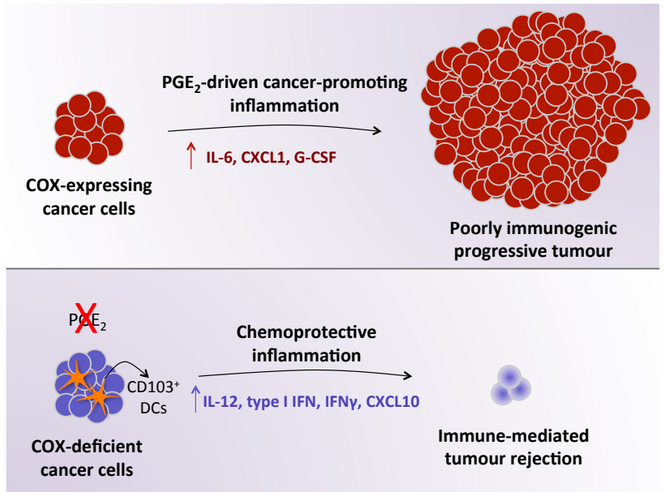
These observations have prompted the classification of tumours into 'hot' T cell-inflamed tumours, and 'cold' non-inflamed tumours lacking T cell infiltration, and stimulated the study of the principles and rules that account for the establishment and maintenance of these two tumour phenotypes. Indeed, the signals, factors and pathways controlling the priming of, recruitment to and function of, T cells within tumours in natural conditions or following therapy are still poorly defined. This knowledge is essential to the design of novel interventions to enhance the efficacy of cancer therapy. In this context, our group actively investigates the signals that drive innate and adaptive immunity against malignant cells aiming to distinguish factors that accelerate cancer progression from those that mediate tumour elimination.

Tumour-protective versus tumour-promoting inflammation

Inflammation is a common feature of most, if not all, clinically apparent tumours. This type of inflammatory response, however, commonly associated with the presence of myeloid cells such as macrophages or specific subsets of regulatory T cells, typically fuels tumour growth, aggressiveness and can drive resistance to cancer treatment, including therapies aimed at boosting the anti-tumour immune response. Thus, rather than tumours being inflamed or not inflamed, they exhibit quantitative and qualitatively different cellular and molecular inflammatory profiles. Tumour-infiltrating inflammatory cells, such as macrophages, T cells, neutrophils, dendritic cells and other white cells can be found in various numbers and displaying a wide spectrum of differentiation status. All these cells can have a dual role impacting favourably or negatively on cancer progression depending on their number, relative composition and activation phenotype.

Building on from a recent study where we uncovered a fundamental role for prostaglandin E_2 (PGE_2) in cancer immune evasion (Zelenay et al. *Cell* 2015), our group investigates the basis for how different types of inflammation are established or manipulated by a growing tumour and to what extent manipulation of the local inflammatory response constitutes an opportunity for therapeutic intervention. In this proof of principle study we demonstrated that melanoma, colorectal or breast cancer cells rendered unable to produce PGE_2 by genetic means are greatly impaired in their ability to form tumours and frequently completely regressed. Cancer cells, impaired in their ability to produce PGE_2, failed to grow in immunocompetent animals while grew progressively, as their parental counterparts, in immunodeficient hosts highlighting an essential role for PGE_2 in immune escape. Moreover, tumours formed by cancer cells deficient in cyclooxygenase (COX) 1 and 2, the rate limiting enzymes for PGE_2 production, showed a drastic shift in the local inflammatory signature characterised by lower expression of cancer-promoting factors and a concomitant increase in mediators normally associated with T cell-dependent tumour control (Figure 1). Thus, our findings suggest that manipulation of the type of inflammation and subversion of immune surveillance by a growing tumour is essential for its ability for progressive growth. Our current working model postulates that tumour-derived PGE_2 allows cancer immune escape by shifting the intra-tumoural immune environment from one favourable to anti-tumour immunity to one that fuels tumour growth (Figure 1; Zelenay & Reis e Sousa *OncoImmunology* 2016).

Figure 1
Cyclooxygenase (COX)-driven production of the inflammatory lipid PGE_2 by cancer cells fuels tumour-promoting inflammation and allows progressive tumour growth. Cancer cell-specific COX-deficiency alters the inflammatory profile at the tumour site increasing anti-tumour mediators and enabling immune-dependent tumour control and eradication.



We are currently further investigating the underlying basis for this dominant and essential role of PGE_2 in immune evasion, combining the use of in vitro assays with the analysis of genetically engineered cancer mouse models that very closely reflect the genetics and pathology of human cancer. Furthermore, our current analyses of publically available cancer patient datasets underscored a notable conservation of the COX-driven mouse inflammatory signature and extended our original early findings in human cutaneous primary melanoma to advanced metastatic melanoma and to other cancer types, suggesting that COX-activity might be a driver of immune suppression across different malignancies.

Manipulating inflammation to raise cancer immunogenicity

An additional key finding from this study on which most of our initial research questions are based was that reduction of PGE2 levels using COX inhibitors synergised with immune checkpoint blockade in promoting immune-dependent tumour growth control in pre-clinical models. Indeed, combining the COX-inhibitor aspirin, one of the most widely used drugs, with anti-PD-1 blockade, promoted much more rapid adaptive immune-mediated tumour eradication than either treatment alone. Thus, in a project of great translational and clinical significance, we are further characterising the role of COX-inhibitors in potentiating anti-tumour immune responses following conventional and immune-based cancer therapies. These activities included the design of a clinical trial to test these combinations in cancer patients in collaboration with oncologists from The Christie NHS Foundation Trust and a research agreement with a pharmaceutical company to evaluate the efficacy of improved newly developed COX-2 inhibitors as potential additions to immunotherapies based on immune checkpoint blockade. Similarly, we are investigating whether combination with COX inhibitors may be useful to a broader range of cancer therapies, where their efficacy rely, at least in part, on the stimulation of anti-tumour immunity, such as chemotherapy and radiotherapy.

Finally, using an approach that combines once again in vitro assays with the analysis of in vivo cancer models and human samples and datasets, we embarked on a project specifically designed to identify other potential immunomodulatory and immunosuppressive factors that, similarly to PGE_2, act in a dominant manner. These factors, unlike recessive mechanisms of immune escape, constitute ideal therapeutic targets to unleash anti-cancer immunity and to enhance the efficacy of standard and immune-based therapy.

Publications listed on page 58

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CELL DIVISION

Group Leader
Iain Hagan

Associate Scientist
Agnes Grallert

Postdoctoral Fellow
Lenka Halova

Scientific Officers
Ben Hodgson
Kuan Yoow Chan

The inappropriate proliferation of cancer cells can arise from unchecked cell division, a failure to engage cell death pathways, or simultaneous changes in both. Understanding how the diverse cues are integrated to co-ordinate cell division and death therefore sits at the heart of our need to understand the biology of cancer. Furthermore, DNA damaging and anti-mitotic therapies owe much of their success to the checkpoint pathways that ensure that transition through the cell division cycle only occurs when genome integrity is guaranteed.

We therefore study the targets of two of these therapeutically important checkpoint pathways: the commitment to, and the exit from, the physical process of genome segregation, mitosis. Because the regulatory networks that control cell division are highly conserved, we study the simple, unicellular, fission yeast to identify the key questions to ask of the analogous controls in the much more complex context of human cell division.

In a typical cell division cycle the G1 gap phase precedes DNA replication in Synthesis (S phase), before a second gap phase, G2, separates S from genome segregation in Mitosis (M phase). Growth, developmental and environmental cues regulate the G1/S and G2/M transitions to control the rate of proliferation. Passage through these key transitions is driven by the activation of distinct CDK-Cyclin complexes. The G2/M transition is a critical safeguard of genome integrity; incomplete DNA replication or DNA damage triggers checkpoint pathways that block the G2/M transition to ensure that chromosomes are not segregated when incomplete or damaged. The G2/M transition is driven by activation of the Cdk1-Cyclin B protein kinase. Wee1-related kinases phosphorylate the catalytic subunit, Cdk1, to inhibit the complex during interphase. This phosphate is removed by Cdc25 phosphatases to promote mitotic entry. Cdk1-Cyclin B activation promotes a positive feedback loop that boosts Cdc25 and inhibits Wee1 activities to ensure that mitotic commitment is a rapid and irreversible bi-stable switch (Figure 1). In humans and fission yeast, this feedback control exploits polo kinase to determine the timing of mitotic entry.

The observation that active Cdk1-Cyclin B appears on human centrosomes before propagating throughout the cell has been consolidated by other data to suggest that the centrosome provides a specific microenvironment for the activation of Cdk1-Cyclin B to trigger the G2/M transition. Our studies of the fission yeast centrosome equivalent, the spindle pole body (SPB), provide molecular insight into how this switch may operate. We have been able to exploit the malleability of yeast to show that release of Cdk1-Cyclin B or Polo kinase activity at the SPB, but at no other location in the cell, triggers the G2/M transition. This mechanistic insight suggests that the correlative observations of Cdk1-Cyclin B activation in metazoa reflect a true functional relationship between the centrosome and the activation of Cdk1-Cyclin B to initiate mitosis. Furthermore, our studies of the SPB scaffold Cut12 provide insight into just how great an impact centrosomal control can have. Simply blocking the recruitment of protein phosphatase 1 (PP1) to the SPB enables us to delete the *cdc25*$^{+}$ gene without impacting upon viability. The basis for this bypass of the requirement for this essential mitotic inducer appears to lie in the influence that the Cut12/PP1 axis holds over Polo kinase activity. Polo activity shows a direct, inverse, correlation with PP1 recruitment to the SPB indicating that the SPB appears to act as a catalyst to release Polo activity throughout the cell (Figure 1). Inappropriate elevation of Polo activity probably removes the requirement for Cdc25 because it inappropriately represses Wee1 activity, as it would ordinarily do during a normal mitotic commitment.

The importance of signalling from the SPB is re-enforced by the counterintuitive observation that the signalling network that controls the timing and execution of cytokinesis also relies upon anchorage to the SPB. This network is anchored by a second scaffolding molecule; Sid4. We recently found that signalling from Sid4 can compensate for mitotic commitment deficiencies when Cut12 function is compromised. Thus, dialogue between the mitotic exit and mitotic commitment scaffolds determines when division will be initiated. This dialogue provides a plausible rationale for the use of the centrosome as a signalling hub: convergence of signals from multiple pathways to a limited number of neighbouring scaffolds, clustered together on the SPB, can integrate the inputs from the different pathways to generate a coherent signal that sets the flux through outgoing signalling cascades. We are pursuing the molecular basis for this dialogue and the function of the equivalent kinases and similar centrosomal scaffolds in human cells.

Anti-mitotic drugs, such as the microtubule stabilising derivatives of Taxol, arrest mitotic progression because the lack of chromosomal alignment on the mitotic spindle stimulates the spindle assembly checkpoint (SAC). SAC activation blocks the destruction of Cyclin B that is required for mitotic exit. Prolonged SAC dependent arrest triggers apoptotic cell death. As the genomic instability of tumour cells places a disproportionate demand upon SAC function, further load on the SAC by anti-mitotics is sufficient to trigger apoptosis in the tumour cells, while simply delaying exit in non-transformed cells that have a normal karyotype. However, anti-mitotics only work in certain tumour settings, as cells can "slip" out of mitosis from the mitotic arrest without completing either cell division or triggering death. Slippage occurs when Cdk1-Cyclin B activity falls below a threshold level that is required to maintain the mitotic state. There is therefore great interest in finding therapeutic routes to block slippage and so reinforce the impact of anti-mitotics to trap slippage-prone cells in mitosis. A simple approach would be to block the phosphatase activities that counteract Cdk1-Cyclin B activity to set the Cdk1-Cyclin B slippage threshold.

PP1 and protein phosphatases 2A (PP2A) account for over 90% of the serine/threonine directed phosphatase activity in human cells. PP1 is recruited to docking sites from where it dephosphorylates targets. Hetero-trimeric PP2A enzymes comprise single scaffolding and catalytic subunits, alongside one of four different types of regulatory subunit. Multiple, alternatively spliced, genes give the potential to generate hundreds of variants of each type of PP2A complex in humans, whereas fission yeast can live on one of each, or, in the case of PP2A-B55, none. PP2A-B55, PP2A-B56 and PP1 have each been proposed to drive mitotic exit. PP1, PP2A-B55 and PP2A-B56 activities decline upon mitotic commitment.

We found that direct recruitment of PP1 to PP2A-B55 and PP2A-B56 re-activates these PP2A phosphatases to support appropriate mitotic progression/exit in fission yeast. Mitotic inhibition of PP1 arises from direct phosphorylation by Cdk1-Cyclin B. The destruction of Cyclin B subsequently allows PP1 to auto-dephosphorylate and restore its own phosphatase activity. Reactivated PP1 then reactivates PP2A-B55. Polo phosphorylation of the PP1 docking site of PP2A-B56 initially blocks PP1 binding to PP2A-B56. When Polo activity declines in mitotic exit, PP2A-B55 dephosphorylates the Polo phosphorylation site on B56 to allow PP1 to reactivate PP2A-B56 (Figure 2). Dr Anja Hagting in Professor Jonathan Pines' lab (Institute of Cancer Research, London) found a relationship between PP1 and PP2A-B56 that mirrors our findings in yeast. We are now extending these studies in human cells and using yeast to identify the PP1 target in the PP2A complexes and will characterise the fundamental properties of PP2A phosphatases. Our findings will impact upon cancer research beyond cell cycle control as PP2A components and regulators are frequently mutated in cancer.

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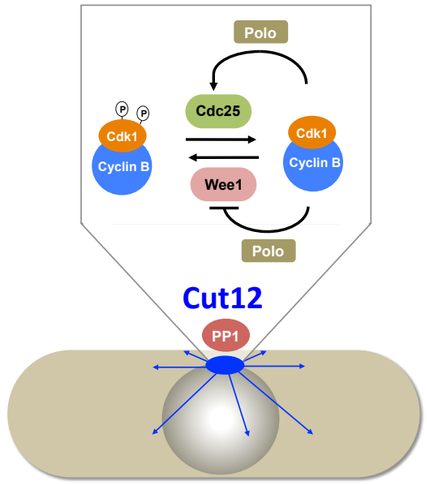

Figure 1: The Spindle pole body acts as a signalling hub to trigger Cdk1-Cyclin B activation
Cdk1-Cyclin B activity is held in check in interphase as a consequence of phosphorylation of Cdk1 by Wee1. Cdc25 removes the inhibitory phosphate to trigger mitosis. This trigger level of Cdk1-Cyclin B then activates polo kinase to further boost Cdc25 activity and inhibit Wee1 to make this transition a bi-stable switch between two distinct fates. The affinity of PP1 for Cut12 sets the threshold for the activation of Polo kinase. When PP1 is tightly bound to Cut12, Polo activity cannot rise to threshold levels that trigger the feedback controls that drive division. If PP1 is absent, Polo activity rises inappropriately to inhibit Wee1 and remove the need for Cdc25.

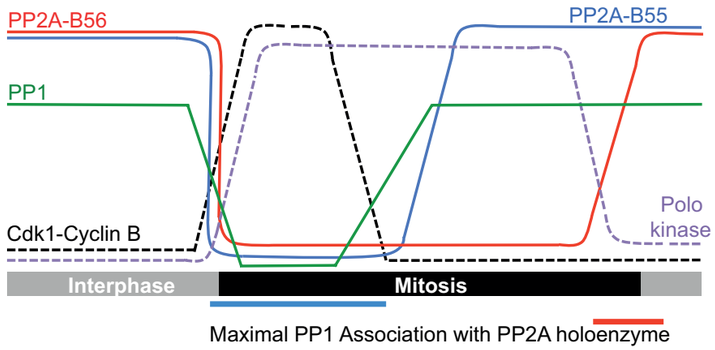

Figure 2: The mitotic PP1-PP2A phosphatase relay
PP1 and PP2A activities are all repressed upon entry into mitosis. The mode of PP2A repression is unclear, however, it is well established that Cdk1-Cyclin B phosphorylation represses PP1 activity. Cyclin B destruction then allows PP1 to auto-catalytically remove this inhibitory phosphate from itself. As PP1 is bound to the B55 regulatory subunit of PP2A-B55 at this time, PP1 reactivation immediately restores PP2A-B55 activity. In contrast, PP2A-B56 is unable to recruit PP1 because Polo kinase phosphorylates a residue within the PP1 docking site on the regulatory B56 subunit. Once Polo activity declines at the end of mitosis, PP2A-B55 can overcome Polo activity towards this site and remove the inhibitory phosphate from the PP1 docking site of B56. Consequently PP1 can be recruited to PP2A-B56 and this second PP2A activity is reactivated at the end of mitosis. Reprinted by permission from Macmillan Publishers Ltd: Nature 517:94-98, copyright 2015.

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CELL REGULATION

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The stress-activated kinase pathways play an essential role in the cellular response to many different extracellular and intracellular signals and as a result regulate many key biological processes including cell proliferation and cell death. Cancer cells are exposed to various stress conditions such as oncogenic stress and oxidative stress and it is not surprising therefore that these pathways have been implicated in cancer development and progression. Their involvement appears to be complex and can either promote or inhibit tumour growth in a context-dependent manner. Our goal is to better understand the role and molecular function of these pathways in particular human cancer types.

We have predominantly focused on the JNK stress-activated kinase pathway and one of its downstream targets, namely the transcription factor ATF2. The role of JNK in tumourigenesis has been studied in a variety of mouse tumour models and in most cases shown to be tumour-suppressive. We have shown that JNK suppresses Ras-dependent tumour formation in an orthotopic mouse model of liver cancer and that this effect is mediated through its regulation of ATF2 activity. Tumour suppression by JNK is lost in the absence of ATF2 or by mutation of the key residues in ATF2 that are phosphorylated by JNK. We identified a novel ATF2-driven transcriptional programme activated in response to stress stimuli in a JNK-dependent manner and showed a striking down-regulation of this programme in several human tumour types (including breast, lung and ovarian) compared to normal tisues. These studies strongly indicate that ATF2 and its upstream regulator JNK play an important tumour supressive role in human cancer development. Furthermore, these observations are supported by the presence of inactivating mutations in several kinases lying upstream of JNK in a number of human cancers.

The role of stress-dependent transcription in prostate cancer

Prostate cancer is often a stable disease with modest symptoms that can be managed conservatively. In fact, the great majority of men are diagnosed with relatively benign, indolent tumours that remain localised to the prostate gland. On the other hand, a minority of patients will develop aggressive tumours that have the potential to metastasize, and endanger life. However, the molecular mechanisms that determine whether a tumour remains indolent or progresses are only poorly understood, and this impedes the development of better tools for the management and treatment of prostate cancer patients.

We have analysed the expression of ATF2 target genes in prostate tumours at different stages of development using several publically available datasets. The results indicate that many ATF2 targets are strikingly under-expressed in metastatic versus primary tumours. By this criterion, impairment of ATF2 function occurs in all metastatic prostate tumours and is not restricted to tumours with specific genetic lesions. Furthermore, we find that in primary tumours, reduced expression of ATF2 targets is related to Gleason score. Analysis of two independent patient cohorts reveals a clear trend, with low grade tumours having the highest, and high grade tumours the lowest, expression of ATF2 targets. This suggests that ATF2-dependent gene expression is lost initially during development of the primary tumour, and that impaired ATF2 function is associated with increased risk of metastasis.

Given that following radical prostatectomy, disease recurrence is caused by the presence of small metastatic lesions, we hypothesised that the expression of ATF2 targets should correlate with clinical outcome. Accordingly, we find that low expression of several ATF2 target genes strongly correlates with a high risk of recurrence. Based on these findings, we used a bioinformatics approach to derive a prognostic gene signature based solely on ATF2 target genes. The resulting prognostic index, which utilises expression information from 18 ATF2 targets, is significantly associated with risk of disease recurrence in independent patient cohorts – supporting the notion that progressive disease is associated with impaired expression of stress-responsive genes.

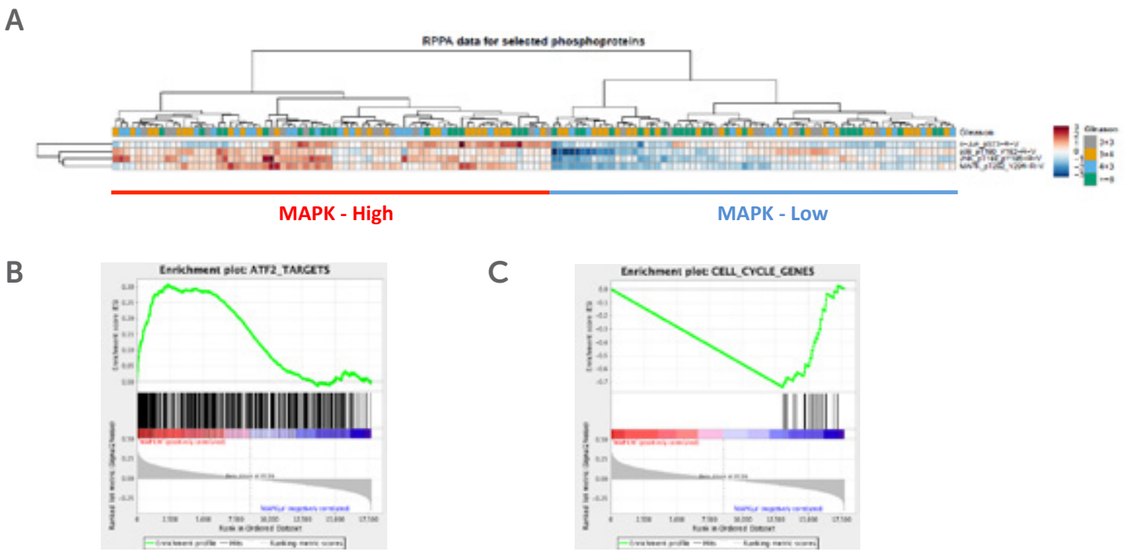

Figure 1: The status of stress-response pathways in primary prostate cancers.
A) Analysis of reverse-phase protein array data from TCGA was used to quantify phosphorylation of the three major MAP kinases (JNK, ERK and p38). Hierarchical clustering reveals two groups of tumours possessing either high or low MAPK activation. B & C) Gene set enrichment analysis reveals distinct patterns of gene expression associated with MAPK activation status; whereas expression of ATF2 targets correlates with elevated MAPK activation in prostate tumours (B), genes associated with cellular proliferation are highly expressed in tumours with relatively low MAPK phosphorylation (C).

Since ATF2 drives transcription in response to its phosphorylation by MAPKs, we asked whether the expression of ATF2 targets was correlated with the activation status of these kinases in prostate tumours. Focusing on JNK, p38 and ERK, we performed hierarchical clustering on RPPA (Reverse-phase protein array) data available in the TCGA PRAD dataset. This approach reveals that the activation status of all three kinases is strikingly similar, and that primary tumours are readily organised into two classes having either high or low activation of MAPKs. Furthermore, gene set enrichment analysis of these two clusters reveals that expression of ATF2 targets is significantly associated with MAPK activation. Stratifying the RPPA dataset by Gleason score reveals a clear trend towards reduced MAPK activation in high grade tumours. Therefore our data indicate that high risk lesions are characterised by a loss of MAPK signalling which results in impaired expression of ATF2 targets.

3-4 % of prostate tumours harbour homozygous loss of the chromosomal locus 2q31, with the peak of deletion occurring in the vicinity of the ATF2 gene. Although the number of tumours lacking ATF2 is relatively low, our analysis indicates that they possess an aggressive phenotype. The presence of these deletions correlates with a high risk of disease recurrence following radical prostatectomy, and these tumours are characterised by high expression of markers of cellular proliferation. These observations suggest that genetic loss of ATF2 may alter the course of the disease, promoting the outgrowth of highly proliferative tumours that possess increased metastatic potential.

To elucidate the function of ATF2 in prostate cells we used CRISPR-based gene editing to ablate ATF2 expression in the cell line PNT2. When cultured in low serum concentrations, ATF2 KO cells continue to incorporate BrdU more readily than their WT counterparts, revealing a function for ATF2 in the regulation of proliferation. Furthermore, we find that the expression of ATF2 targets is negatively correlated with markers of cellular proliferation in several datasets. Thus, tumours displaying high levels of MAPK activation (and ATF2 target expression), are characterised by low expression of cell-cycle genes. Taken together, these data suggest a role for ATF2 in the negative regulation of proliferation in prostate tumours.

Characterisation of tumour suppression by MEKK1-MKK4 signalling

Characterisation of various mouse models provides compelling evidence for a tumour suppressive role of JNK signalling in human cancer. Whilst JNK is not recurrently mutated in tumours it is clear that genes encoding several upstream kinases of the pathway do harbour missense mutations at a reasonably high frequency. Of particular interest are the MAP3 kinase, MEKK1 and the MAP2 kinase, MKK4. In breast and ovarian tumours, these genes are subject to inactivation by either deletion or missense mutation, and intriguingly, the presence of an inactivating mutation in one of these kinases is almost always exclusive of a mutation in the other suggesting that they function together to suppress tumour formation. To understand their function in suppression we have established and are characterising appropriate mouse models in which floxed alleles of either MEKK1 or MKK4 are deleted in a tissue-specific manner.

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Recurrent mutations and overexpression in tumours and cell lines implicate the small GTPase RAC and its activators, the guanine nucleotide exchange factors (GEFs), in the development and spreading of a range of human cancers. Furthermore, the effects of deleting genes encoding RAC proteins or RAC GEFs in mouse or of inhibiting RAC-GEF/RAC interactions with drugs strongly suggest that targeting RAC signalling could constitute a cancer treatment. However, to be effective, only RAC-dependent effects that promote tumour growth and progression should be targeted, while those functions that antagonise tumour progression or are essential in healthy cells should be spared.

RAC, a member of the family of RHO-like GTPases, cycles between a GDP- and a GTP-bound state. When GTP-bound, it binds to various effector molecules that stimulate downstream responses including, notably, actin cytoskeletal reorganisation. Multiple mechanisms control RAC activity including nucleotide binding and hydrolysis regulated by GEFs and GTPase Activating Proteins (GAPs) respectively, subcellular localisation, modulation of RAC protein levels, and post-translational modification including isoprenylation and, as we and others have demonstrated, ubiquitylation and SUMOylation (Castillo-Lluva et al. *Oncogene* 2013; Castillo-Lluva et al. *Nat Cell Biol.* 2010).

RAC GEFs are typically large proteins containing multiple protein–protein interaction domains. Besides stimulating guanine nucleotide exchange, GEFs function as molecular scaffolds targeting active RAC to particular subcellular locations and potentially increasing the local concentration of selective effector molecules, thereby influencing downstream processes. Through influencing selectivity in RAC signalling, GEFs could therefore perform unique signalling roles that could be important for tumourigenesis. Indeed, mice deficient for the RAC GEF TIAM1 are resistant to the formation of skin tumours induced by chemical carcinogens that target H-Ras and the few resulting tumours grow very slowly (Malliri et al., *Nature* 2002). Thus TIAM1, we infer, plays a unique role in mediating RAS transformation that the Cell Signalling group is elucidating, focusing currently on RAS-driven lung cancer.

TIAM1 antagonises malignant progression via regulating cell–cell adhesion

Despite their slower growth, skin tumours arising in *Tiam1*-deficient mice progressed more frequently to malignancy, suggesting that retention of TIAM1 impedes malignant progression, consistent with down-regulation of TIAM1 in skin malignancies in the mouse chemical carcinogenesis model (Malliri et al., *Nature* 2002). One mechanism by which TIAM1 and RAC suppress malignant progression is through stimulating cell–cell adhesion. Previously, we identified β2-syntrophin, a component of the dystroglycan adhesion complex, as a TIAM1 interacting partner. Our study (Mack et al. *Nat Cell Biol.* 2012) uncovered a novel role for this complex in promoting tight junction formation and the development of apicobasal polarity through generating a RAC activity gradient in the membrane region encompassing these junctions.

Malignant progression can entail the loss of cell–cell adhesion. Over-expression of activated RAC or TIAM1 promotes the formation of adherens junctions (AJs) and the associated generation of an epithelial-like phenotype in mesenchymal cell lines (Malliri & Collard, *Curr Opin Cell Biol* 2003). Moreover, TIAM1 is required for both the formation as well as the maintenance of cadherin-based adhesions (Malliri et al., *J Biol Chem* 2004). The oncoprotein SRC, a non-receptor tyrosine kinase, targets AJs for disassembly. Previously, we revealed that SRC phosphorylates TIAM1 inducing its cleavage by Calpain and its depletion from adherens junctions. Blocking

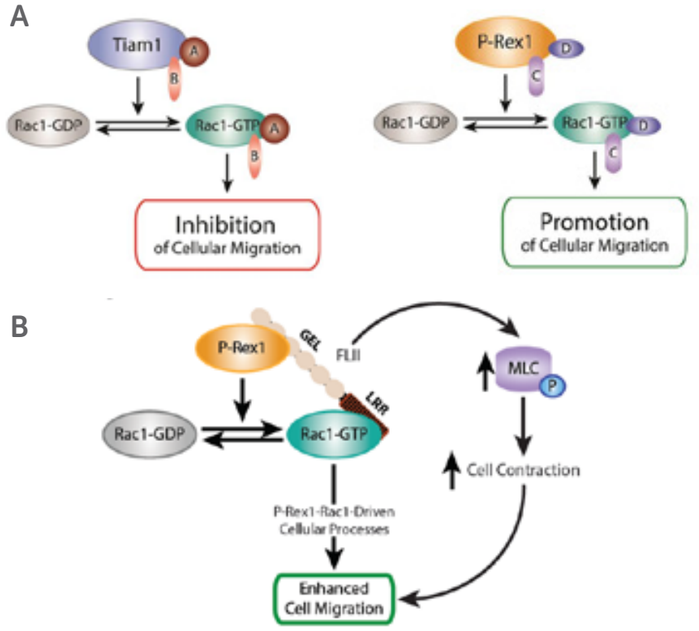

Figure 1: **(A)** RAC GEFs regulate RAC signalling via serving as RAC activators as well as scaffolding proteins. The RAC GEFs TIAM1 and P-REX-1 lead to different effects on cell migration via regulating the RAC interactome. **(B)** Schematic representation of the P-REX1-RAC-FLII signalling cascade. Activation of RAC by P-REX1 results in RAC binding to FLII. P-REX1, via its scaffolding ability, binds to FLII through its GEL domain and brings it in close proximity to active RAC further stimulating the RAC-FLII interaction via the LRR domain of FLII. Through this interaction, P-REX1 induces phosphorylation of MLC (pMLC) thereby enhancing cell contraction in a FLII-dependent manner. This cascade accounts, in part, for P-REX1-RAC-driven cell migration.

phosphorylation of TIAM1 by SRC suppressed AJ disassembly (Woodcock et al., *Mol Cell* 2009). In another study from our laboratory (Vaughan et al., *Cell Reports* 2015), we found that TIAM1 is ubiquitylated and degraded upon treatment of cells with hepatocyte growth factor (HGF), a cytokine that is abundant in cancer and promotes invasion of cancer cells. We mapped the ubiquitylation site on TIAM1 and also identified the responsible E3 ligase as being the HECT family member HUWE1. Moreover, we showed that interfering with TIAM1 ubiquitylation by depleting HUWE1 or mutating the ubiquitylation site retards the scattering and invasion of cells through delaying AJ disassembly. HGF and HUWE1 are plentiful in lung cancer. We showed that HUWE1 and TIAM1 expression are inversely correlated in lung cancer specimens and significantly that HUWE1 promotes lung cancer invasion by degrading TIAM1. Potentially, drugs capable of disrupting the HUWE1-TIAM1 interaction could antagonise invasion of lung and other cancer cells, reducing the risk of metastasis.

TIAM1 localises in the nucleus of colorectal cancer cells and inhibits their migration and invasion

Previously, using recombinant mouse models, we showed that TIAM1 cooperates with WNT signalling during the initiation of colorectal cancer (CRC) but then antagonises CRC tumour progression (Malliri et al., *J Biol Chem.* 2006). However, how TIAM1 influences CRC initiation and progression remained obscure. To further address the influence of TIAM1 on CRC and increase our understanding of its clinical role, we probed a tissue microarray comprising 650 samples, including Dukes stages A-C, from a well-characterised patient cohort using a TIAM1 antibody. Intriguingly, we detected TIAM1 not only in the cytoplasm, but also in cell nuclei and showed that nuclear and cytoplasmic TIAM1 staining intensity decreased with advancing Dukes stage. Thus, TIAM1 expression is negatively associated with colon cancer progression, consistent with our previous finding that TIAM1 antagonised progression of intestinal tumours in $Apc^{MIN/+}$ mice. Further analysis of these data also revealed that patients with high nuclear TIAM1 had significantly better survival than those with low nuclear TIAM1. Subsequent experiments into the mechanism by which TIAM1 antagonises CRC progression showed that TIAM1 localises in the nucleus of CRC cells via a functional nuclear localisation signal. These experiments also identified TIAM1 as a critical antagonist of CRC migration and invasion through inhibition of TAZ and YAP transcriptional co-activators. Thus, our findings have identified a critical role for TIAM1 in colon cancer progression through regulating TAZ/YAP activity and showed that high levels of nuclear TIAM1 could serve as a good prognostic factor for CRC patients.

GEFs determine differential RAC signalling

Activation of RAC can lead to contradictory migratory phenotypes— cell–cell adhesion versus motility— raising the possibility that targeting RAC in a clinical setting could worsen tumour progression. This calls for the identification of factors that both influence the selection of RAC-driven cellular processes as well as mediate RAC's effects. In a recent study (Marei et al., *Nat Commun.* 2016), we demonstrated that two RAC GEFs TIAM1 and P-REX1 promote RAC-driven cell–cell adhesion and RAC-driven cell migration and invasion respectively, through regulating the RAC interactome. While TIAM1 promotes the association of RAC with proteins that stimulate the formation and maintenance of cell–cell adhesions and consequently inhibits migration, we established that P-REX1 stimulates migration through augmenting the interaction between RAC and the actin-remodelling protein Flightless-1 homolog (FLII), to modulate cell contraction in a RHOA-ROCK independent manner. Thus, we provided direct evidence that RAC GEFs are determinants of selectivity in signalling events downstream of RAC and also identified FLII as a novel mediator of RAC-driven migration and invasion, which now needs evaluation as a factor promoting tumour metastasis.

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Clinical and Experimental Pharmacology has two major research goals: firstly to discover, develop, validate, and implement biomarkers to support personalised medicine for cancer patients as we expand the Manchester Centre for Cancer Biomarker Sciences; and secondly to develop a patient relevant platform in order to discover and test novel therapies for small cell lung cancer that can be translated to the clinic. Highlights this year included: (i) the initiation of the first international collaboration within the 'Cancer Moonshot' programme between CRUK and the USA NCI to develop early detection biomarkers based on single circulating tumour cell analysis; (ii) development of the first DNA based liquid biopsy for SCLC that anticipates chemoresponse; (iii) studies that revealed the functional significance of vasculogenic mimicry for SCLC spread and drug resistance; (iv) the translation of a novel drug combination for SCLC to the clinic based on data from our CTC patient derived explant models; and (vi) leadership of the personalised medicine theme in the newly awarded Manchester Biomedical Research Centre.

Highlights from the Preclinical Pharmacology Team

SCLC CTC and drug development

This team, led by Kris Frese and Kathryn Simpson, expanded our panel of SCLC CDX from 17 to 35 models, including seven matched pairs representing disease at chemonaive baseline and then again at progression after treatment with platinum/etoposide standard of care chemotherapy (SOC). Our panel now recapitulates the spectrum of patient responses to SOC. The panel is being used to explore mechanisms of inherent and acquired chemoresistance using both CRISPR-based forward genetics as well as computational bioinformatics, and putative mediators of chemoresistance are undergoing validation. A key part of our strategy has been the development of CDX ex vivo cultures that can be genetically modified, and re-implanted back into mice (Figure 1). Importantly, CDX cells maintain their transcriptional profiles and chemosensitivity throughout this process, making this a suitable platform for functionally validating candidate mediators of chemoresistance.

In collaboration with Pharma partners, we are testing new therapies in CDX with parallel biomarker development in CDX and in CTCs in order to rapidly translate promising treatments to early phase trials at The Christie NHS Foundation Trust. p53 aberrations render the G1 checkpoint compromised in all SCLC patients thus placing increased reliance on the G2 checkpoint to stall the cell cycle and allow DNA damage repair. In collaboration with AstraZeneca, we completed testing of the combination of their Wee1 G2 checkpoint kinase inhibitor AZD1775 and their DNA damage repair inhibitor olaparib (a PARP inhibitor) in several SCLC CDX models. This combination promoted durable tumour regression in a chemosensitive CDX model with complete tumour regression for up to a year after final drug combination treatment. Efficacy was maintained if the novel combination was administered after standard of care but was less durable. The response to olaparib/AZD1775 was less impressive in baseline chemorefractory CDX, but still outperformed cisplatin/etoposide. By testing in a baseline/progression CDX matched pair, we learned that the new combination was most effective at baseline with efficacy lost at progression, arguing for rapid introduction of the combination after first line chemotherapy. These exciting data contributed to AstraZeneca's decision to take this drug combination to clinical trial in SCLC in 2017 with The Christie NHS Foundation Trust as one of the clinical trial sites.

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Vasculogenic mimicry in SCLC

Our research on vasculogenic mimicry (VM), (Williamson et al. *Nature Communications* 2016; see Research Highlights) describes the ability of tumour cells to adopt endothelial characteristics and form fluid conducting channel-like structures independent of host vasculature. We showed that VM occurs in SCLC and correlates with worse patient overall survival. VM was observed in CDX thus enabling further study. The endothelial marker VE-Cadherin co-localised with VM channels in CDX and via copy number analysis of laser capture micro-dissected CDX tissue and disaggregated single cells, we confirmed that VM cells are of tumour origin. We identified sub-populations of VE-Cadherin expressing CTCs and in collaboration with Prof. Peter Kuhn at The University of Southern California, we isolated single VE-Cadherin expressing CTCs and confirmed their tumour origin using their High Definition Single Cell Assay (HD-SCA) (the 'Cancer Moonshot' technology) (Figure 2). Knock down of VE-Cadherin in a xenograft model reduced the intratumoural delivery of cisplatin consistent with a VM-mediated improved blood supply. However, subsequent drug response was reduced, implicating VM signalling in chemoresistance. The molecular regulation and role of VM in metastasis is under investigation.

Use of CDX models to develop biomarkers

Pharmacodynamic biomarker development in CDX runs in parallel with testing of novel targeted therapies with a view to conversion to CTC based assays for the clinic. We have focused on the DNA-damage response pathway, frequently aberrant in SCLC, using a combination of immunohistochemical and immunofluorescence-based single and dual staining methods. Of considerable interest is our recent finding that CDX cells are capable of metastasis in the mouse with the same tropism seen in the donor patient (Figure 3).

Biomarkers to support clinical trials

In the past 12 months, our biomarkers' portfolio supported 36 clinical trials (26 academic sponsored, 10 pharmaceutical company sponsored and 12 NIHR badged), and 23 experimental medicine studies. Our focus has remained the development and application of liquid biopsies - specifically CTCs and circulating DNA (ctDNA) - that reduce reliance on invasive - and not always feasible or repeatable - tumour biopsies.

Highlights from the Cells and Proteins Biomarkers Team

This team, led by Jonathan Tugwood, developed and validated several new CTC based assays and enumerated CTCs to good clinical practice (GCP) standards using CellSearch (EpCam based CTC capture) in 1,615 blood samples within 13 clinical trials. Cognisant of the need to evaluate EpCAm positive and negative CTCs, we evaluated three marker independent CTC platforms (Parsortix, Clearbridge Spiral Chip and RareCyte AccuCyte/CyteFinder) where CTCs are enriched on the basis of cell size, buoyancy and/or deformability and biobanked resultant CTC enriched samples. A versatile 'liquid staining' method for CTC characterisation is in the final stages of validation and will be deployed to enumerate and characterise our biobank of CTC enriched samples in order to better capture CTC heterogeneity. During 2016, we have initiated several exciting new collaborations with key external partners including Amgen (SCLC CTC based biomarkers), Merck (NSCLC CTC based biomarkers), Carrick Therapeutics (biomarkers to support development of their CDK7 inhibitor in breast cancer), and AstraZeneca (biomarkers to support therapeutics targeting DNA damage repair in lung cancer).

New academic collaborations have been set up with Professor Charles Swanton at University College London, for CTC based biomarker analysis on DARWIN trials as sub-studies of the CRUK Lung Cancer Centre of Excellence flagship TRACERx (TRAcking non-small cell lung Cancer Evolution through therapy [Rx]) programme, and with Alastair Greystoke (CEP alumnus) within the Newcastle ECMC to assess CTCs in thyroid cancer. We installed and qualified the Aushon CiraPlex system for multiplex ELISA analysis, and this platform has been used to analyse proof of concept biomarkers in several trials of anti-angiogenic therapies.

Highlights from the Nucleic Acids Biomarkers Team (NAB)

The Nucleic Acids Biomarkers (NAB) team, led by Ged Brady, continued their development and application of molecular profiling methods suitable for monitoring tumour status from a simple blood sample.

Molecular Analysis of CTCs

A major focus is the genomic analysis of individual CTCs with bioinformatic analysis of CTC copy number alterations (CNA) to establish if patterns of CNA are linked to response to therapy. We established that CNA patterns identified in SCLC CTCs obtained prior to patient treatment correlated with response to standard of care chemotherapy (Carter et al. *Nat Med.* 2016; see Research Highlights). Examination of CNA profiles obtained from patients' CTCs following treatment and relapse indicated that the genetic basis for failure to respond to initial treatment differs from that seen at relapse after an initial response. These observations are the first to establish a link between genetic changes in CTCs and clinical outcomes.

The NAB team also examined the genetic status of single CTCs from patients with primary

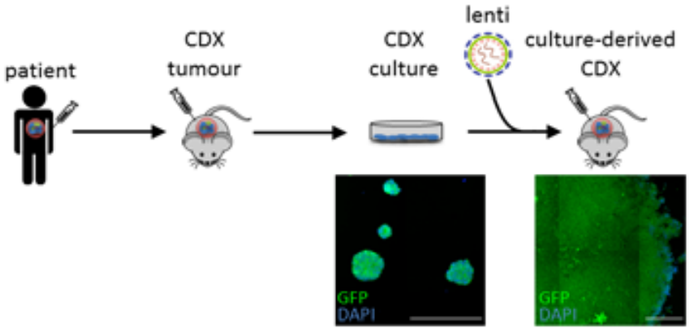

Figure 1: CTCs enriched from patient blood are implanted into mice to generate CDX models. Cells from these CDX can subsequently be cultured ex vivo and genetically modified using lentiviruses before re-implanting into mice. These culture-derived CDX maintain the original characteristics of the donor tumour.

non-small cell lung cancer (NSCLC) and, as part of the TRACERx (TRAcking non-small cell lung Cancer Evolution through therapy [Rx]) study led by Charles Swanton, compared CTC and tumour profiles. Initial results identified both CNA patterns and mutations that are shared between CTCs and the matched tumour as well as changes that differ. The data indicate that the combined single cell CTC and tumour analysis identifies additional layers of heterogeneity that would not be seen by either approach alone and that this will further our understanding of how tumour clonal heterogeneity impacts upon therapeutic outcomes.

The established ability within NAB to genetically profile single CTCs as well as micro-dissected cell subpopulations was exploited to compare split sample matched CDX tumours and CTCs. In a case study of a NSCLC patient, a CDX was generated despite lack of detectable CellSearch CTCs. Molecular analysis of this CDX and matched size enriched CTCs revealed predominantly mesenchymal phenotypes in both with common somatic mutations confirming the tumour origin of these CTCs (Morrow et al. *Ann Oncol*. 2016).

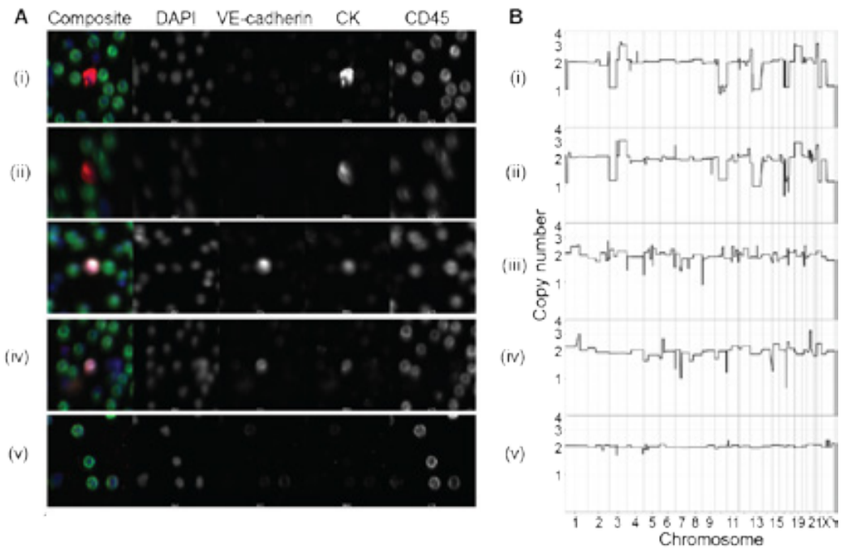

Figure 2: Single Cell CNA analysis of VE-Cadherin negative and VE-Cadherin positive CTCs from a SCLC patient indicate two genetically distinct sub-populations of cells. **A** CTCs were identified using the HD-SCA assay whereby cells were stained for DAPI (blue), VE-Cadherin (white), CKs (red), CD45 (green) and identified DAPI+/VE-Cadherin-/CK+/CD45- CTCs (i) and (ii) and DAPI+/VE-Cadherin+/CK+/CD45- CTCs (iii) and (iv). A reference white blood cell (wbc) is included which is negative for all markers except CD45 (v). **B** CNA analysis of cells from A shows the two populations of CTCs based on VE-Cadherin expression also have different genomic architecture, VE-Cadherin- CTCs are clonal (i) and (ii) whereas VE-Cadherin+ CTCs show non-clonal arrangements (iii) and (iv). Representative images and profiles are shown.

ctDNA analysis - the TARGET initiative and SCLC

In collaboration with Richard Marais and with Andrew Hughes, Matthew Krebs, Emma Dean and Natalie Cook at The Christie Early Clinical Trials Unit, and Andrew Wallace and William Newman at the Manchester Centre for Genomic Medicine, the NAB team is conducting extensive analysis of ctDNA within the TARGET precision medicine initiative that seeks to identify the optimal allocation of a patient to an available Phase I trial. We have processed ctDNA from the first 100 TARGET patients using a workflow based on a sensitive next generation sequencing (NGS) approach that enables parallel sequencing of >600 cancer associated genes selected to cover a wide range of actionable drug targets. A dedicated QA scientist is currently working with us to ensure our ctDNA NGS assays become GCP compliant to allow clinical decision making. The extended feasibility phase of the project demonstrated that blood samples could be processed and NGS data generated with a turnaround time suitable for routine feedback to the TARGET Molecular Tumour Board. A comparison of the cfDNA NGS data to the patient's archival tumour biopsies profiled using a standard Oncocarta gene panel showed impressive overlap in detected mutations. Ongoing development aims to increase ctDNA assay sensitivity and improve classification of ctDNA mutations not covered or identified by tumour analysis.

The NAB team is also examining ctDNA obtained from plasma of patients with SCLC to establish NGS mutation profiles using the same process developed for the TARGET initiative as well establishing ctDNA CNA profiles. This will allow us to compare ctDNA and CTC CNA patterns and establish the utility using either readout alone or whether the combination of both provides an improved biomarker readout (Figure 4).

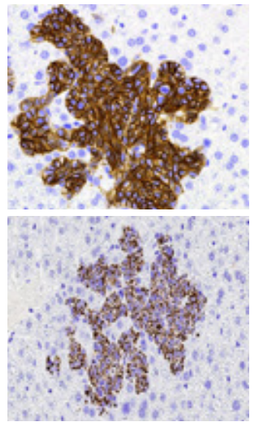

Figure 3: CDX cells are capable of metastasis with the same tropism as observed in the donor patient. IHC for the neuroendocrine biomarker CD56 (left panel) and anti-human mitochondria (right panel) positive cells in liver of a CDX bearing mouse where the model was derived from a patient with liver metastases.

The Quality Assurance Team

In addition to supporting on-going Good Clinical Practice (GCP) related activities within CEP, much of the activity for the QA team in 2016 centred on the re-implementation of internal auditing processes, the development and implementation of a non-conformance process, the roll-out of refresher GCP training & Quality Management System (QMS) training to longer serving members of staff and maintaining up to date knowledge in an increasingly changing regulatory environment. Implementation of harmonised risk-based internal audit and non-conformance processes were delivered to provide evidence of on-going assurance of compliance with GCP and a framework for continual improvement to both local CEP management and The University of Manchester Research Compliance Committee. Four internal audits of laboratory processes and 14 audits of laboratory notebooks were conducted alongside 28 internal data audits to assure the accuracy of reported data and compliance with GCP, sponsor requirements and local procedures, allowing us to address issues arising and prevent reoccurrence. Refresher GCP training and QMS training were also developed and implemented to ensure awareness of regulatory requirements in addition to the underlying principles and rationale for GCP Laboratory Quality Management Systems. The QA team also maintained external links and collaborated with The Christie QA team, ECMC Quality Assurance in Translational Science network and the newly formed Manchester Corridor QA/GxP Network, sharing knowledge, experience and practices. Awareness of current regulatory issues and hot topics within the GCP arena was also maintained by attendance at NIHR refresher GCP training, a North of England Regional Forum in October 2016 and the Annual Research Quality Association (RQA) Conference in November 2016.

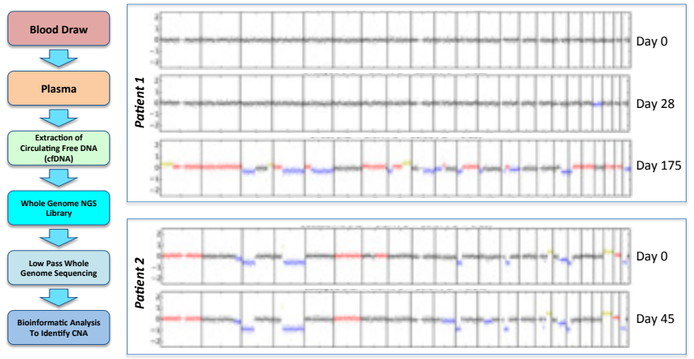

Figure 4. Workflow and Examples of Whole Genome Copy Number Alterations (CNA) seen in Plasma cfDNA from two patients with Small Cell Lung Cancer (SCLC):
The workflow to the left summarises the entire process from blood collection to the generation of CNA patterns. CNA data is presented by chromosome order (1-22); regions with copy number gains coloured red/yellow (yellow indicating a higher degree of copy number gain) and regions with copy number loss coloured blue. In the three sequential time-points analysed for Patient 1 the Day 0 and Day 28 samples show little or no Copy Number Changes, whereas prominent changes are seen in the Day 175 sample indicating low levels of circulating tumour DNA (ctDNA) within the 1st month, followed by an increase in ctDNA reflecting disease progression. In contrast, for Patient 2 both time-points show similar pronounced CNA patterns indicating the presence abundance of ctDNA at both time points.

The new Clinical Informatics team and the iDECIDE Programme

The Clinical Informatics team of the Manchester Centre for Cancer Biomarker Sciences is being established. Its first research programme, iDECIDE is a five year collaboration between four strategic partners/leaders in oncology research: the Manchester Centre for Cancer Biomarker Sciences; The University of Manchester Faculty of Biology Medicine and Health; The Christie NHS Foundation Trust Clinical Trials Unit; and AstraZeneca. The iDECIDE programme commenced in January 2016 supported by AstraZeneca (£11.5M).

The overarching iDECIDE vision is to enable better, early clinical trial decision making that directly benefits the patient. Specifically, iDECIDE will:

- enable all experts, including patients, to work together as a team, striving to evolve the science of cancer medicine
- transform clinical trials through precision science: delivering treatments that work for people, healthcare and society
- empower patients to become active collaborators and co-scientists in our clinical trials to make personalised medicine a reality

The newly established team (currently 11 staff) brings a broad range of skills sets including clinical, business analysis, clinical informatics, patient centricity, software/enterprise systems and biomarker development. The team has now formal collaborations in place with the Manchester Royal Infirmary (Nephrology) and AstraZeneca Research & Development Informatics with further collaborations planned in 2017. The Joint Steering Committee was also initiated and formally agreed to provide robust governance.

The key goals for 2017 are the delivery of:

- new independent iDECIDE Microsoft Azure environment supporting the deployment of our principal systems – Real-time Analytics for Clinical Trials (REACT) and Patient Reported Outcomes About Clinical Tolerability (PROACT)
- the REACT Patient Tracker component (patient summaries)
- the PROACT Protocol in studies at The Christie - this protocol will be sponsored by The Christie and paid for using the iDECIDE grant
- new integrated REACT exposure, biomarker and efficacy visualisations PoC in H1 2017

Publications listed on page 60

DRUG DISCOVERY

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Head of Bioscience
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Dominic James
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Graeme Thomson
Adnana Tudnose
Graeme Walker
Mandy Watson

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Daniel Mould

Undergraduate Students
Shaun Johns[2]
Klaudia Milc[1]

[1]joined in 2016
[2]left in 2016

2016 has been a year of considerable success for the Drug Discovery Unit (DDU) and has seen the achievement of several significant milestones. In April, we disclosed for the first time our novel tool compounds against the DNA repair target PARG at the AACR Annual Meeting in New Orleans. In October, we announced that the lead series of compounds developed for our collaborative programme with GlaxoSmithKline (GSK) had been returned to them for accelerated progression toward pre-clinical candidate selection. Most notable, however, was the declaration of our first pre-clinical candidate compound, arising from our RET inhibitor project.

The aim of this latter project, which is partially funded by the venture capital company 6th Element Capital (6EC), is to develop new treatments for lung cancer patients whose tumours harbour activating gene fusions in RET, a receptor tyrosine kinase. This particular genetic alteration is found in approximately one percent of all lung cancers but has recently been associated with sixteen percent of non-small cell lung cancer cases in non-smokers. The selection of a pre-clinical development candidate is a first for the team as a whole and represents a major achievement for a group of our size. Importantly, this nomination has also triggered the availability of extra funds from 6EC, supporting both the additional work required to take the candidate drug through safety testing and into clinical trials and the development of a back-up series. Work to deliver this back-up series is primarily being undertaken in the labs here at the CRUK Manchester Institute. We are also working closely with our local experts in The Christie NHS Foundation Trust Clinical Trials Unit to develop efficient ways of identifying suitable patients, in order to help facilitate the design of future clinical trials for these compounds.

Our second success stemmed from our ongoing epigenetics collaboration with GSK in Philadelphia (US). Following a very successful drug discovery collaboration running in parallel at both sites, the Drug Discovery Unit has now transferred the lead chemical series (which was designed, synthesised, tested and developed here in Manchester) to GSK. This is the first time we have transferred a late-stage programme to a pharmaceutical partner and in doing so, the team triggered a success payment being made to CRUK. Again, this direct appreciation of the value created in a Manchester-based project represents a significant acknowledgement of our success and capabilities. This collaborative project remains highly active within GSK, who are now driving the lead series towards the clinic in no less than three disease areas, including oncology. The DDU team here in Manchester are now focusing upon the delivery of a chemically distinct back-up inhibitor compound series for this challenging target.

Whilst the past year has seen significant focus on these two major projects, other projects within the team continue to make good progress:

Our PARG programme recently disclosed the first ever cell-active tool compounds against this challenging target, both as published research articles and at the American Association for Cancer Research meeting in New Orleans in April. This disclosure was the subject of two podium presentations and two very busy posters. These tool compounds are now available to our collaborators and the wider community, as we seek to expand upon the understanding of this critical but poorly understood component of DNA repair. We hope that these investigations will suggest new therapeutic applications for PARG inhibitors and will catalyse further efforts in the area. The project is also the subject of late-stage partnering discussions.

Alongside this, our SMARCA2 project continues to progress. This biological target has been the subject of intense interest over the past two years or so from many organisations but it has, to date, proved difficult to find good chemical start points. There will be significant capability build over the coming months as the group undertakes full scale phenotypic cell based screening for the first time.

Further strengthening our portfolio of projects have been several advanced discussions which we anticipate will bring new drug discovery targets and new partnerships with the pharmaceutical industry into the DDU in the coming months.

The Future

The primary aim of the DDU in this quinquennium was to deliver our first candidate drug into pre-clinical development, through our own efforts or in partnership with a collaborator. Having achieved this highly significant milestone, our primary focus is now to ensure the long term sustainability of our project portfolio. We are investing significant effort in the identification and validation of the next generation of drug discovery targets for the Unit to work on, to ensure continued delivery of further candidate drug molecules.

Publications listed on page 62

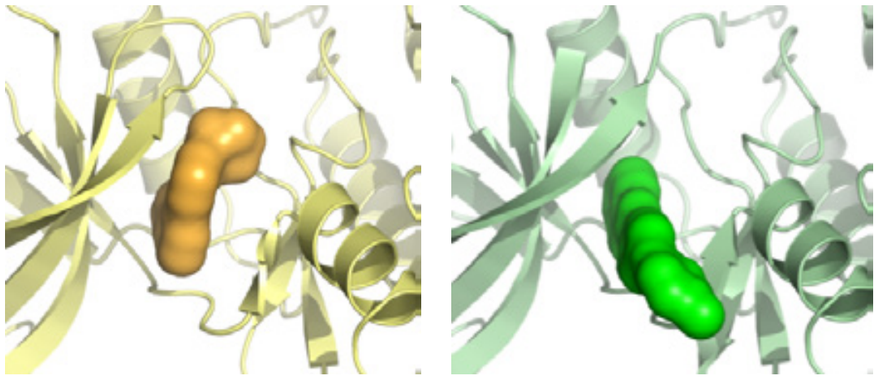

Figure 1:
Novel chemical startpoints, bound to the kinase domain of RET, are being developed toward alternate lead compounds. Ligand-protein structures were obtained through collaboration with the Cancer Research UK Structural Biology Accelerator Award.

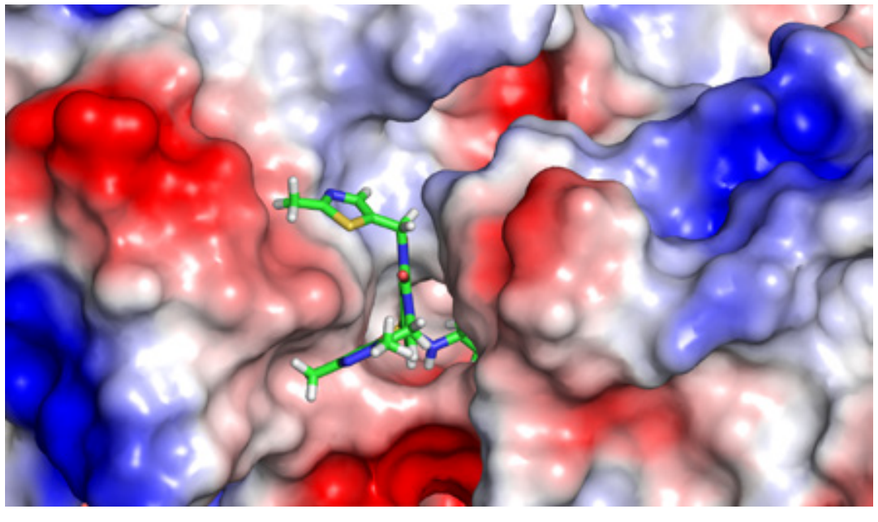

Figure 2:
Docked model of the PARG chemical probe compound PDD00017273 bound to the human PARG protein.

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LEUKAEMIA BIOLOGY

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[1]joined in 2016

A key focus of the laboratory is the translation of our basic science discoveries into the clinic aiming for future patient benefit. In 2012 we reported in *Cancer Cell* that LSD1 (for lysine-specific demethylase 1) contributes to the differentiation block that is the core pathologic feature of acute myeloid leukaemia (AML). This led to our collaboration with Oryzon Genomics, a Spanish pharmaceutical company with a first-in-class inhibitor of LSD1. In turn, this led to a first-in-man Phase 1 trial of Oryzon's advanced lead compound ORY-1001 in the clinical setting of AML, with The Christie NHS Foundation Trust a major recruiting centre. Data from this trial were presented for the first time at the American Society of Hematology in December 2016, and show that, as in our pre-clinical models, treatment of patients with ORY-1001 promotes differentiation of AML blast cells in blood and bone marrow. These exciting translational findings serve as a platform for further clinical development of a novel differentiation therapy for patients with AML.

A major milestone in 2016 for the group has been the completion, in collaboration with Oryzon Genomics, of a first-in-man, first-in-class Phase 1 clinical trial of ORY-1001, a tranylcypromine-derivative inhibitor of lysine-specific demethylase 1 (LSD1, also known as KDM1A). The data arising from the study were presented at the American Society for Hematology meeting in San Diego in December 2016. This trial follows on from a pre-clinical study from our lab which was originally published in *Cancer Cell* in 2012. In that report we demonstrated that LSD1 contributes to the differentiation block in acute myeloid leukaemia (AML) (in particular in the Mixed Lineage Leukaemia (MLL) molecular subtype of the disease) and is targeted by novel derivatives of tranylcypromine to promote differentiation of leukaemic blast cells. A block in myeloid differentiation is one of the core pathologic features of AML.

The Phase 1 trial was funded by a co-operative grant from the European Union EUROSTARS scheme awarded in late 2013 to Oryzon Genomics and The University of Manchester. The trial, which in its first phase was a standard dose escalation design, demonstrated that ORY-1001 was well tolerated by patients without unexpected side effects. Importantly, and as predicted by our previously published pre-clinical studies, in the extension phase of the trial (which was focused in particular on patients with MLL leukaemias), patients showed morphological and pharmacodynamic features of blast cell differentiation following drug treatment. This indicates that, at least for certain patients with AML, inhibition of LSD1 represents an entirely new approach to differentiation therapy. These exciting findings are now being taken forward in collaboration with Roche, which has formed a partnership with Oryzon Genomics. Further clinical trials with ORY-1001 are underway in the clinical setting of small cell lung cancer (SCLC), where novel therapies are desperately needed. In addition to AML, a strong pre-clinical signal for LSD1 inhibition has been observed in SCLC.

Understanding the mechanism by which pharmacological inhibition of LSD1 promotes differentiation in AML (Figure 1) is key to optimising future use of ORY-1001 and related drugs in patients. LSD1 was initially identified as a core component of an RCOR1 (CoREST) histone deacetylase (HDAC) transcription corepressor complex and later found to have histone tail lysine-specific demethylase activity. Indeed LSD1 was the first protein described to have histone demethylase activity. Prior to this some had argued that histone tail methylation was an irreversible phenomenon. LSD1 is a flavin adenine dinucleotide (FAD) dependent homologue of the amine oxidase family with an ability to demethylate monomethyl or dimethyl lysine 4 (K4) of histone H3.

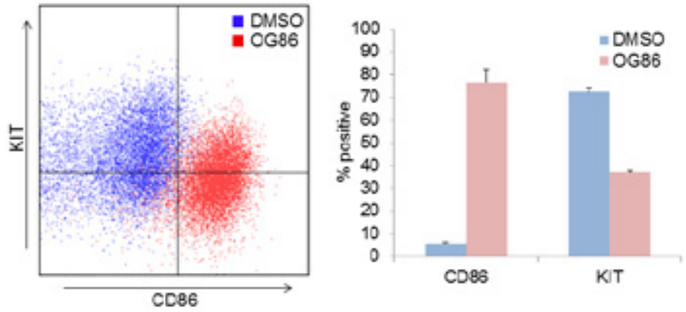
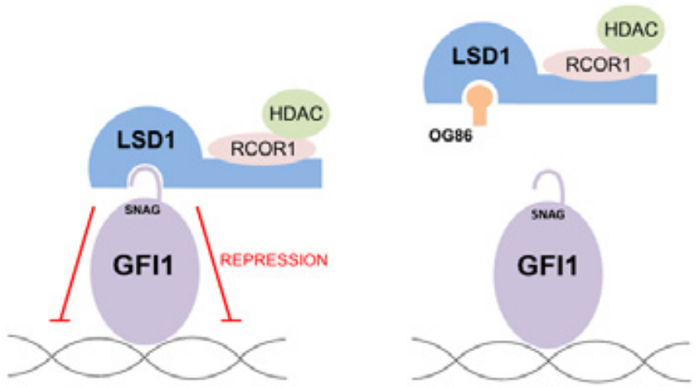


Figure 1:
Effect of treatment of THP1 AML cells with the tranylcypromine-derivative LSD1 inhibitor OG86.
THP1 AML cells were treated with 250nM OG86 or DMSO vehicle for 24 hours in semi-solid culture. Exemplar flow cytometry plot (left panel) and bar chart (right panel) indicate change in expression of the monocyte/macrophage differentiation marker CD86 and the stem cell marker KIT. Error bars refer to SEM; n=4.

In view of the high level of expression of *LSD1* in a range of poor prognosis sub-groups of prostate, lung, brain and breast cancer, as well as in certain haematological malignancies, there has been much interest in the potential of LSD1 inhibitors as novel therapies in cancer. Given the known enzymatic activity of LSD1, the assumption in the field has been that LSD1 contributes to gene repression by removing monomethyl and dimethyl histone marks from lysine 4 of histone H3 and that this is the key activity targeted for potential therapeutic effect. However, LSD1 also interacts with multiple transcription factors raising the possibility that other mechanisms may be significant.

In in vitro experiments we observed that rapid, extensive drug-induced changes in transcription occurred without accumulation of the histone modifications targeted for demethylation by LSD1, and that a demethylase-defective mutant rescued *LSD1* knockdown AML cells as efficiently as wild-type protein. These experimental findings called into question the hypothesis that it was the enzymatic activity of LSD1 that is targeted by inhibitors of LSD1.

Figure 2:
Mechanism of action of LSD1 inhibitors.



It is also well established that LSD1 physically interacts with SNAG domain transcription factors, and that this interaction is absolutely required for the function of these factors as transcription repressors. Following analysis of the transcriptome of drug treated leukaemia cells, we found that LSD1 inhibition mimicked knockdown of the SNAG domain transcription factor GFI1. This led us to discover that LSD1 inhibitors target the protein:protein interaction of LSD1 with GFI1, promoting the physical separation of one from another, and also more generally the physical separation of LSD1 from chromatin (Figure 2). Using an elegant inducible fusion transcription factor system developed by Gary Spencer in the lab we went on to demonstrate that the differentiation resulting from treatment of AML cells with LSD1 inhibitors depended upon physical separation of LSD1 from GFI1. The consequence of disruption of the GFI1:LSD1 interaction following LSD1 inhibition was a localised increase in histone acetylation at the surrounding chromatin, consistent with a model whereby GFI1/LSD1-repressed (or primed) enhancers become activated following drug treatment. Thus, unexpectedly, pharmacological inhibitors of LSD1 are effective through disrupting a protein:protein interaction rather than, as expected, through disrupting catalytic activity.

Our studies highlight GFI1 and its interaction with LSD1 as critical targets for differentiation therapy in human AML with *MLL* translocations, a class of leukaemia typically distinguished by monoblastic/monocytic morphology. Our findings emphasise that compounds developed through drug discovery programs focused on optimising inhibition of the demethylase activity of LSD1 unexpectedly block both structural and catalytic activities. Our data illustrate a paradigm for epigenetic therapy whereby, through disruption of the protein:protein interaction between a transcription repressor and an epigenetic regulator, repression is released and dynamic enhancer acetylation and gene expression ensue. Further, they refine the role of LSD1 in leukaemic haematopoiesis as an epigenetic reader of transcription factor binding sites, in addition to its role elsewhere as an eraser of histone methylation marks.

These functional studies, which have revealed an unexpected mechanism of action of LSD1 inhibitors, are being taken forward in the lab by Gauri Deb and Alba Maiques-Diaz, who are trying to uncover novel genes and cellular pathways which, when inhibited or blocked, collaborate with LSD1 inhibition to promote myeloid differentiation. Such cellular co-targeting strategies may hold out a prospect for future patient benefit in clinical trials.

Publications listed on page 63

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MOLECULAR ONCOLOGY

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Sally Wood[2]

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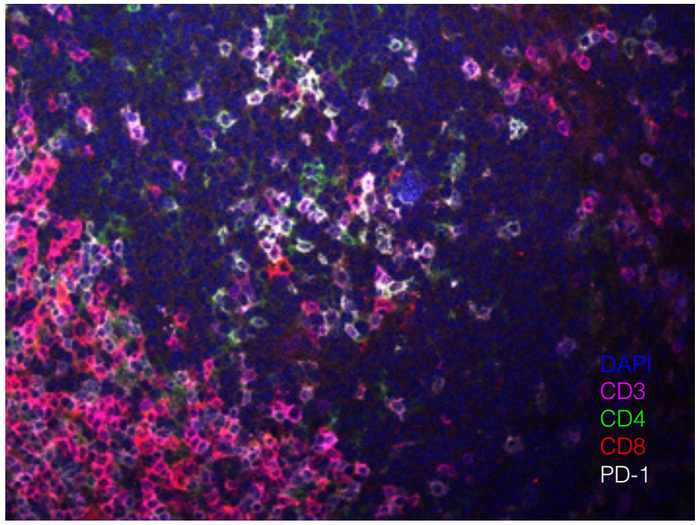
Denys Holovanchuk

My group studies cancer biology and we use the new knowledge we generate to improve cancer patient care. We are developing new anti-cancer drugs, and are developing precision medicine protocols to tailor treatments to individual patients. Our aim is to develop multi-disciplinary teams of scientists and clinicians to develop new diagnostic tools and new treatments for cancer patients.

During the last decade, the treatment of melanoma has advanced rapidly and led to significant improvements in patient survival. The discovery that the *BRAF* gene is mutated in about half of melanomas, has led to the development of drugs that inhibit the mutant protein and slow the growth of the tumours to provide significant extensions of patients' lives. In parallel, drugs have been developed to induce the immune system to attack the tumours, and these also prolong patient survival and in some cases even lead to cures. Despite these remarkable advances, many patients' tumours develop resistance to targeted therapies, and many patients do not respond to immunotherapies, and as a consequence, most patients with metastatic melanoma still die of their disease. A major challenge for the field is therefore to develop approaches that can get the best out of these remarkable new drugs. We need to learn how to combine and schedule the drugs to achieve the best responses for patients as a whole and also how to personalise the treatment to get the best for individual patients. For this we require prognostic and predictive biomarkers so that each patient receives the best first line treatment, and that their treatment is adapted as the tumours evolve to try to escape the first and each subsequent line of treatment.

There is great complexity in the mechanisms that underpin the development of resistance to targeted therapy, and in the last year we described two studies that focused on metabolism. Through a collaboration with Dr Beloueche-Babari at the ICR in London, we reported that BRAF inhibition caused a decrease in glycolytic activity that resulted in reduced extracellular lactate and a build-up of intracellular glucose and glutamate, consistent with a reduction in glucose consumption when BRAF is inhibited in BRAF mutant melanoma cells (Delgado-Goni et al, 2016). This data may allow us to image cells to measure their response to these drugs. In a related study, we demonstrated that acquired resistance to BRAF inhibitors is associated with a switch in metabolism from glucose to glutamine (Baenke et al, 2016). Despite this, there is increased oxidative metabolism and the cells show a greater dependence on glutamine and mitochondrial function for proliferation and survival. Consequently, the resistant cells are more sensitive to mitochondrial poisons and inhibition of glutaminolysis, providing exciting therapeutic opportunities to explore.

The sort of complexity described above as melanoma cells adapt to the presence of the various drugs creates enormous clinical challenges for patient care, and last year we described a platform of approaches that can be used to allow patient treatment adaptations as this occurs (Girotti et al, 2016). We used next generation sequencing to reveal the landscape of genomic changes in patients' tumours and to follow how these change over time. We developed patient-derived xenografts (PDX) by growing individual patient tumours in immuno-compromised mice, and used these to test novel treatment strategies and provide proof-of-principle of effectiveness so that we can begin to develop new hypothesis-driven clinical trials. While PDX technology is a powerful approach to test targeted and chemotherapies, it cannot be used to test immunotherapies because these experiments are conducted in immuno-compromised mice. We have therefore refined the BRAF/UVR driven mouse melanoma model that we reported in 2014 to test immunotherapies, because the tumours develop in fully immuno-competent genetically-engineered mice and, as in humans, are driven by ultraviolet light. To understand how these tumours respond to specific treatments, we have established techniques to monitor how different drugs affect the infiltration of specific cells of the immune system (illustrated in Figure 1), and over the next few years we will use our models to test various combinations of targeted and immuno-therapies, and investigate how best to combine and schedule these treatments for the best outcomes for patients.



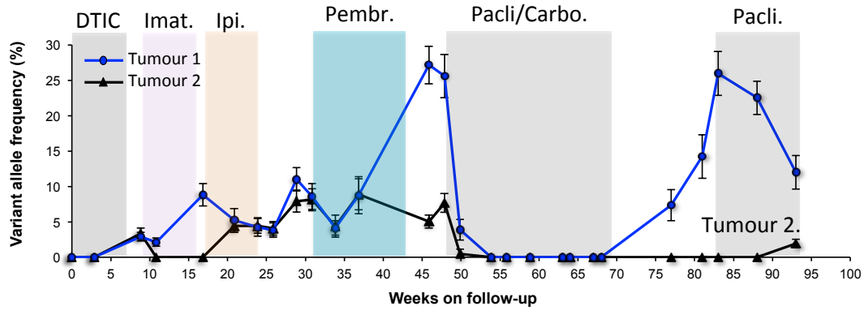
Erasmus student
Monika Wensing[1]

[1]joined in 2016
[2]left in 2016

Figure 1:
Multiplex staining of cells of the immune system in the spleen of an immuno-competent mouse. The image shows the presence of CD3+ (purple), CD4+ (green) and CD8+ (red) T cells, and also shown is staining for PD-1. DAPI is used as a counter-stain for the nucleus.

One of the other major clinical challenges with the new treatments is to know precisely how patients are responding and when they are about to relapse. In this setting, an early warning of impending treatment failure will allow better adjustment of the various treatment modalities, and also allow second and third-line treatments to be applied earlier and with more precision. Last year we reported that circulating tumour DNA (ctDNA) can be used to meet this challenge (Girotti et al, 2016). Several studies have established that ctDNA is released from tumours into patient blood and although it is technically challenging to detect, it can be used to monitor how tumours are responding to treatment. In a further elaboration of this approach, we performed whole exome sequencing on the ctDNA from a patient from whom we were unable to obtain tumour biopsies (Gremel et al, 2016). We discovered a small number of mutations, and analysed those across the patient's treatment cycles with targeted therapies, immunotherapies and chemotherapies. We discovered that the patient had two tumours that responded differently to each of these treatment modalities (Figure 2), and this study demonstrates the power of ctDNA. We are now elaborating those studies by developing clinical trials that will use this approach to determine the optimal time for switching between targeted and immuno-therapies in individual patients.

Figure 2:
A patient with two tumours with distinct responses to therapy is revealed by ctDNA. The image shows longitudinal analysis of ctDNA from a melanoma patient who was treated sequentially with decarbazine (DTIC), imatinib (imat.), ipilimumab (ipi.), pembrolizomab (Pembr.), and paclitaxol (Pacli) with or without carboplatin (Carbo). The data show distinct responses by the tumours to imatinib, pembrolizomab, and paclitaxol, revealing the complexity of responses that can occur with targeted, immuno and chemo-therapies.



Although our studies are largely focused on melanoma, we are using the approaches we have developed for melanoma to investigate the biology of other cancers. In 2016, we reported the use of whole genome sequencing in a chronic myeloid leukaemia (CML) patient whose tumour had developed resistance to the 3rd generation ABL inhibitor ponatinib. Our analysis revealed that by combining the BCL2 inhibitor navitoclax (ABT-263) with ponatinib, we could inhibit growth of the patient's tumour cells. Last year we elaborated this study in B-cell acute lymphoblastic leukemia (B-ALL), an aggressive disease that is lethal in ~50% of adult patients. We observed that inhibition of the protein kinase MEK synergised with inhibition of the BCL-2/BCL-XL anti-apoptotic proteins to induce death of B-ALL cells are driven by various genetic abnormalities, suggesting the potential of this combination in this disease.

We have developed several powerful approaches that can be used to test hypothesis-driven treatments with the aim of translating these into the clinic to improve patient care. Our biomarker studies in particular have given us the confidence to use our approaches to examine other cancers, and in the coming years we will be reporting on how we are using these approaches to examine the genomic landscape of prostate cancer in order to determine how we can use our approaches to impact the care of other types of cancer.

Publications listed on page 63

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PROSTATE ONCOBIOLOGY

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Prostate cancer (PCa) is a very heterogeneous disease both clinically and biologically. Amongst men in the UK, PCa is the most commonly diagnosed cancer, and the second leading cause of cancer-related death. Although androgen deprivation therapy (ADT) is initially effective, most patients inevitably progress to castration-resistant prostate cancer (CRPC). In the last decade considerable progress has been accomplished in understanding the molecular events that lead to PCa, although identification of the cell type(s) involved in neoplastic transformation is still not clear with contradictory reports published in the literature. Understanding the multistep process of prostate neoplastic transformation would undoubtedly facilitate the development of new diagnostic and prognostic markers as well better therapies.

The prostate gland is comprised of basal cells (which separate luminal cells from basement membrane), luminal secretory epithelial cells, and scattered rare neuroendocrine cells. Luminal cells produce most of the components of the seminal plasma, such as the Prostate Specific Antigen (PSA) that have revolutionised the diagnosis and follow-up of PCa. The function of neuroendocrine cells in prostate biology is unknown although it has been linked to aggressive prostate cancer. The role of the basal cells is also poorly understood in homeostasis and disease. Indeed, PCa progression is characterised by a reduction of basal cells to undetectable levels.

Understanding the cell distribution in a given population under homeostasis, androgen-deprived (castration), and regeneration conditions may facilitate further understanding of tumour initiation, and tumour evolution towards the castration-resistant stage. Unlike basal cells, which are largely insensitive to androgen deprivation, the majority of luminal cells undergo apoptosis during castration, although a small proportion remain castration-resistant (CR). Prostate basal cells possess multipotent stem cell activity and can differentiate into luminal cells and serve as a cell-of-origin of PCa, as revealed by transplantation and injury-repair assays. However, lineage-tracing studies using luminal-specific mouse models demonstrate that the prostate luminal lineage in adults is largely self-sustained by luminal cells. In particular, these studies support the existence of CR multipotent and unipotent luminal progenitor (LP) cells that repopulate the luminal lineage upon androgen-induced regeneration. Furthermore, luminal cells have been shown as the preferred cell-of-origin of PCa. Lineage-tracing experiments also reveal that PCa may have a basal origin; however, basal cell-derived PCa exhibits a longer latency, due to initial differentiation of basal cancer cells to luminal cancer cells. The recently developed organoid culture system has allowed detection of multipotent or unipotent LPs in vitro from both human and mouse origins. Despite these efforts, the identity of CR prostate cells in vivo, particularly CR luminal cells, and their contribution to CRPC remain largely unresolved.

To address this gap in knowledge, we utilised a novel Fluidigm multiplex quantitative PCR (qPCR)-based single cell expression analysis platform to interrogate expression profiles of individual prostate cells sorted from hormone-naïve (HN) and castrated mice, and coupled the analysis with organoid culture and in situ lineage-tracing. By unsupervised clustering analysis, we observed that prostate epithelial cells within the luminal lineage appeared more heterogeneous than those in the basal lineage. We found that cells of the luminal lineage from HN mice could be separated into at least five subsets. *Krt8*^{high}-subsets are characterised by differential mRNA expression patterns of differentiation genes, prostate stem/progenitor marker genes; and some subsets express basal

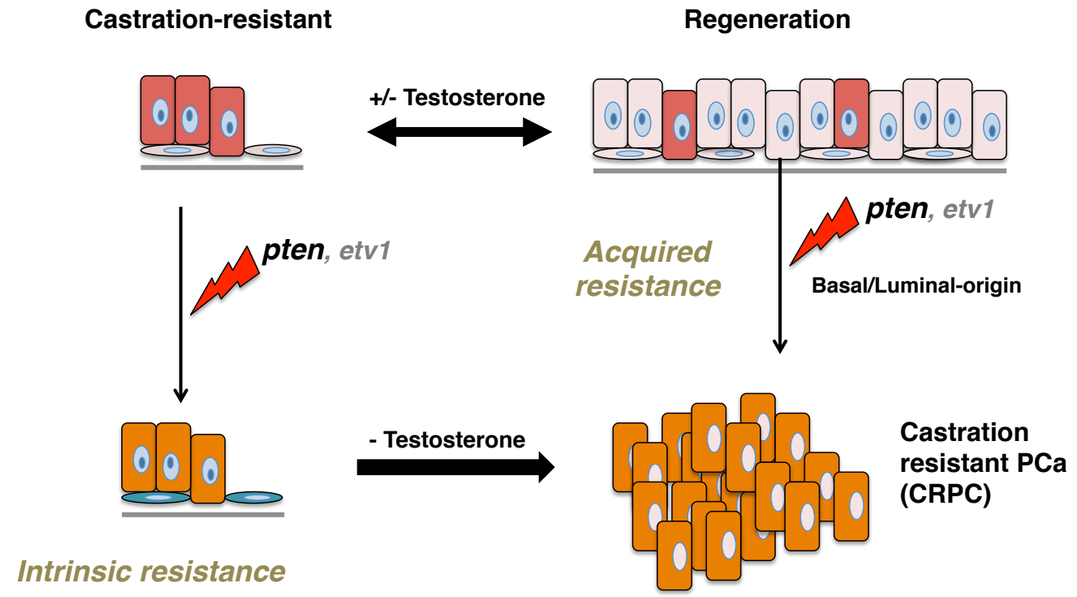

Figure 1:
Schematic prostate duct in the hormone-naïve, castrated and regenerated states. Most luminal cells undergo apoptosis during castration, whereas most basal cells survive; hence, the process of regeneration primarily produces luminal cells. Different cell types of origin in the lineage hierarchy might then generate distinct tumour subtypes following oncogenic transformation.

genes. We have identified expression of a novel potential marker for prostate luminal progenitors that has not been implicated in prostate biology. To test this hypothesis, we have evaluated those prostate epithelial subpopulations from HN mice for organoid culture, recently established in the laboratory for in vitro characterisation of stem/progenitor cells, particularly luminal progenitors. We found that this newly defined prostate subpopulation has a higher organoid-forming capacity, and are multipotent in vitro forming organoids from distinct morphologies and cellular content, including solid mass organoids, acinar organoids composed of a lumen surrounded by multiple layers of cells, and translucent organoids with a large and hollow lumen surrounded by a thin layer of cells. Of note, we found that they formed organoids in an androgen-independent manner.
Conditional genetic marking permits both lineage and temporal control of genetic alterations, thereby allowing initiation of PCa specifically in CR or HN luminal cells. By comparing the phenotypes of PCa arising from different luminal cells of origin, we obtained evidence to support that tumour lesions originating from CR luminal cells are more advanced. Indeed, PCa patients expressing high RNA levels of the inherently-CR markers at diagnosis correlates with aggressive disease and disease recurrence, and more importantly, with development of CRPC. In parallel, these novel cell-surface markers are under evaluation in human prostate cells and patient tissue microarrays, and we will move towards the isolation of human tumour subpopulations to assay directly for tumour-initiation/propagation capacity.

To assess how mutations in the CR stem/progenitor subpopulations might promote tumour initiation/progression to CRPC, we are currently characterising mouse and human models with several genetic alterations such as Pten deficiency and ETS factors overexpression (i.e ETV1). We will test the requirements for selected ETV1/PTEN inflammation-associated targets for tumour growth and immune cell recruitment using mouse tissue and human PCa primary cell organoid co-culture. We anticipate that understanding the role of CR stem/progenitor cells in tumourigenesis and the interactions with their niches may lead to identification of novel targets to tackle propagation of prostate cancer.

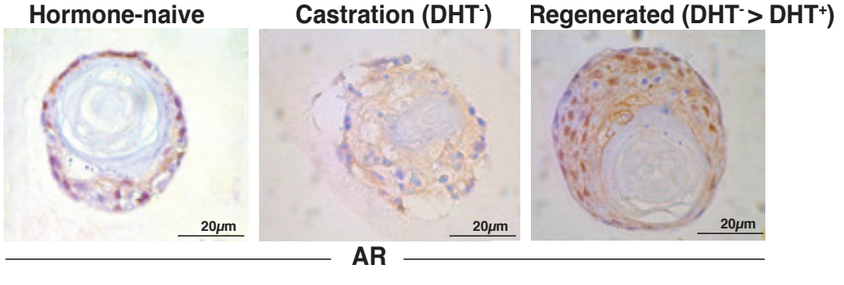

Figure 2:
Regeneration capacity ex vivo of prostate epithelial organoids from lineage-marked luminal cells in response to AR. Mouse prostate organoids in hormone-naïve conditions showing nuclear AR staining, after castration/androgen deprivation (-DHT, without dihidrotestosterone stimulation) showing cytoplasmic AR staining. Ex-vivo regeneration capacity was induced by stimulation with DHT (DHT- >DHT+) and evaluated by nuclear AR staining.

RNA BIOLOGY

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The RNA Biology group is interested in why less than 2% of the human genome encodes amino acids, and what this noncoding sequence does differently in cancer. By studying these parts of the genetic code, we aim to uncover new processes regulating how tumours grow and develop. A major focus of our work is directed at the study of noncoding RNAs. These are transcripts that are expressed, but never translated into proteins. The group is highly interdisciplinary, and uses computer science, mathematics, and computational biology to generate new hypotheses that we explore experimentally at the bench.

Although less than 2% of the human genome encodes amino acids, about 70% of it is transcribed into RNA, even though these transcripts are never translated into proteins. This pervasive transcription is a relatively recent discovery, enabled by rapid advances in microarray and deep sequencing technologies. As a result, the vast majority of these transcripts have yet to be assigned a function. Those that have been studied, however, have been shown to perform a wide variety of roles, acting in all compartments in the cell and functioning through a broad range of interactions with proteins, with DNA, and with other RNA molecules.

A major challenge when studying noncoding RNAs (ncRNAs) experimentally is that the wide variety of functions that they can perform is accompanied by an increasingly diverse set of possible experiments that might be used to characterise them. We are developing novel computational approaches to help us make predictions about how a noncoding RNA might function, and to predict which biological processes it might be involved in. This allows us to narrow down the set of experiments we might then perform to study an interesting ncRNA in more depth. By combining these approaches with the analysis of RNA-sequencing data from patient samples, we are able to prioritise our analyses towards ncRNAs of most relevance in cancer.

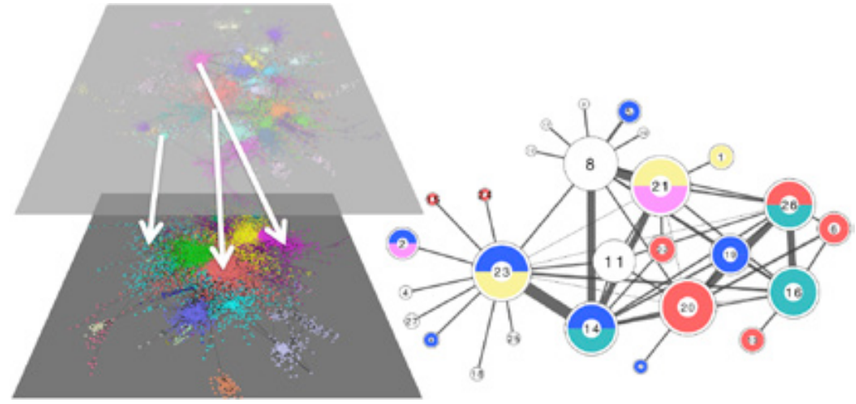
Having identified candidate ncRNAs through our in silico work, we then pursue them experimentally at the bench. Approximately 50% of the group is now bench-based. The bench work within the group has always been highly collaborative, with members of the group embedded in with collaborators in other parts of the Institute. As the group has grown, it became appropriate to bring everyone together, and this year we were able to move into our own lab space.

The majority of our work is focused on lung cancer, through our involvement in the CRUK Lung Cancer Centre of Excellence, and through our computational work, we also have significant collaborations with other groups in the Institute, including an extensive collaboration with the Clinical and Experimental Pharmacology group (page 20). Recently Ron Rodrigues Pereira joined us as a PCUK funded graduate student to study the role of noncoding RNAs in prostate cancer, in collaboration with the Prostate Oncobiology group (page 30).

Computational approaches to long noncoding RNAs

A major class of ncRNAs are long intergenic noncoding RNAs (lincRNAs). These are >200 nucleotides in length and expressed from genes that are spatially distinct from protein coding genes. Many of these transcripts are 5' capped, spliced, 3' poly(A)-tailed and differentially expressed. We are using mathematical techniques from graph theory to build complex networks, and to provide a principled way in which to analyse changes in lincRNA expression. These techniques from Big Data analysis, more commonly applied to social networks, have allowed us to start to predict lincRNA function, helping when we select them for detailed mechanistic study at the bench. We are also developing novel approaches to investigate RNA structure, since a major determiner of noncoding transcripts' behaviour is the way they fold in three dimensions. We are combining these with techniques from machine learning and using these to infer the molecules with which lincRNAs interact.

Figure 1:
Using Complex Networks, to interrogate expression data from patient-derived lung adenocarcinoma samples (left) provides a principled mathematical framework with which to identify modular regulatory systems and pathways (right) that are perturbed in tumours. When long noncoding RNAs are included in these models, these approaches help predict biological function for un-annotated transcripts.



High Performance Computing

Many of the analyses we perform are computationally demanding, and we make substantial use of the Institute's ~2,000 core High Performance Computing (HPC) facility to perform these intense calculations. We are developing novel software that makes use of MapReduce to parallelise calculations across the HPC system, while other computational biologists in the group are exploiting access to the large amounts of RAM (> 2TB) provided on some of our compute nodes to build enormous data structures and explore them efficiently.

Alternative splicing in hypoxia

When a gene is transcribed, the nascent RNA is often processed to systematically remove certain portions of the transcript, called introns, and to splice together the remaining sections, known as exons. This process allows genes to produce more than one transcript, depending on which introns are spliced out of the sequence, and to express different splice-variants at different times, and in different conditions. The majority of human protein-coding genes are alternatively spliced, potentially adding considerable functional diversity to the set of proteins in a cell's repertoire.

We are interested in how splicing patterns might change in tumours and, in particular, how they are altered as cells respond to a lack of oxygen (a condition known as hypoxia). This is important because the majority of solid tumours are hypoxic; a condition that is associated with poor patient outcome and resistance to radiotherapy. Hypoxia has a major influence on the cell, and affects numerous aspects of cancer biology including down regulation of DNA repair pathways, promotion of pro-survival phenotypes and increased proclivity for invasion and metastasis. In a study published this year we showed using cell lines that tumour hypoxia is associated with genome-wide changes in alternative splicing (Memon et al. 2016). Interestingly, many of the genes that changed were associated with the DNA damage response, and switched from expressing a protein coding mRNA to expressing a noncoding splice variant instead. In many cases, this happened through a process of intron retention, in which an intron that is usually removed from the transcript is no longer spliced out. We then showed that this allows cells to shut down protein expression, revealing for the first time that alternative splicing into a noncoding isoform can act as a novel way of regulating gene expression. This has the potential to increase the number of regulatory pathways that impinge on a gene, and to change the kinds of changes we look for when we are studying the effects of cancer on the expression patterns within a tumour.

We then looked at RNA-sequencing data from patient samples from The Cancer Genome Atlas, by building transcript models from scratch and using these to ask whether we saw the same patterns in real tumours as we had seen in the cell lines. These data revealed widespread changes from coding to noncoding transcription, and the same characteristic pattern, in which many DNA damage response genes switch to expressing a noncoding RNA as their major isoform. This was associated with poor patient outcome.

Publications listed on page 64

SIGNALLING NETWORKS IN CANCER

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Cancer genomic sequencing has significantly impacted our understanding of the temporal and spatial genetic alterations that lead to tumorigenesis. This information enables the development of targeted therapies that result in durable and less toxic responses in patients. In regard to kinases, the biomedical community has focused research efforts on approximately 200 kinases among the 538 kinases present in the human kinome, yet siRNA screens and cancer genomic studies indicate that the vast majority of these unexplored kinases (approximately 300) are implicated in cancer and harbour putative driver mutations.

The major focus of our research is to elucidate novel cancer-associated kinases in the unexplored kinome, guided by bioinformatics and functional genomic approaches, with an overarching aim of understanding the molecular mechanisms utilised by these kinases to promote tumorigenesis. Through use of in vivo patient derived xenograft mouse models, we will translate these findings to the clinic and encourage drug development programs focused on these novel targets. The overall goal of our research is to provide a platform for transformational research to identify novel druggable drivers so that the vast majority of cancer patients can begin to benefit from precision medicine based targeted therapies. Collectively this research should identify new genetic drivers, targets for therapeutic intervention, and novel mechanisms of tumorigenesis.

The lab utilises a multitude of strategies to identify critical pathways required to promote tumorigeneis. These include high-throughput bioinformatics and structural modelling, siRNA screening, and precision genome editing to establish various functional genomic approaches to identify novel drivers. Utilising bioinformatics we identify novel kinases enriched for functional mutations to hone in on activated enzymes that can serve as drug targets. We then assess the structural consequences of a subset of mutations in the respective kinases, where crystal structures are available, to determine if the mutations likely increase or decrease catalytic activity. These approaches have been successful in identifying kinases with activating mutations in lung cancer (ABL1 – Testoni et al *EMBO Mol Med.* 2016), as well as novel tumour suppressing kinases in colon and lung cancer that include MLK4 and DAPK3. In a second approach we use genetic dependency screens to identify mutationally activated drivers of lung cancer. Targeted genetic dependency screens are an effective way to uncover low frequency oncogenes that can serve as targets for therapeutic intervention for tumours of any origin. Specifically we identified FGFR4, PAK5, and MLK1 as kinases that harbour novel gain of function (GOF) mutations in lung cancer patients and these mutations result in hyperactivation of the MEK/ERK pathway. The mutation frequency for the genes we identified ranged from 2-10% of lung cancers; given the frequency of lung cancer in the population, these targets could be exploited by pharmaceutical companies for drug discovery development.

Recurrent MLK4 loss-of-function mutations suppress JNK signalling to promote colon tumorigenesis

The lab is also interested in MLK4, which is a member of the mixed-lineage family of kinases that regulate the JNK, p38, and ERK kinase signalling pathways. MLK4 mutations have been identified in various human cancers, including frequently in colorectal cancer, where their function and pathobiological importance have been uncertain. We assessed the functional consequences of MLK4 mutations in colon tumorigenesis. Biochemical data indicated that a majority of MLK4 mutations are loss-of-function (LOF) mutations that can exert dominant-negative effects. In seeking to understand the abrogated activity of these mutants, we elucidated a new MLK4 catalytic domain structure. To determine whether MLK4 is

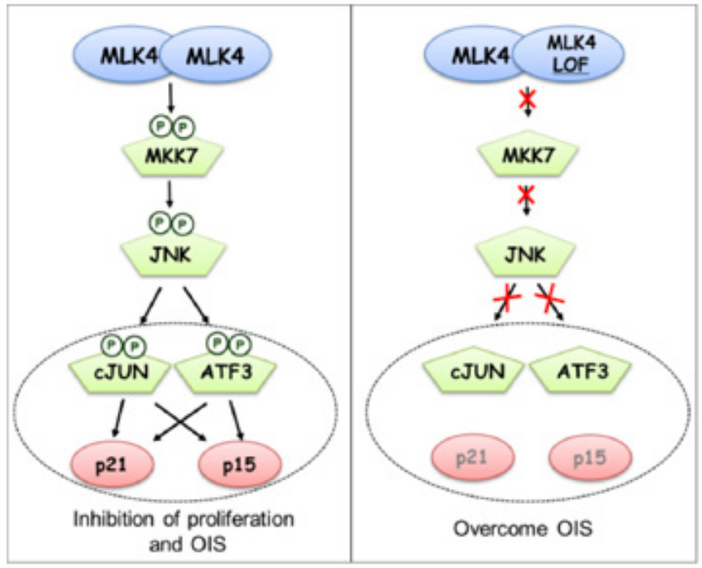

Figure 1:
Diagram showing JNK signalling pathway in colon cancer cells expressing MLK4-WT versus cells expressing MLK4-LOF mutant.
MLK4-WT activates JNK signalling and downstream targets including p21 and p15, which might lead to inhibition of proliferation or OIS (oncogene-induced senescence). LOF mutations in MLK4 block MLK4-WT activation and JNK pathway signalling, which results in overcoming OIS and increased proliferation of colon cancer cells.

required to maintain tumorigenic phenotypes, we reconstituted its signalling axis in colon cancer cells harboring MLK4-inactivating mutations. We found that restoring MLK4 activity reduced cell viability, proliferation, and colony formation in vitro and delayed tumour growth in vivo. Mechanistic investigations established that restoring the function of MLK4 selectively induced the JNK pathway and its downstream targets, cJUN, ATF3, and the cyclin-dependent kinase inhibitors CDKN1A and CDKN2B. Our work indicates that MLK4 is a novel tumour-suppressing kinase harbouring frequent LOF mutations that lead to diminished signalling in the JNK pathway and enhanced proliferation in colon cancer.

Somatically mutated ABL1 is an actionable and essential NSCLC survival gene

The lack of actionable mutations in patients with non-small cell lung cancer (NSCLC) presents a significant hurdle in the design of targeted therapies for this disease. We identified somatically mutated ABL1 as a genetic dependency that is required to maintain NSCLC cell survival. We demonstrated that NSCLC cells with ABL1 mutations are sensitive to ABL inhibitors and we verified that the drug-induced effects on cell viability are specific to pharmacological inhibition of the ABL1 kinase. Furthermore, we confirmed that imatinib suppresses lung tumour growth in vivo, specifically in lung cancer cells harboring a gain-of-function mutation in ABL1. Consistent with structural modelling, we demonstrated that mutations in ABL1 identified in primary NSCLC tumors and a lung cancer cell line increase downstream pathway activation compared to wild-type ABL1. Finally, we observe that the ABL1 cancer mutants display an increased cytosolic localisation, which is associated with the oncogenic properties of the ABL1 kinase. In summary, our results suggest that NSCLC patients with ABL1 mutations could be stratified for treatment with imatinib in combination with other therapies.

We are continuing to focus on novel druggable drivers of lung cancer with a focus on resident genes on the 3q amplicon in lung squamous cell carcinoma and novel genetic dependencies in KRAS positive lung adenocarcinomas.

Publications listed on page 65

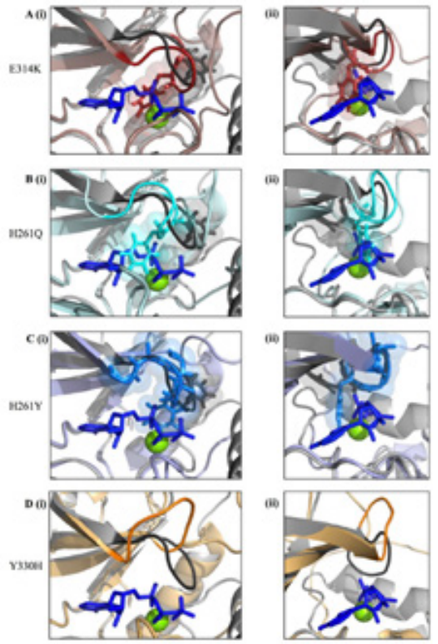

Figure 2: Structural analysis of the effects of movements in the glycine rich motif.
The likely position of ATP (dark blue sticks) and Magnesium (green sphere) within MLK4 as determined by aligning the structures to human CDK2 crystalised with ATP present (pdb accession code: 1HCK). Images show the change in position of mutants (A) E314K (shown in red), (B) H261Q (shown in pale blue), (C) H261Y (shown in blue) and (D) Y330H (shown in orange) compared to the WT structure (shown in black). Facing view (i) is rotated 90° counter clockwise to give side view (ii). Images produced using PyMol.

www.cruk.manchester.ac.uk/Research/CRUK-MI-Groups/Skin-Cancer-and-Ageing/Home

SKIN CANCER AND AGEING

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Skin cancer predominantly affects the ageing population and the majority of skin cancer deaths affect people who are older than 50. Although most deaths are due to melanoma, skin cancers of other cell origins also affect overwhelmingly the aged population. This subgroup of patients has a particularly poor outcome and the reasons underlying this adverse prognosis are poorly understood.

The Skin Cancer and Ageing group studies the mechanisms that drive poor outcome in the elderly population. Skin cancer morbidity and mortality represents a critical current problem in health care. We focus our research on understanding the changes in aged skin that promote skin cancer progression and the biology of aggressive disease that affect the elderly to identify new strategies of adjuvant therapy.

Unique features of skin cancer in the aged population

Cancer is more common in elderly patients and melanoma incidence continues to rise predominantly in this population. Approximately 80% of melanoma deaths in the UK occur in patients who are older than 50 years of age, and mortality is specifically increasing in the elderly. Melanomas in this patient group more frequently present as aggressive primary lesions - thick primary tumours that predominantly comprise the high-risk primary melanoma stages (Stage IIB-IIC). The overall survival for stage IIB-IIC patients of all ages at 5 years is 60% and 45%, respectively, despite being localised to the skin and non-metastatic at diagnosis. There is a gradual decline in 5-year survival with increasing decades of life, with an almost 20% decrease from ages 60 to 69, up to ages greater than 80 years. Additional characteristics of poor prognosis, such as ulceration and elevated mitotic rate, are also more common in the elderly. However, even after taking the main prognostic factors into account there is a survival discrepancy between elderly and young patients, and age is the strongest independent adverse prognostic factor together with tumour thickness. Importantly, older patients are more likely to suffer from multiple melanocytic and non-melanocytic skin cancers, which underscore the need for tailored, specialist care in this high-risk group.

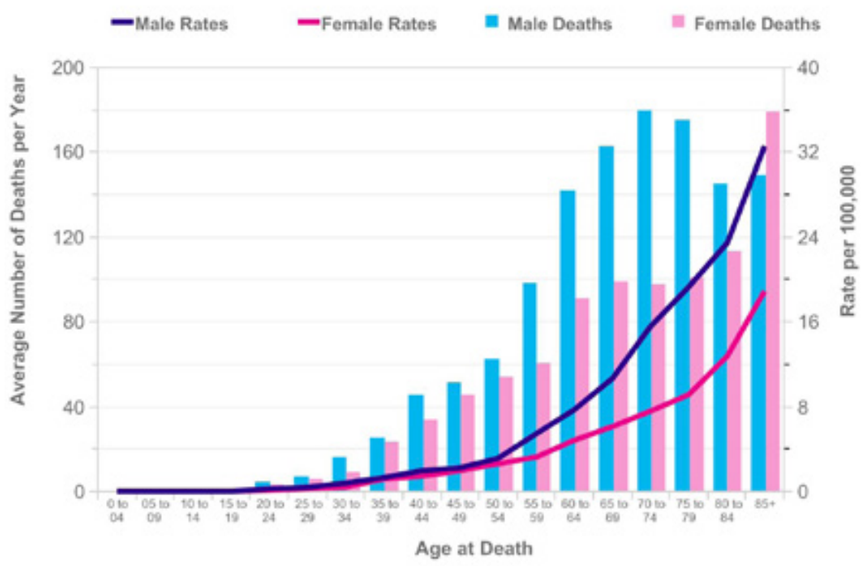

Figure 1:
Age-specific melanoma mortality rates. *Data prepared by CRUK*

Susceptibility to skin cancer in the elderly host

The environmental risk factor for skin cancer is ultraviolet radiation (UVR), which increases risk to neoplasia of keratinocytic lineage in a linear association to lifetime UVR exposure. By contrast, the relationship between UVR and melanoma is multifaceted, with a less clear-cut correlation between total UVR exposure and higher incidence of disease. Importantly, UVR confers higher risk of melanoma progression once the disease is established. As elderly patients are the least likely to survive skin cancer, we are addressing how the interaction between aged skin and the environment modulates skin cancer initiation and progression.

Biology of tumours and adjuvant care of elderly patients

The salient features that define a poor outcome in tumours from aged patients are currently poorly understood. We are defining the epidemiological, clinical, microenvironmental and genetic features that characterise high-risk tumours to better stratify disease, guide prevention and follow-up care. Old patients who have had a primary melanoma removed and are non-metastatic are at high risk of disease progression, but there are currently no approved approaches of adjuvant therapy for this cohort. We are developing approaches of secondary prevention to delay or inhibit skin cancer recurrence to improve the care of elderly patients, who are at highest risk of disease progression.

STEM CELL BIOLOGY

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Genes encoding the AML1/RUNX1 transcription factor and its cofactor CBFβ are frequently rearranged or mutated in human leukaemias such as acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL). Consistent with its implication in leukaemia, RUNX1 has also been shown to be critical for haematopoietic development.

Similarly, the transcriptional co-activator MOZ is involved in recurrent independent myeloid chromosomal translocations in human leukaemia. Our group studies the function of RUNX1 and MOZ in haematopoietic development and maintenance in order to better understand how alterations of these functions might lead to leukaemogenesis.

Clinical needs for blood cell populations

Bone marrow transplantations are well-established cellular therapies for the treatment of a variety of haematopoietic malignancies, leukaemia or genetic haematopoietic disorders. The success of these transplantations relies on a rare population of haematopoietic stem cells (HSCs), which can reconstitute the entire blood and immune system cells. However, a major restriction to the wider application of these curative treatments is the difficulty, or even the possibility, of finding a compatible source of donor tissue. The scarcity in matched donors could potentially be overcome in the future by the provision of unlimited and renewable sources of HSCs from pluripotent stem cells such as embryonic stem cells (ESCs) or patient-derived induced pluripotent stem cells (iPSCs). Furthermore, ESCs- or iPSC-derived HSCs could represent a cellular source to generate various specific immune cells for adoptive cancer immunotherapy. The fulfillment of these promises relies on a better understanding of the molecular and cellular mechanisms underlying the development of the haematopoietic system.

New insights into the birth of haematopoietic stem cells

Recent studies have established that the first haematopoietic stem cells originate in the intra-embryonic aorta-gonad-mesonephros (AGM) region from a specialised endothelium, i.e. a haemogenic endothelium (HE) through an endothelial to haematopoietic transition (EHT). The EHT process is characterised by the loss of endothelial identity concomitant with the acquisition of a round cellular morphology and the gain of haematopoietic cell surface marker expression. In the AGM, the EHT process results in the generation of haematopoietic clusters in the dorsal aorta containing cells with a HSC phenotype. More recently, HE cells have been shown to also give rise to blood generated in the yolk sac, a process that is recapitulated in vitro during ESCs culture system. Although HE has now been clearly established as the cellular source of the first HSCs in vivo, the molecular and cellular mechanisms orchestrating this intriguing trans-differentiation remain largely unknown. One important clue in understanding this process was provided by the observation that the transcription factor RUNX1 is critical for the generation of blood cells by EHT. In the absence of this transcription factor, HE cells do not lose their endothelial identity nor do they acquire a haematopoietic fate.

Taking advantage of this critical role of RUNX1 in the EHT process, we identified the transcriptional repressors GFI1 and GFI1B as direct transcriptional targets of RUNX1 during the EHT. To investigate the relevance of these two proteins in the EHT, we evaluated their ability to rescue this transition in *Runx1*$^{-/-}$ HE cells. We observed that ectopic expression of *Gfi1*, or *Gfi1b*, restored many features of the EHT process. The cells acquired a round non-adherent morphology and silenced the expression of endothelial markers. To confirm the association of GFI1 and GFI1B with the EHT in vivo, we analysed in detail their expression in the mouse AGM region. We observed that *Gfi1* and *Gfi1b* were successfully expressed during the EHT process giving rise to haematopoietic clusters (Figure 1 top). Furthermore, we observed that transplantation of the endothelial cells expressing Gfi1 resulted in long-term repopulation of irradiated recipient mice directly demonstrating their HSC potential. Although these findings suggest the importance of GFI1 and GFI1B in the EHT, none of the respective knockouts recapitulated the early block in haematopoietic development and early

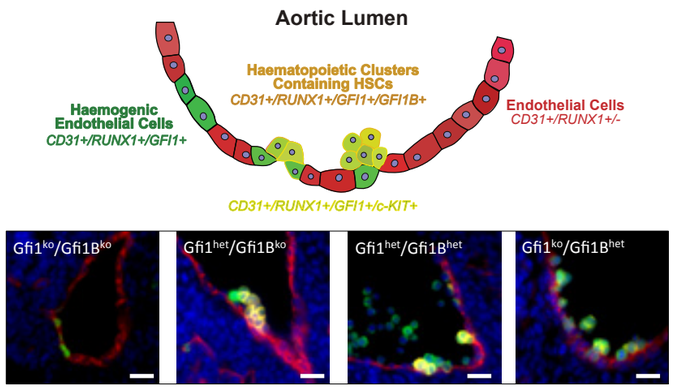
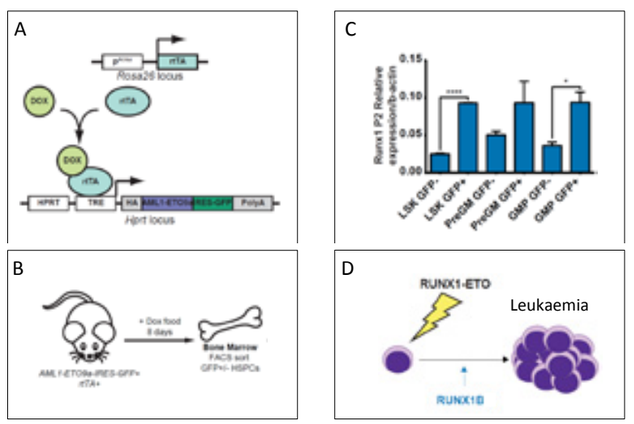


Figure 1: GFI1 and GFI1B are critical for the EHT giving rise to HSCS. Top. Schematic representation of the expression of RUNX1, GFI1, GFI1B and c-KIT during the formation of haematopoietic clusters in the dorsal aorta. Bottom. Immunohistochemistry on *Gfi1*ko/*Gfi1b*ko,*Gfi1*het/*Gfi1b*ko, *Gfi1*het/*Gfi1b*het and *Gfi1*ko/*Gfi1b*het E10.5 AGMs for CD31 (red), GFI1 and GFI1B (green) and c-KIT (yellow).

embryonic lethality observed in the absence of RUNX1. We hypothesised that the lack of an early phenotype might be due to a functional compensation for the loss of one gene by the other. Indeed, the two GFI1 and GFI1B proteins exhibit very high levels of homology in their functional domains and were previously shown to be functionally interchangeable in the adult haematopoietic system. We therefore examined the consequences of deleting both proteins using *Gfi1* and *Gfi1b* GFP knock-in mice. In the double knockout embryos, strong defects in the EHT were observed; yolk sac derived blood cells were absent from the circulation and haematopoietic clusters were not observed in the AGM (Figure 1 bottom). Instead, we found GFP^{+} cells accumulating in the yolk sac vasculature or embedded within the endothelial lining of the dorsal aorta, demonstrating the critical requirement for GFI1 and GFI1B in the EHT.

GFI1 and GFI1B have been shown to repress transcription in a MEL (murine erythroleukemia) cell line, by recruiting the chromatin regulatory CoREST complex containing the histone demethylase LSD1 (KDM1A). To investigate if this complex was involved in EHT, we examined the consequences of LSD1 inactivation on this transition. LSD1 genetic deletion or inhibition impaired the emergence of round non-adherent haematopoietic cells. Finally to identify direct transcriptional targets of GFI1 and GFI1B, we compared changes in global gene expression profiles upon LSD1 inhibition with lists of genes

Figure 2: RUNX1B might promote pre-leukaemia. A. AML mouse model expressing a doxycycline-inducible AML1-ETO9a linked to a GFP. B. Induction of AML1-ETO9a for 8 days and FACs sort of cell population. C. Upregulation of RUNX1 P2 expression in AML1-ETO9a (GFP+) expressing cells. D. RUNX1B might be involved in the persistence and amplification of pre-leukaemic cells.



bound by GFI1 and/or GFI1B. Notably, the resulting list of candidates contained genes involved in cardiovascular development, blood vessel maintenance and remodelling. Altogether our findings suggest that during the emergence of HSCs, GFI1 and GFI1B epigenetically silence the endothelial programme to allow the acquisition of a round non-adherent cellular morphology and the release of newly formed blood cells into the circulation.

RUNX1 isoforms in normal and malignant haematopoiesis

As with other mammalian *Runx* genes, *Runx1* has two promoters, *P1* (distal) and *P2* (proximal), which generate the distinct protein isoforms RUNX1C and RUNX1B respectively. The activities and specific relevance of these two promoters in adult haematopoiesis remain to be fully elucidated. Utilising a dual reporter model we demonstrated that the distal *P1* promoter is broadly active in adult haematopoietic stem and progenitor cell (HSPC) populations. By contrast the activity of the proximal *P2* promoter, and RUNX1B expression, is more restricted and its upregulation in both the immature Lineage-Sca1^{high} cKit^{high} (LSK) and bipotential Pre-Megakaryocytic/Erythroid Progenitor (PreMegE) populations coincides with a loss of erythroid specification. Accordingly the PreMegE population can be prospectively separated into "pro-erythroid" and "pro-megakaryocyte" populations based on *P2* activity. Prospective isolation of these two populations provides the opportunity to further investigate and define the molecular mechanisms involved in megakaryocytic/erythroid (Mk/Ery) cell fate decisions.

Recent studies have revealed that in addition to a tumour suppressor role, WT AML1/RUNX1 is also required for the promotion of leukaemogenesis in certain leukaemia subtypes. Notably, AML1-ETO-driven AML appears to be dependent on maintaining some WT RUNX1 activity to protect the leukaemic cells from apoptosis. However, it is unclear whether AML1-ETO oncogene expression promotes the expression of one Runx1 promoter over another. To address this question, we utilised a novel AML mouse model expressing a doxycycline-inducible AML1-ETO9a linked to GFP (Figure 2) and studied the impact of AML1-ETO9a on Runx1 isoform expression in vivo by isolating AML1-ETO-expressing (GFP^{+}) and non-expressing (GFP^{-}) bone marrow. We analysed LSK, PreGM and GMP cell populations as they contain the leukaemia propagating cell fraction in numerous AML patient samples. In all populations, expression of AML1-ETO was correlated with a specific upregulation of Runx1 P2 expression. Our results suggest that RUNX1B activity generated by RUNX1 P2 promoter might specifically participate and support a pre-leukaemic phenotype in emerging AML cells.

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STEM CELL HAEMATOPOIESIS

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During vertebrate ontogeny, blood, endothelium, smooth muscle and cardiac tissue develop from the mesoderm germ layer to form the cardiovascular system. Whilst we are starting to understand better the molecular mechanisms underlying this developmental process, the overall network of transcription factors and signalling pathways governing cell fate decisions during cardiovascular development remains largely unknown. The research in our laboratory aims at further understanding the regulatory network that orchestrates this developmental process. Many transcriptional regulators implicated in haematopoietic specification during embryogenesis are also linked to leukaemogenesis events, often as a result of aberrant expression. Through a better understanding of the function of these transcriptional regulators at the onset of haematopoietic specification, we hope to gain insights into their potential role in the initiation and maintenance of haematological malignancies. Our work on the transcription factor SOX7 revealed that its aberrant expression in B cell acute lymphoblastic leukaemia is linked to enhanced proliferation and dissemination of the leukaemic cells.

The SOX family of transcription factors is highly conserved throughout evolution and in mouse comprises 20 members divided into subgroups based on sequence similarities. During embryogenesis, SOX factors are essential for the regulation of many developmental processes. In addition to their critical roles during embryogenesis, SOX factors are also often implicated in the maintenance and identity of stem cell populations. SOX7 and its two close homologues, SOX17 and SOX18, belong to the SOXF subgroup and play important roles in cardiovascular development. The SOXF factors have also been implicated in the development of the haematopoietic system, the embryonic origin of which is closely linked to endothelium development. We and others have demonstrated that the ectopic expression of SOXF factors in embryonic haematopoietic cells leads to dramatic alterations in the balance between proliferation and differentiation. Our group showed that *Sox7* expression was up-regulated in mesoderm precursors at the onset of blood specification and down-regulated as differentiation progresses to committed blood lineages. The enforced expression of SOX7 in embryo-derived cells, or in in vitro differentiated embryonic stem cells, was shown to promote the self-renewal of early blood progenitors harbouring endothelial-like features and to block further differentiation to committed lineages.

SOX7 expression promotes the expansion of adult blood progenitors and blocks B cell development

Given the potential of SOXF factors in maintaining the self-renewal properties of embryonic blood progenitors, we hypothesised that the ectopic expression of SOX7 may also confer a proliferative or survival advantage to adult haematopoietic cells. Using a transgenic inducible mouse model, we explored the consequences of *SOX7* ectopic expression on adult haematopoiesis. First, we showed that the enforced expression of *Sox7* ex vivo in adult bone marrow cells dramatically increases proliferation and impairs the differentiation of B lymphocytes. The enforced expression of *Sox7* in bone marrow cells led to the ex vivo maintenance and expansion of a progenitor population able to give rise to myeloid, erythroid and lymphoid lineages and to provide short-term in vivo engraftment. Genome-wide analysis revealed that the enforced expression of *Sox7* in adult bone marrow cells impairs B cell maturation while promoting a stem/progenitor signature. Given the impact of *Sox7* expression in ex vivo cultures, we investigated whether Sox7-enforced expression in vivo affects the homeostasis of the haematopoietic system. Our findings demonstrated that in vivo the ectopic expression of *SOX7* led to B lymphopoiesis impairment and expansion of haematopoietic progenitors in the bone marrow associated with fibrosis, splenomegaly and extra-medullary hematopoiesis.

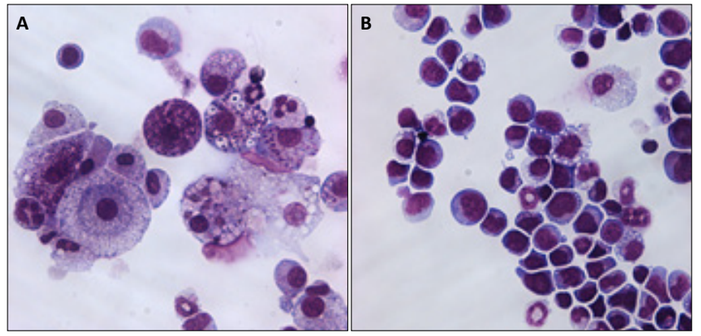

Figure 1:
SOX7-expressing bone marrow cells retain an immature morphology
(A) Cells isolated from wild type bone marrow present the expected heterogeneous morphologies of myeloid and erythroid cells. (B) Most bone marrow cells expressing SOX7 present a homogenous immature and blast-like morphology.

When ectopically expressed in blood progenitors, all SOXF factors were shown to alter the balance between proliferation and differentiation. Given their known degree of redundancy, it is very likely that all three SOXF factors activate a similar transcriptional program that promotes proliferation at the expense of lineage differentiation. Based on the known functions of the SOXF factors, two possible explanations can be suggested for these observations: i) the transcriptional program activated by all SOXF in blood cells is similar to the one activated by SOX17 in foetal haematopoietic stem cells which promotes the active proliferation of these cells; SOX7 and SOX18 are able to activate this programme when ectopically expressed in a blood-specific cell context; ii) All three SOXF factors are expressed in haemogenic endothelium and might in this cellular context induce a transcriptional programme promoting proliferation, which can be induced in a blood-specific cell context upon ectopic expression. The transcriptional targets specifically activated by the SOXF factors to regulate self-renewal are still currently unknown; this will be an important avenue of investigation for future studies.

SOX7 promotes the maintenance and proliferation of B cell precursor acute lymphoblastic cells

Acute lymphoblastic leukaemia (ALL) is the most common cancer in children and comprises neoplastic precursor cells committed to the B cell (BCP-ALL) or the T cell (T-ALL) lineages. BCP-ALL represents the majority of ALL, accounting for up to 85% of childhood ALL and 75% of adult ALL. Genes involved in B cell development such as *EBF1, IKZF1, PAX5* or *PBX1* are frequently found mutated in BCP-ALL. Additional cytogenetic abnormalities are frequently detected and often define specific subtypes of the disease with unique prognostic features. While childhood BCP-ALL is curable in most cases with a survival rate approaching 80%, the rate of cure for adult patients presenting with BCP-ALL is only 40%. Despite progress in the treatment of BCP-ALL, intensive chemotherapy regimens cause life-threatening complications and the outcome of those who relapse is poor. There is a great need for the development of less toxic compounds and novel therapies for the treatment of relapsed and specific subgroups of BCP-ALL patients with poor prognosis.

The deregulation of *SOX* gene expression has been widely documented in cancer, and a few studies have identified a direct involvement of SOX factors in tumorigenesis. The expression of *SOX7* is frequently down-regulated in solid tumours such as prostate, colon and endometrial cancers, but the functional relevance of these findings remains unknown. A recent study has also revealed a tumour suppressor role for SOX7 in acute myeloid leukaemia. In our study, we established that *SOX7* is specifically and frequently expressed in human BCP-ALL, without an obvious association with specific chromosomal abnormalities or cytogenetic subtypes. Furthermore, our data demonstrated that the down-regulation of *SOX7* in BCP-ALL human cell lines induced a significant decrease in proliferation, even though *SOX7* transcript levels were only decreased by 50%. Finally, we showed that in vivo the down-regulation of *SOX7* delays the onset of BCP-ALL and decreases the leukaemic burden, establishing the critical contribution of SOX7 expression in maintaining the high proliferative status of the leukaemic cells.

Together, our study suggests that *SOX7* expression in BCP-ALL is an important factor contributing to leukaemia. It will be important in future work to define the downstream programme regulated by SOX7 in BCP-ALL and to identify the key pathways regulating the proliferative potential controlled by SOX7. They might represent potential novel druggable targets for the treatment of poor prognosis and relapsed BCP-ALL. Alternatively, the identification of small molecules that can interfere with SOX7 transcriptional activity either through SOX7-DNA interaction or through protein complex formation will represent interesting avenues of investigation with potential therapeutic benefits.

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SYSTEMS ONCOLOGY

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The main aim of the Systems Oncology group is to understand how signal transduction mechanisms are deregulated in tumour cells, with a specific focus on delineating the role of the microenvironment. It is widely appreciated that solid tumours contain a multitude of infiltrating 'normal' host cells in addition to the malignant cancer cells. Such host cells form the cellular basis of the tumour stroma and consist of immune cells, endothelial cells as well as fibroblasts.

Interactions between tumour cells and stromal cells have been shown to modulate a number of key processes such as tumour progression, metastasis, metabolism and response to therapy. However, while the functional importance of the tumour stroma is reasonably clear, the mechanisms whereby tumour cells co-opt stromal cells and the means by which coerced stromal cells support tumour cells to facilitate malignant progression are not well understood. Delineating these mechanisms is essential to our understanding of the basic biology of tumour progression, but also for the development of therapeutic strategies co-targeting the tumour stroma.

Understanding how tumour cells escape the vascular system during metastasis

Tumour cell metastasis accounts for 90% of cancer related deaths. As tumour cells escape the primary tumour, enter the vascular system and exit to proliferate at the metastatic site, they interact with a number of host cells. In particular, very little is known of the mechanisms whereby tumour cells in the vascular system adhere to and cross the endothelial monolayer at the metastatic site. To address this, we developed a co-culture system where tumour cells are plated on top of an endothelial cell monolayer. As cells interact, receptors between the two cell-types engage to regulate signalling pathways in a cell-specific manner. Building on our previous experience with cell-specific labelling (Jorgensen et al, *Science* 2009), we applied methods to differentially label the proteome of tumour and endothelial cells with isotopomeric versions of amino acids (SILAC labelling, Ong et al, *Mol Cell Proteomics* 2002). We then conducted an analysis of differentially regulated signalling pathways in tumour cells that were either co-cultured with endothelial cells or left in solution (Locard-Paulet et al, *Sci Signal* 2016).

This approach enabled the identification of numerous receptors and signalling pathways that were specifically regulated in endothelial and tumour cells as they interact (Figure 1).

We observed that the receptor tyrosine kinase EPHA2 displayed a decrease in the level of phosphorylation of the activation loop (suggesting a decrease in the activity) specifically in tumour cells engaging with endothelial cells. Interestingly, ablating EPHA2 in tumour cells, or blocking the interaction between EPHA2 and its cognate ligand Ephrin-A1, increased the ability of tumour cells to adhere to and migrate across an endothelial monolayer. This was confirmed in vivo, where cells deficient in EPHA2 displayed increased retention in the lung parenchyma, a typical site of metastasis. Together, these and other results led us to propose a model for EPHA2-dependent regulation of transendothelial cell migration: contact between tumour and endothelial cells leads to EPHA2 activation and a repulsion of tumour cells. However, metastatic tumour cells have developed mechanisms where EPHA2 is rapidly inactivated and switches repulsion into adhesion thus facilitating extravasation.

Tumour-stroma signalling in Pancreatic Ductal Adenocarcinoma

Pancreatic Ductal Adenocarcinoma (PDAC) has a dismal prognosis with a median survival below six months and an average five-year survival below 5%. This is due to the aggressive nature of the cancer, a lack of effective therapy and late diagnosis. The most frequently occurring genetic mutations have been identified with activating mutations in the oncogene KRAS and inactivation of the tumour suppressor CDKN2A in more than 90% of all cases and loss of SMAD4 and TP53 function occurring in 55% and 85% of all cases respectively. A hallmark of PDAC is a

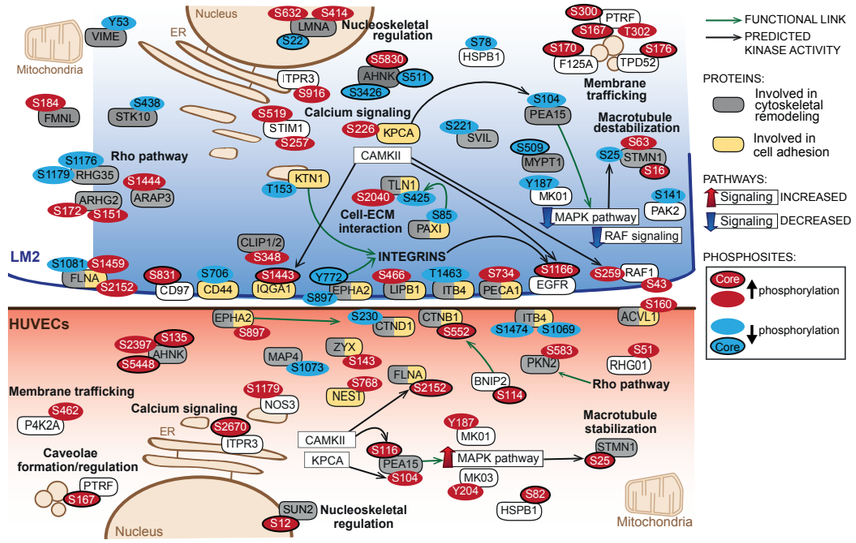

Figure 1:
Contact-initiated signalling between tumour cells (blue, top) and endothelial cells (red, bottom) regulate a number of signalling pathways controlling processes such as cell adhesion, cell migration and cell shape. The receptor tyrosine kinase (EPHA2) is specifically regulated in the tumour cells upon endothelial cell interaction, where the activation loop phosphorylation is decreased (Locard-Paulet et al, *Sci Signal* 2016).

perverse stromal infiltrate that makes up to 80% of the tumour volume. This desmoplastic reaction consists of a pathological remodelled extracellular matrix and influx of fibroblasts and immune cells. The microenvironment has been shown to contribute to therapeutic resistance, immune tolerance and tumour progression. Importantly, very little is still known about the mechanisms whereby the tumour cells co-opt hosts cells to establish this hostile environment. Delineating these mechanisms is therefore important and may lead to the identification of novel therapeutic targets in both the tumour and stromal cells.

How do co-opted stromal fibroblasts regulate tumour cells?

To address how pancreatic cancer cells (PDAC cells) co-opt resident fibroblasts, the pancreatic stellate cells (PSC), we used a co-culture system where PDAC cells with an inducible mutant KRAS (G12D) were directly co-cultured with naïve PSCs (Tape et al, *Cell* 2016). Using our recently implemented and optimised system for long-term cell-specific labelling (Tape et al, *Mol Cell Proteomics* 2014) we then discerned cell-specific changes in tumour cell signalling as a consequence of PSC co-option. Initially we observed that activated KRAS in the tumour cells leads to increased activation of the MEK-MAPK pathway, but not of the PI3K-AKT pathway. In contrast, inclusion of PSCs permitted tumour cells to activate additional pathways, where the number of regulated phosphorylation sites doubled. This included the activation of the PI3K-AKT pathway. Interestingly, tumour cells expressing KRAS secrete abundant levels of the morphogen sonic hedgehog (SHH), but are themselves insensitive to the ligand. However, PSCs are highly responsive to SHH and elicit a response that includes increased production and secretion of ECM proteins and the growth factors IGF-1 and GAS6. These ligands then engage cognate receptors on the tumour cells and activate the PI3K-AKT pathway. Critically, PDAC cells grown in the presence of PSCs also deregulate their metabolic pathways, where the proteomic composition of the mitochondria and ensuing function shifts dramatically. In addition, tumour cells gain the ability to grow under anchorage independent conditions and display decreased levels of apoptosis. Blocking the signals exchanged between tumour cells and fibroblasts (SHH, GAS6 and IGF-1) normalises the tumour cell function, suggesting that these pathways are context-dependent targets. More importantly, what these data highlight is that the tumour cell function is drastically regulated by stromal elements (such as the fibroblasts) and that these elements should be included in our model systems to gain a better understanding of putative therapeutic targets.

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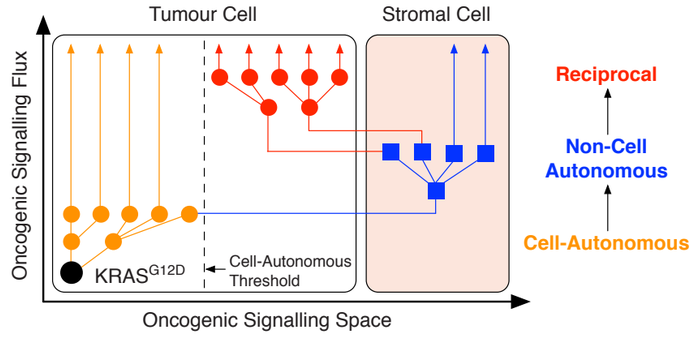
Figure 2:
Establishing a reciprocal signalling axis between pancreatic cancer and stromal cells (fibroblasts). Expression of oncogenic KRAS in tumour cells leads to a cell autonomous activation of MAPK signalling and secretion of SHH, which engage fibroblasts in a non-cell autonomous manner. Coerced fibroblasts in turn elicit a reciprocal signal (GAS6 and IGF-1) to engage additional signalling cascades in the PDAC tumour cells (Tape et al, *Cell* 2016).



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TRANSCRIPTIONAL NETWORKS IN LUNG CANCER

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[2]joined in 2016

Lung cancer causes the most cancer-related deaths in the world and the main obstacle to a cure is chemoresistance. The main interest of our group is to identify the causes behind lung cancer development and resistance to chemotherapy. Over the last decade, a growing number of non-coding transcripts (ncRNAs) have been found to have a pivotal role in gene regulation and cell biology. The most well- known ncRNAs are microRNAs (miRNAs), single stranded RNAs of 19–25 nucleotides in length, which negatively regulate gene expression by translational inhibition or degradation of the mRNA targets. miRNAs are differentially expressed in almost all types of human cancers versus the normal tissue counterpart and are key players in cancer onset and progression, functioning as tumour promoters (TP) or tumour suppressors (TS). The application of miRNAs to cancer therapeutics and diagnostics is emerging as an important field of gene therapy. Thus far both miRNA replacement and miRNA inhibition strategies have been successfully used to restore normal gene networks in vitro and in vivo, evidencing the huge potential of microRNAs in the fight against cancer.

PDGFRs axis

Lung cancer ranks first in cancer morbidity and mortality rates globally. The most frequently diagnosed histological sub-type, NSCLC, accounts for 80–85 % of cases, with a disappointing five-year survival rate of 15.9%. During the last decade, several effective targeted therapies have been developed; Erlotinib, Gefitinib and most recently Osimertinib and crizotinib/ceritinib for patients with EGFR activating mutations and ALK/EML4 translocations, respectively. However, *EGFR*-mutant and *ALK*-rearranged cancers constitute less than one-fifth of all NSCLC cases and patients that initially respond well to therapy inevitably relapse a few months later. Thus, identification of other potential molecular targets and novel therapeutic approaches is of primary importance. It is now accepted that NSCLC is not a singular entity but a heterogeneous disease and therefore requires targeted therapies. In this regard, a pathway that holds significant promise involves platelet-derived growth factors (PDGFs) which bind to PDGF receptors resulting in the activation of a downstream signalling cascade that promotes cellular proliferation, migration and survival. There are five different isoforms of PDGF that activate cellular response through receptors alpha (PDGFR-α) and beta (PDGFR-β). Both PDGF ligands and the receptors have been detected in lung cancer cells but not in normal cells and are markers of worse prognosis. Targeting the PDGFR-α/β axis has proven useful for the therapy of those cancers with alterations in such genes including point mutations and gene fusions that result from chromosomal rearrangements. In both cases, these alterations activate the kinase domain in the absence of the ligand, giving rise to a permanent signal for cell proliferation. Most gastrointestinal tumours (GISTs) have activating mutations in the KIT receptor tyrosine kinase or PDGFR-α and respond well to imatinib. However, in NSCLC, PDGFRs lack the genetic change that activates the kinases, and as a consequence they do not respond to treatment with imatinib. Therefore, further understanding of the molecular mechanisms involved in the PDGFR axis may provide the crucial information necessary to improve its targeting strategies and to develop new drug families with synergistic effects. Our group is focusing on PDGFR-modulated microRNAs to better define this pathway and identify new targets for drug development and targeted therapy.

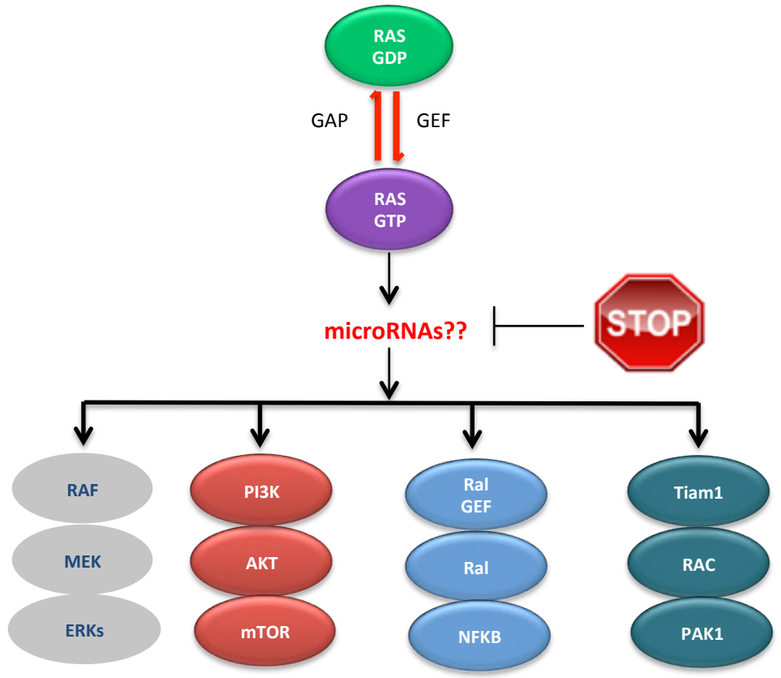


Figure 1: KRAS-modulated microRNAs.
KRAS activation or overexpression induces the upregulation of microRNAs with oncogenic function. Modulating KRAS-regulated microRNAs can stop KRAS-driven lung tumourigenesis.

KRAS

The proto-oncongene *RAS* encodes three different GTPases: *HRAS*, *NRAS* and *KRAS*. RAS proteins switch between 'on' and 'off' conformations that are mediated by the binding of GTP and GDP, respectively. The transition between these two states is regulated by guanine nucleotide exchange factors (GEFs), which stimulate KRAS activation through GDP for GTP exchange, and by GTPase-activating proteins (GAPs), which catalyse the hydrolysis of GTP to GDP to switch off KRAS signalling (Figure 1). Mutations in KRAS are very frequent in NSCLC (~30%) and are markers of worse prognosis in lung adenocarcinoma harbouring K-Ras mutations, but so far no specific drug has demonstrated efficacy. One of our current interests is to identify K-RAS-regulated microRNAs that, by targeting molecules involved in the RAS pathway, can be used as therapeutic tools in lung cancer (Figure 1). Enforced expression of either $KRAS^{WT}$ or KRAS harbouring the G12D mutation (with an amino acid substitution at position 12 leading to constitutive activation of KRAS signalling) in NSCLC cells induced the expression of several microRNAs. We are defining the molecular mechanisms through which these KRAS-modulated microRNAs might be involved in lung tumorigenesis using in vitro experiments and in vivo mouse models.

CANCER RESEARCH UK MANCHESTER INSTITUTE

RESEARCH SERVICES

RESEARCH SERVICES

Chief Laboratory Officer
Stuart Pepper

Maintenance of cutting edge core facilities requires constant acquisition of new equipment as technologies continually develop new capabilities. The following section describes some of the new equipment that has been bought over the last year, but also highlights a focus on continual development of how services are provided to meet the needs of the Institute.

Chief Laboratory Officer
Stuart Pepper

Over the last three years the Biological Resources Unit (BRU) has seen a shift towards a more collaborative environment where work is carried out by BRU and research staff side by side. This brings benefits both in an enhanced work environment and also in the efficient use of the facility. As reported below we have seen a significant increase in the volume of work that the facility can support. A similar approach has now been implemented in the FACS facility, with training offered to scientists so that they can run complex FACS experiments alongside facility staff. Again this brings the joint benefit of enhancing communications, and increasing the volume of work that can be accommodated in the facility.

Elsewhere we have seen changes that reflect the increasing importance of support for data analysis. The laboratory based facilities are benefitting from closer integration with the Scientific Computing (Sci Com) and Computational Biology Support teams managed by Crispin Miller. In particular the Mass Spectrometry team are working closely with bioinformaticians in the development of automated analysis pipelines for data sets generated by the facility. For users of the facilities this collaborative approach allows seamless integration between generation and analysis of data sets. In the Imaging facility one post is now fully dedicated to support image analysis; with the increased use of automated imaging systems some of the major challenges are now connected to the collection, storage and processing of images. By providing more support for image analysis, and working with Sci Com on aspects of data management, the facility is able to obtain the maximum benefit from the imaging systems available to researchers on site. This in turn allows us to fully leverage the benefit of development work described below that has been carried out in the Histology facility.

Towards the end of 2015 a new facility was created to allow the development of novel transgenic mouse lines. This facility is now fully operational with several clones in development. Alongside the two other animal facilities (breeding and experimental), the Institute is now able to carry out all aspects of in vivo research from generation and breeding of novel transgenic lines through to dosing, surgery and imaging protocols as required by research programs.

A final mention goes to the continual development of Next Generation Sequencing (NGS). NGS is established as a supremely powerful tool in many areas of biology and protocols for routine sequencing of DNA and RNA are well established. More recently robust protocols for sequencing of single cells have become available and this is expanding the possibilities for this technology to map genomic and transcriptomic differences between individual cells.

Advanced Imaging and Flow Cytometry

Steve Bagley, Jeff Barry (Deputy Manager, Flow Cytometry), Toni Banyard, Helen Carlin, Abi Johnson, Isabel Peset Martin, Kang Zeng, Heather Woodhouse[1], Bogdan Potereas[1]

[1]joined in 2016

The Imaging and Cytometry facility's remit is to provide state of the art tools for both basic and translational cancer studies (from molecular interactions in primary cells through to tissue-wide responses) to develop new modalities and to train scientists to apply these techniques to their research. The technologies available cover: flow cytometry cell sorting and analysis; widefield and confocal microscopy; two-photon microscopy; high content screening; super resolution imaging; X-ray sources; image processing and analysis; and support for in vivo imaging.

Over the year, new equipment has been introduced in response to the scientific demand of our research groups for increasingly more novel imaging processes and to be able to extend or advance pre-existing methods, including an additional automated scanning system for high resolution histology (Leica Versa), a supplementary high content screening system (Perkin Elmer Operetta) for 3D organoid-based studies, and an automated flow cytometry analysis system (BD Canto).

Method development has been a major requirement this year to support new technologies including single cell sorting for molecular biology applications, serial section reconstruction for 3D histology, establishing major screens of up to 70 plate assays, assistance in the application of CYTOF, high multiplexing for automated quantitative histology analysis, development of in vivo analysis routines (photons, acoustics and CT), screening tools for the analysis of 3D cultures and confetti imaging.

As researchers ask more complex phenotypic (cell behaviour, gene and protein expression, interaction, morphology and function) questions of clinical samples, the requirement for multiple labels in flow cytometry analysis increases. In response, a critical part of the facility's role is to advise on the appropriate use of combinations of markers.

The team has been complemented by the appointment of Heather Woodhouse who manages the day to day running of high content screening. These techniques have expanded considerably over the last five years as eighteen research groups now utilise three systems. The resulting complex data sets require sophisticated mathematical modelling of single cells and sub-populations for drug screening, 3D cultures and biomarker discovery applications. Working in collaboration with Scientific Computing we now have software systems in place for the statistical analysis of phenotype across many millions of cells.

Within the facility, there is an increasing demand for in vitro applications that better mimic in vivo conditions hence the escalated requirement for confocal and multi-photon microscopy. As research projects move from 2D cellular studies to 3D, and over time to 4D, there is a necessity to develop tools for imaging thick samples in an environmental context. Tissue sections approaching a thickness of 1mm have become commonplace for in vitro assay functions such as phenotypic screens for drug discovery.

Working in collaboration with the Histology facility, tissue screening and its analysis has been a huge undertaking over the year as automated histology imaging via cameras and spectrophotometry techniques has resulted in the processing of over 12,000 slides. As a consequence, the Imaging and Cytometry facility has responded by introducing a consultancy service for the analysis and informatics of tissue to enable researchers to apply suitable analysis routines and to collate the subsequent data using informatics software.

Training has been another main feature of this year's efforts; included in the training regime has been:

a) Over thirty staff trained in the image processing and analysis of histology data using contrast based methods
b) Implementation of flow cytometry sorting advanced users' course for 'after hours' usage
c) Over forty researchers trained in flow cytometry analysis using traditional and imaging methods
d) Over thirty users trained in image processing and analysis to aid high content screening applications
e) Twenty-five researchers trained in the application of cell irradiation.

As more complex techniques are being applied to the equipment, the facility members have also undergone several training courses and meetings including screening, flow cytometry panel design, imaging cytometry, management and good laboratory practice.

This year our work on the development of high content screening techniques was presented at the American Association for Cancer Research Annual Meeting and two chapters have been published discussing phenotypic screening and microscopy. The facility also took part in over thirty lab tours for Cancer Research UK supporters and five talks were given to the general public. Data from the facility was also featured in many fundraising and research engagement projects.

As the facility both introduces and develops new technologies, its success is reflected in how the equipment has been employed over the year. Analysis shows that we have engaged with over 270 users, in 240 different projects, where the equipment was in operation for over 32,000 hours, which in turn generated over 120 Terabytes of data, leading to more than twenty five papers featuring data generated in the facility.

Biological Mass Spectrometry Facility

Duncan Smith, Yvonne Connolly, John Griffiths[1]

[1]left in 2016

Our remit is to facilitate the access of research groups to protein characterisation by mass spectrometry (MS). The use of MS, and affiliated technologies, has become widespread in biological research in recent years due to its analytical strengths when analysing protein biochemistry. MS approaches can be employed to ask questions about many aspects of a protein's life in the cell, including modulation of protein levels and post-translational modification (such as phosphorylation, acetylation and ubiquitylation), site assignment and quantification. The dynamic nature of the proteome makes it an ideal candidate for study in cancer progression as all molecular events leading to disease biology are executed at the protein level.

The facility has been used by the majority of groups in 2016 with the identification of protein binding partners, phosphorylation site mapping and label free protein profiling being particularly popular. We benefit from having access to two world-class LCMS platforms (a Thermo Orbitrap Fusion and Sciex 6600) that complement each other and allow us to perform proteomics experiments in a wide applications area.

During the year, we implemented the migration of data analysis pipelines onto high performance virtual machines in collaboration with the Scientific Computing and Computational Biology Support teams. This will facilitate an enhanced level of data processing in addition to significant amounts of automation. The pipeline migration will continue into 2017.

The facility has spent a significant amount of time investigating the utility of automating our gel based sample preparation workflow this year. The process was successfully concluded at the end of 2016 with the purchase of a new robotic liquid handling system. The new system should be installed in Q1 2017 and will significantly improve throughput and facilitate the development of new protein preparation workflows of the future.

Biological Resources Unit

Transgenic Breeding
Team Leader: Kim Acton

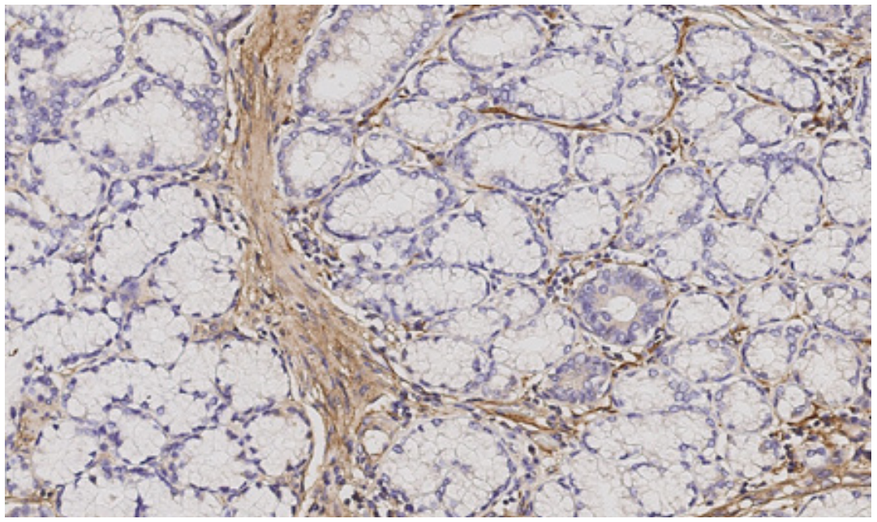
The Biological Resources Unit (BRU) transgenic mouse breeding facility is at The University of Manchester's Incubator Building. Only newly imported lines are now held at the Paterson Building, until re-derivation (via embryos) to the Incubator Building is complete. Live mice are not brought into the Incubator Building directly to maintain the high health status of the facility and avoid possible contamination.

Approximately 37 new lines were set up at the Incubator Building during 2016, mostly by crossing existing lines, but included nine newly imported lines and seven lines created by embryo microinjections. Cryopreservation of embryos and sperm (twice weekly) has resulted in the archiving (closing of live colonies) for 47 lines during 2016. A further 22 live colonies were closed in 2016 (cryopreservation not required).

Currently, the Transgenic Breeding facility has around 145 breeding lines of genetically altered (GA) mice, cared for by 10 staff, for the Institute's scientists. Although the numbers of lines have decreased, the number of cages is increasing (currently at approximately 2,300). This is due to smaller (maintenance) lines having been closed, with increased breeding for the newer lines, to establish stock of appropriate genotypes for experimental use. Additional services include timed mating set up and the import and export of strains (exports in 2016 included Australia, Greece, Portugal and Argentina).

The facility manages the genotyping service, where all samples are sent to an external company (Transnetyx, USA) and genotype results are downloaded into the stock records.

Prompted by an increase in numbers of GA animals produced and used in recent years across the UK, the Home Office Animals in Science Regulation Unit (ASRU), in consultation with breeding experts and establishments (including the BRU Transgenic Team), drafted an assessment framework. As a guide for establishments to self-assess their GAA breeding practices and identify strengths and areas for improvement, the team have been included in a 'pilot' (with five other establishments) with ASRU. Within an initial meeting and facility tour in May and outcome review in October, of 51 points raised across 10 focus areas, including colony management, genotyping, team working etc., 28 were rated as satisfactory, 20 rated as 'examples of excellent practice that can be shared' and three 'relatively minor areas' where some improvement could be made – for which plans are being put in place. This excellent outcome is a great reflection on the organisation and functioning of the unit.



Pancreatic tumour stained with a stromal cell marker. *Image supplied by Colin Hutton (Systems Oncology)*

Biological Resources Unit

Experimental Services
Team Leader: Lisa Doar

The last year has been an exciting one for the experimental team with a steady increase in the number of people working in the facility and the number of projects completed. We have seen a ~20% increase in the number of cages in the unit. One of the biggest challenges for this year has been coping with and processing the number of training requests we have received from new licensees joining the Institute. We have established a new system and database to help manage the requests more efficiently and so far this process is handling the increased training requirements well.

We have had another piece of imaging equipment installed into the unit this year – a micro-CT scanner. This enables us to visualise tumour growth in the lungs and bones and is now used on a weekly basis by two of the lung cancer groups. As with all the other imaging equipment in the facility, staff in the experimental team have been trained so that we can offer a quality service to the researchers.

As part of our on-going commitment to keep the service up to date, members of the experimental team have attended several conferences and symposia again this year. We are also proud that one of our technicians won the Institute's Annual 3Rs' Prize (aimed at the replacement, reduction and refinement of animal work) with her poster on how she has improved practices for monitoring of ovarian tumours, which is a fantastic achievement for the team. Another important project that has been carried out by one of our other technicians is determining whether cable-tie 'swings' hung from the cage bars are a good form of enrichment for mice. The results showed that our most commonly used strain of mouse interacted with the swing 40% of the time during the monitoring sessions, which represents a significant refinement and development for the welfare of our mice.

Our plan for the coming year is to ascertain whether we can refurbish our quarantine facility to improve collaboration with other institutes that have a different health status for working with PDX models. These improvements will aim to provide access opportunities to alternative imaging modalities based at other institutes in the local area.

Transgenic Production Facility

Natalia Moncaut, Mark Willington, Athina Papaemmanouil[1]

[1]joined in 2016

The Transgenic Production Facility generates new genetically modified mice working closely with the research groups and according to their specific needs. The service is responsible for the entire process of generating a transgenic mouse model: from its initial design by employing the most appropriate targeting strategy, to the generation of all required components: microinjection, mouse surgery, screening of targeted events and breeding of founder mice. Depending on the project type and its complexity, we offer two approaches to target a locus of interest. The first involves the co-delivery of CRISPR-Cas reagents into embryonic stem (ES) cells and the second approach employs the direct microinjection of CRISPR-Cas components into one-cell stage mouse embryos.

It has been an exciting and challenging first year for the team. Since its inception we have established a new molecular biology laboratory and an ES cell culture room in the Paterson

Building. The animal work, based at The University of Manchester's Incubator Building, has also been reorganised to ensure regular provision of mouse embryos. During 2016 we initiated seven fully customised projects incorporating the different approaches. With the increase in workload, a new member of staff was recruited to the team in September with responsibilities in the molecular biology and cell culture area.

New targeting technologies are changing completely the field of mouse production, and consequently the field is evolving rapidly. In order to keep on top of these new developments the Transgenic Production Facility team attended the annual International Society of Transgenic Technologies meeting as well as several national conferences, including a CRISPR Conference at the Wellcome Trust Sanger Institute.

Histology

Garry Ashton, Caron Abbey, Adam Burke, Emma Watson, Usman Mahmood[1], Deepti Wilks (Haematological Malignancy Biobank)

[1]joined in 2016

The Histology facility underpins the research activities of a large number of both basic and translational research groups within CRUK MI. It allows the adoption of tissue-based experimental approaches to all research programmes. The facility's remit is to offer a full range of both routine and advanced histological services for oncology research. In 2016, Histology continued to develop and expand. As the range and complexity of services offered continues to grow, the training and continued professional development of staff has ensured the facility continues to offer a comprehensive and flexible service at all times. Space has been acquired and equipment purchased that has allowed us to begin establishing a laboratory that can operate to GCP standards. A basic sample barcoding and tracking system (for nucleic acid extraction across research groups and core facilities) was also implemented to complement our existing tissue labelling and barcoding system.

The facility routinely processes, embeds and sections both human and mouse tissue in addition to organotypic assays, spheroids, agar plugs and cell pellets. Vibratome sections from fresh tissue (50–250µm) have also been prepared for the set up of ex vivo cultures of tumours and to evaluate and develop three dimensional studies. A number of research groups have used several special stains, including Masson Trichrome, PAS and reticulin.

The high throughput routine immunohistochemistry service, troubleshooting and antibody validation services have experienced exceptional demand this year. In addition, the availability of the new Leica and Roche platforms has also allowed us to continue to evaluate, develop and automate both mRNA in situ hybridisation and multiplex immunohistochemistry. We have offered these platforms as services and incorporated sophisticated labelling techniques into several groups' research programs. These techniques allow researchers to gain more information about the relationships between cells from different lineages on a single tissue sample or to obtain gene expression information in the context of tissue or cell morphology.

In a collaborative project, a 6plex immunohistochemistry panel was evaluated, whereby percentage staining in single-multiple protocols was compared. Cell counts from five areas on each slide were used to form comparisons using linear regression for each single-multiple signal. The results indicate that antibody order may have an effect on staining quality in multiplexed immunohistochemistry and, therefore, while multiplexed immunohistochemistry is a promising concept, this effect needs to be borne in mind in experimental design.

One interest of the Stem Cell Biology group is to study the development of the blood system in the mouse and to identify new regulators of this process. mRNA in situ hybridisation is currently being used to study the expression of these genes. The group are also investigating the role of RUNX1 in prostate cancer. The Histology facility has provided extensive support in the development of multiplexed immunohistochemistry panels to study the expression of RUNX1, in the context of other established markers, on patient tissue micro-arrays constructed in the facility. Multiplex immunohistochemistry is also being used for the Molecular Oncology group to define subpopulations of lymphocytes infiltrating frozen mouse melanoma tissue.

The Prostate Oncobiology group have used immunohistochemistry initially to characterise the lymphocyte antigen 6 complex, locus D progenitor subpopulation in the mouse prostate. Following this, multiplex IHC is being used to characterise both FFPE and frozen organoid samples (basal lineage, luminal lineage, proliferation marker and functional response to stimuli by androgen receptor). Evaluation of the potential role of this novel marker in prostate cancer patients' outcome, using multiplex IF on prostate cancer patients' tissue-microarrays is ongoing in order to stratify patients and predict their clinical outcome.

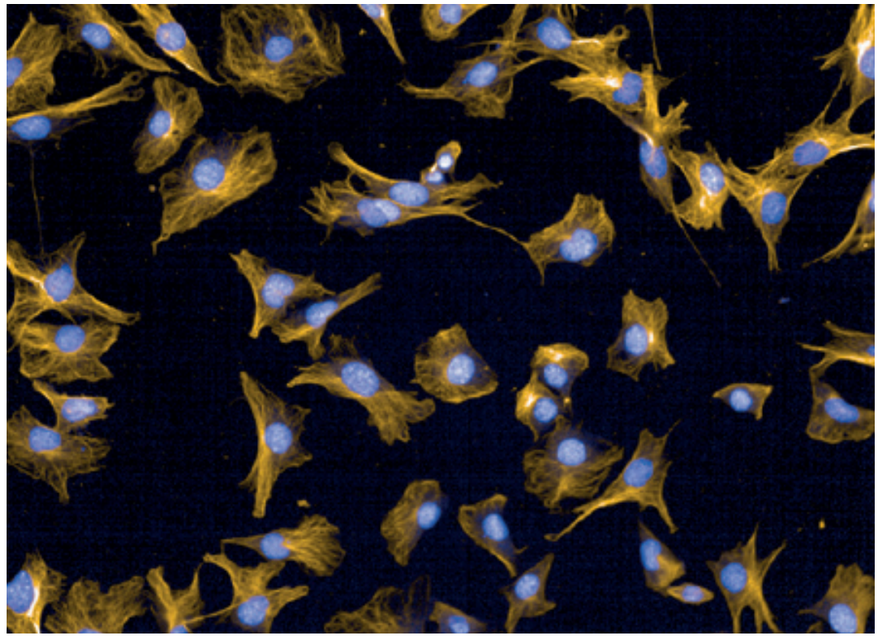
The microdissection/macrodissection of tissue and downstream extraction of both RNA and DNA, sufficient in quantity and quality for NGS from relatively small amounts of material is now routine. In a recent project with the Molecular Oncology group, following pathological evaluation DNA/RNA was extracted from 282 individual prostate samples from 36 patients. The quality was high, with median DIN 6.4 (2.4-8.7) and median RIN 7.2 (0-9) and the amounts obtained were more than sufficient for downstream analysis; median DNA recovered 1.25ug (0.13-6ug) and RNA recovered 0.37ug (0-4). These results led to very high quality sequencing data, which has in turn led to novel findings in prostate cancer genetics which are currently being prepared for publication.

In a separate project somatic molecular profiling is being performed by NGS on approximately 110 resected thymomas. Thymoma is a rare malignant tumour of thymic gland that constitutes 5 histological subtypes (type A, AB, B1, B2, and B3). Due to the rarity, molecular findings have been scarcely reported in the literature. A further complication is that this group of tumours often contain heavy inflammatory cellular components, which are non-neoplastic in nature, particularly type B1 group in which the vast majority of cellular components are non-neoplastic inflammatory cells. In order to extract optimal tumour DNA for NGS, sections of type B1 thymomas were immunohistochemically stained with pan-cytokeratin using clone AE1/AE3, and positive tumour cells were dissected using laser-capture microdissection.

The use of our existing tissue microarray archive and the construction of new arrays have again proved extremely popular. All TMAs give true sample representation and are of the highest quality. TMAs from disease groups including breast, melanoma, prostate (cores and chips), bladder, lymphoid, small cell and non-small cell lung cancer plus mouse model and cell pellet controls are all available.

The facility continues to be used heavily by the CEP preclinical team and IHC biomarkers teams. CDX models are phenotyped routinely on our automated IHC platforms ensuring consistency, reproducibility and standardisation. The Leica RX platform is also used to stain slides that are scanned on the RareCyte platform. These slides are stained with a cocktail of antibodies to identify circulating tumour cells (CTCs) in patient blood samples. CEP has a collaborative relationship with the Histology facility which acts as an excellent platform for scientific discussion and knowledge exchange.

Alongside the work described above, the Histology facility also has a critical role supporting the MCRC Biobank. The two teams have a close working relationship which means that there is a seamless connection between the collection, storage, quality control and processing of samples held by the Biobank.



Pancreatic stellate cells.
Image supplied by Colin Hutton (Systems Oncology)

Laboratory Services

Mark Craven, Tony Dawson, Corinne Hand, Petra Kubinova[1], Jolanta Organisciak[2], Adriana Tudelo, Christine Whitehurst

[1]joined in 2016 [2]left in 2016

During 2016, Laboratory Services continued to support the research activities within the Paterson and MCRC Buildings along with the Wolfson Molecular Imaging Centre (WMIC). We staff dedicated departments within the Paterson and MCRC Buildings, both containing equipment for glass washing and autoclaving items for the research groups to use.

Our primary role is to supply and deliver sterile glassware, plastics, water and simple buffers to labs. We collect any dirty glassware and plastic ware twice a day and refill labs with new stock each day.

Via our purpose-built clean room within the Paterson Building, we also offer a range of bacteriological media and agar plates across the site. We manufacture these items using standardised recipes to maintain a consistent product but also prepare and adapt bespoke material if required by the research groups. This year, we manufactured a total of 3500 litres of liquid media, 3200 agar plates and sterilised 9500 litres of PBS.

In other roles, we maintain and clean the two dark rooms operating film developers. We also organise the supply of clean lab coats for general and tissue culture work as well as for visitors. The department oversees the monthly pipette clinics and the annual servicing of lab kit such as anemometers and personal oxygen monitors. Additionally within the Paterson Building we monitor and replace first aid and eye wash supplies and ensure all taps are opened as part of the Legionella testing programme each month.

Molecular Biology Core Facility

Wolfgang Breitwieser, Chris Clark, Toni Grady, Emily Hulme[1], Gillian Newton, Leanne Wardleworth, John Weightman

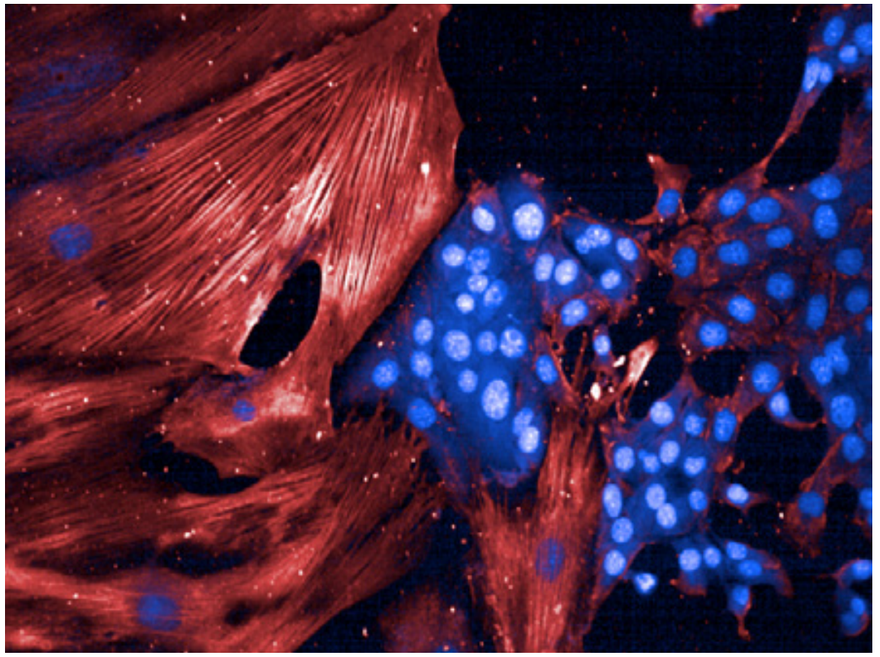
[1]joined in 2016

While in the last year there remained a steady demand for a number of Molecular Biology Core Facility (MBCF) core applications, including Sanger sequencing and microbe testing, requests for other services such as cell line authentication and sample QC (Bioanalyzer and Tapestation) have experienced a significant increase. High throughput instrumentation for quantitative PCR is a backbone of MBCF support and after almost a decade of service, the existing qPCR setup has been replaced by 2 ABI Quantstudio 5 (384-well) and 1 Quantstudio 3 (96-well) instruments. Their impact has been immediately felt by their ease of access and use, and fast and reliable performance.
Introduced in the previous year, the Compound Library Service has seen a steady increase in demand in 2016. Supported by currently four small to medium sized compound libraries, we assist drug screens by high throughput dispensing of compounds using the Labcyte Access/Echo platform. The service is also increasingly used in compound dosing experiments and combinatorial assays.
The last year has also seen a continued rise in demand for Next Generation Sequencing (NGS), which is demonstrated by the fact that over this period all but one of the research groups used this service. To reflect the versatility of the technology we have validated and adapted a number of novel NGS applications, especially RNASeq methods, where we now offer solutions for a wide range of inputs and sample qualities. For example, due to the nature of the fixation process the investigation of nucleic acids from FFPE samples has been difficult. However, improved extraction methods and applications for library preparation have made it possible to exploit FFPE DNA and RNA for NGS. We have therefore validated appropriate methods and added them to routine services for genome and transcriptome analysis.

Another focus of assay development has been on workflows for high throughput RNASeq of FACS sorted single cells. This application allows us to process hundreds of cells in one experiment and is currently used, for example, for cell lineage tracing in haematopoiesis. Of particular importance is the automated plate handling and liquid dispensing at very low volumes. To achieve this we have exploited our assortment automation platforms, e.g. by using the Formulatrix Mantis for cDNA preparation, the Agilent Bravo for magnetic bead clean up steps, and the Labcyte Echo for cDNA quantitation and sequencing library preparation. Consequently the assay miniaturisation provided by the automation also resulted in a significant reduction in the cost of processing per single cells.

Scientific Computing and Computational Biology Support

These two services provide high performance computing and data analysis solutions for the Institute's scientists and are overseen by Crispin Miller.



Pancreatic cancer-associated fibroblast and cancer cell co-culture.
Image supplied by Colin Hutton (Systems Oncology)

Scientific Computing

Marek Dynowski[1], ZhiCheng Wang, Christopher Wirth, Christopher Smowton[2]

[1]joined in 2016 [2]left in 2016

This year Marek Dynowski joined the Institute to lead the Scientific Computing Team. The year has been very productive, with the team introducing a number of new services, and making continued improvements to the stability and efficiency of the high performance computing (HPC) and storage systems. New job management software MOAB has been installed, increasing both the reliability of the system and the efficiency of job scheduling. In cooperation with the Estates team, several changes were made to improve the stability of the system and drive it towards high availability. These measures allow us to fully exploit the potential of our water-cooled machine room and uninterruptible power supply. To improve services when problems arise, or when new software needs to be installed, an IT Support Ticket system has been set up and put into production. This new system decreases response times for users of SciCom services and hardware and helps to optimise the SciCom support.
Two NVIDIA K40 graphic accelerators, which are proving especially useful for molecular dynamics simulations, were integrated into the job management system and are now accessible to all our users.

The first step in creating a fast 10GbE network connection between SciCom and the CRUK MI Core Facilities was taken in cooperation with the IT department. It allows tighter integration of data sources, such as sequencers or microscopes, into the SciCom storage infrastructure – a prerequisite for establishing effective and highly-automated data lifecycle management.

A virtualisation infrastructure was introduced to optimise hardware usage, which led to a reduction in administrative effort and expense, while still providing new services such as a central versioning repository for source code Gitlab. It allows programmers to keep track of their code while ensuring that code developed by CRUK MI is centrally documented as well as backed up and archived.

Several pipelines developed by the Computational Biology Support team, the RNA Biology group and CEP were improved and accelerated through close cooperation with Christopher Wirth and Christopher Smowton.

In the autumn, Snakemake was introduced as our new workflow management system on the HPC cluster Troodon, which, in combination with our new job management system, leads to substantially increased throughput in our data analysis pipelines.

Computational Biology Support

Hui Sun Leong, Sudhakar Sahoo, Samuel Taylor[1], Pieta Schofield[1], Shambhavi Srivastava[1]

[1]joined in 2016

The continual increase in the amount of experimental data derived from high-throughput technologies has caused a paradigm shift in

biological research, with a shift from a traditionally hypothesis-driven to a data-driven approach. Given the size and complexity of these big data, scientists are in need of rigorous and scalable methods to unearth the exciting knowledge captured within the data. The Computational Biology Support (CBS) team helps researchers in the CRUK Manchester Institute meet this challenge by providing expertise in data analysis of next generation sequencing (NGS) experiments, and other large-scale biological experiments that require bioinformatics support.

CBS underwent a major expansion in 2016. With the recruitment of two new computational biologists, the group was able to extend its service provision beyond genomics data analysis to the processing of high-throughput proteomics datasets, as well as supporting the bioinformatics needs of members from the Movember Prostate Cancer Centre of Excellence through the recruitment of an additional Prostate Cancer UK funded member of staff.

During the course of 2016, CBS collaborated with experimental researchers within the Institute on various NGS projects involving RNA-seq, small RNA-seq, ChIP-seq, genome-wide CRISPR/Cas9 knockout screens, whole-exome and whole-genome sequencing. For example, the team have been working closely with members from the Clinical and Experimental Pharmacology group to develop dedicated data analysis workflows for detecting somatic mutations and copy number aberration events in circulating tumour DNA samples and liquid biopsies. Some of the collaborative efforts have contributed to high-impact publications (Carter L et al. Nat Med. 2016; Williamson SC et al. Nat Commun. 2016; Tape CJ et al. Cell. 2016; Draper JE et al. PLoS Genet. 2016).

We have also been actively expanding into new areas to help support scientific advances in other research technologies. For example, we are working closely with the Molecular Biology Core Facility (MBCF) to develop approaches for performing NGS on archival materials derived from formalin-fixed paraffin embedded (FFPE) tumour samples, and to evaluate the potential use of FFPE samples in cancer genomics studies.

In 2017, CBS will be working with the Biological Mass Spectrometry Facility and Systems Oncology group to develop dedicated workflows for analysing label-free quantitative proteomics, SILAC/iTRAQ and SWATH datasets generated by research programmes within the Institute. We will also be collaborating with the Scientific Computing team to develop automated NGS pipelines with improved performance and throughput.

CANCER RESEARCH UK MANCHESTER INSTITUTE

PUBLICATIONS AND ADMINISTRATION

Staining of secretory luminal cells (CK8+) in glands of normal murine prostate.
Image supplied by João Diogo Barros Silva (Prostate Oncobiology)

RESEARCH PUBLICATIONS

Santiago Zelenay (page 12)
Cancer Inflammation and Immunity

Other publications
Zelenay S, Reis E Sousa C. (2016)
Reducing prostaglandin E2 production to raise cancer immunogenicity. ***Oncoimmunology***, 5(5):e1123370.

Iain Hagan (page 14)
Cell Division

Other publications
Grallert A., Hagan I.M. (2016)
Elementary protein analysis in *Schizosaccharomyces pombe*. ***Fission Yeast: a Laboratory Manual CSHLP*** 119-121.

Grallert A, Hagan IM. (2016)
Preparation of Protein Extracts from *Schizosaccharomyces pombe* Using Trichloroacetic Acid Precipitation. ***Fission Yeast: a Laboratory Manual CSHLP*** 122-126.

Grallert A, Hagan IM. (2016)
Small-Scale Immunoprecipitation from Fission Yeast Cell Extracts. ***Fission Yeast: a Laboratory Manual CSHLP*** 127-133.

Grallert A., Hagan I.M. (2016)
Large-Scale Immunoprecipitation from Fission Yeast Cell Extracts. ***Fission Yeast: a Laboratory Manual CSHLP*** 134-140.

Hagan IM, Bagley S. (2016)
Fixed-Cell Imaging of *Schizosaccharomyces pombe*. ***Fission Yeast: a Laboratory Manual CSHLP*** 193-198.

Hagan I.M. (2016)
Immunofluorescence Microscopy of *Schizosaccharomyces pombe* Using Chemical Fixation. ***Fission Yeast: a Laboratory Manual CSHLP*** 199-207.

Hagan IM. (2016)
Chromatin and Cell Wall staining of *Schizosaccharomyces pombe* Using Chemical Fixation. ***Fission Yeast: a Laboratory Manual CSHLP*** 208-212.

Hagan IM. (2016)
Staining Fission yeast Filamentous actin with fluorescent phalloidin conjugates. ***Fission Yeast: a Laboratory Manual CSHLP*** 213-216.

Hagan IM, Grallert A, Simanis V. (2016)
Analysis of the *Schizosaccharomyces pombe* cell cycle. ***Fission Yeast: a Laboratory Manual CSHLP*** 271-282.

Hagan IM, Grallert A, Simanis V. (2016)
Cell Cycle Synchronization of *Schizosaccharomyces pombe* by Centrifugal Elutriation of Small Cells. ***Fission Yeast: a Laboratory Manual CSHLP*** 283-290.

Hagan IM, Grallert A, Simanis V. (2016)
Cell Cycle Synchronization of *Schizosaccharomyces pombe* by Lactose Gradient Centrifugation to Isolate Small Cells. ***Fission Yeast: a Laboratory Manual CSHLP*** 291-294.

Hagan IM, Grallert A, Simanis V. (2016)
Synchronizing Progression of *Schizosaccharomyces pombe* Cells from Prophase through Mitosis and into S Phase with *nda3-KM311* Arrest Release. ***Fission Yeast: a Laboratory Manual CSHLP*** 295-299.

Hagan IM, Grallert A, Simanis V. (2016)
Synchronizing Progression of *Schizosaccharomyces pombe* Cells from G2 through Repeated Rounds of Mitosis and S Phase with *cdc25-22* Arrest Release. ***Fission Yeast: a Laboratory Manual CSHLP*** 300-304.

Angeliki Malliri (page 18)
Cell Signalling

Refereed Research Papers
Myant KB, Cammareri P, Hodder MC, Wills J, Von Kriegsheim A, Győrffy 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. (2016)
HUWE1 is a critical colonic tumour suppressor gene that prevents MYC signalling, DNA damage accumulation and tumour initiation. ***EMBO Molecular Medicine***, Epub 2016 December 21.

Marei H, Carpy A, Macek B, Malliri A. (2016)
Proteomic analysis of Rac1 signaling regulation by guanine nucleotide exchange factors. ***Cell Cycle***, 15(15):1961-74.

Marei H, Carpy A, Woroniuk A, Vennin C, White G, Timpson P, Macek B, Malliri A. (2016)
Differential Rac1 signalling by guanine nucleotide exchange factors implicates FLII in regulating Rac1-driven cell migration. ***Nature Communications***, 7:10664.

Other publications
Marei H, Malliri A. (2016)
Rac1 in human diseases: The therapeutic potential of targeting Rac1 signaling regulatory mechanisms. ***Small GTPase***s, Epub 2016 July 21.

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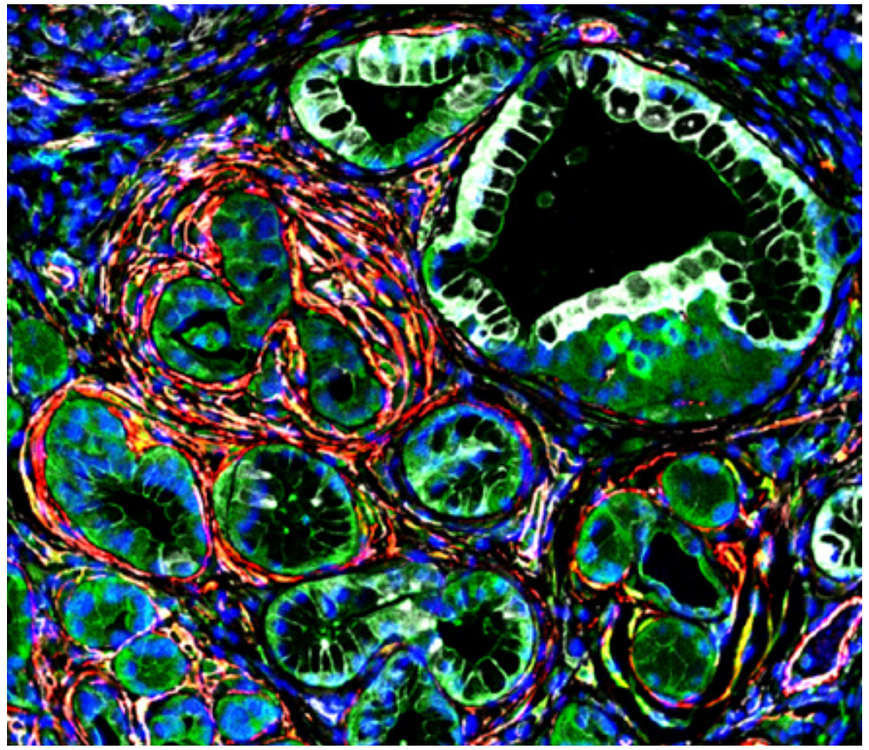
GEFs: Dual regulation of Rac1 signaling. ***Small GTPases***, Epub 2016 June 17.

Porter AP, Papaioannou A, Malliri A. (2016)
Deregulation of Rho GTPases in cancer. ***Small GTPases***, 7(3):123-38.

Mouse pancreatic intraepithelial neoplasms stained with SMA (Red), LOX (Green), PDFR Beta (Grey) and DAPI (Blue).
Image supplied by Haoran Tang (Molecular Oncology)



Caroline Dive (page 20)

Clinical and Experimental Pharmacology

Refereed Research Papers

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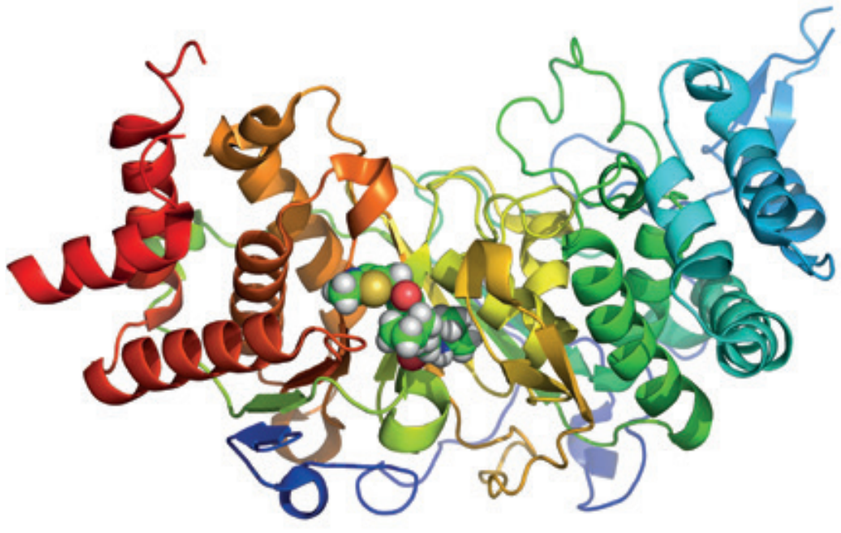

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Donald Ogilvie (Page 24)
Drug Discovery

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Image supplied by Haoran Tang (Molecular Oncology)

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Tim Somervaille (page 26)
Leukaemia Biology

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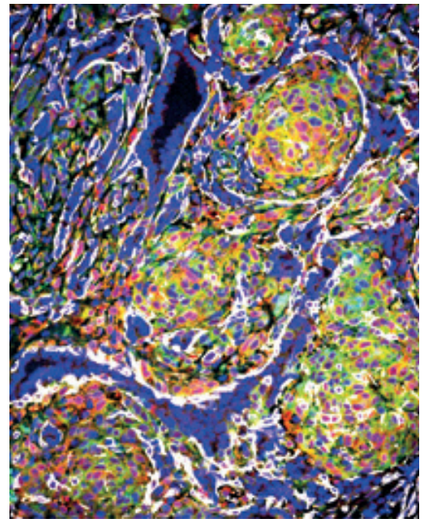

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Crispin Miller (page 32)

RNA Biology

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John Brognard (page 34)

Signalling Networks in Cancer

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Georges Lacaud (page 38)

Stem Cell Biology

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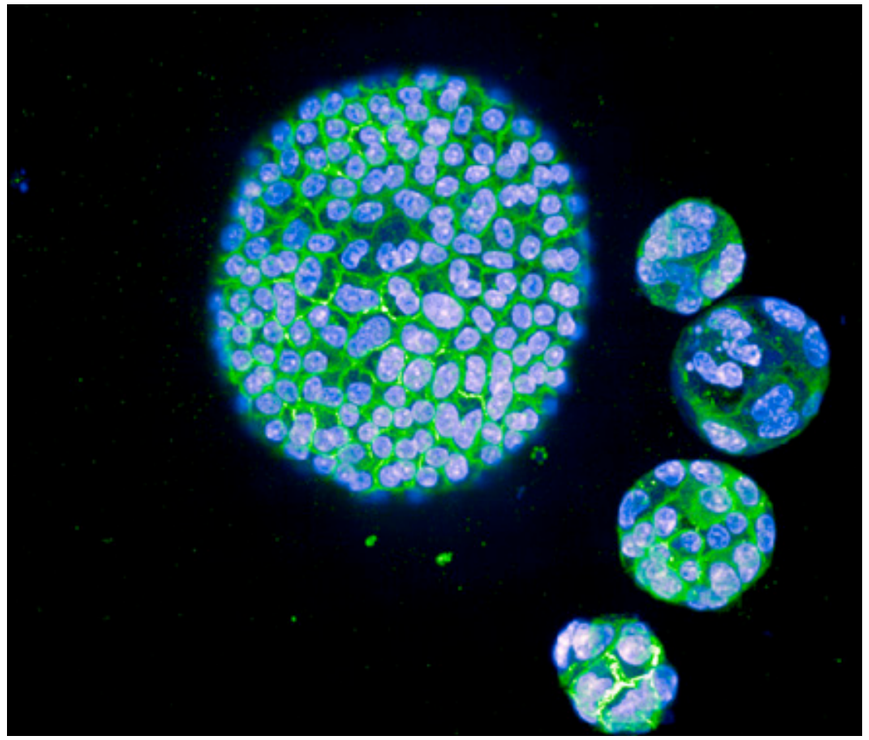
Image supplied by Xiaohong Zhang (Systems Oncology)



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Valerie Kouskoff (page 40)

Stem Cell Haematopoiesis

Refereed Research Papers

Lilly AJ, Costa G, Largeot A, Fadlullah MZ, Lie-A-Ling M, Lacaud G, Kouskoff V. (2016) Interplay between SOX7 and RUNX1 regulates hemogenic endothelial fate in the yolk sac. ***Development***, 143(23):4341-4351.

Obier N, Cauchy P, Assi SA, Gilmour J, Lie-A-Ling M, Lichtinger M, Hoogenkamp M, Noailles L, Cockerill PN, Lacaud G, Kouskoff V, Bonifer C. (2016) Cooperative binding of AP-1 and TEAD4 modulates the balance between vascular smooth muscle and hemogenic cell fate. ***Development***, 143(23):4324-4340.

Nasrallah R, Fast EM, Solaimani P, Knezevic K, Eliades A, Patel R, Thambyrajah R, Unnikrishnan A, Thoms J, Beck D, Vink CS, Smith A, Wong J, Shepherd M, Kent D, Roychoudhuri R, Paul F, Klippert J, Hammes A, Willnow T, Göttgens B, Dzierzak E, Zon LI, Lacaud G, Kouskoff V, Pimanda JE. (2016) Identification of novel regulators of developmental hematopoiesis using Endoglin regulatory elements as molecular probes. ***Blood***, 128(15):1928-1939.

Cuvertino S, Lacaud G, Kouskoff V. (2016) SOX7-enforced expression promotes the expansion of adult blood progenitors and blocks B-cell development. ***Open Biology***, 6(7). pii: 160070.

Cuvertino S, Filiciotto G, Masurekar A, Saha V, Lacaud G, Kouskoff V. (2016) SOX7 promotes the maintenance and proliferation of B cell precursor acute lymphoblastic cells. ***Oncotarget***, Epub 2016 July 7.

Thambyrajah R, Patel R, Mazan M, Lie-A-Ling M, Lilly A, Eliades A, Menegatti S, Garcia-Alegria E, Florkowska M, Batta K, Kouskoff V, Lacaud G. (2016) New insights into the regulation by RUNX1 and GFI1(s) proteins of the endothelial to hematopoietic transition generating primordial hematopoietic cells. ***Cell Cycle***, 15(16):2108-2114.

Eliades A, Wareing S, Marinopoulou E, Fadlullah MZ, Patel R, Grabarek JB, Plusa B, Lacaud G, Kouskoff V. (2016) The Hemogenic Competence of Endothelial Progenitors Is Restricted by Runx1 Silencing during Embryonic Development. ***Cell Reports***, 15(10):2185-99.

Garcia-Alegria E, Iluit M, Stefanska M, Silva C, Heeg S, Kimber SJ, Kouskoff V, Lacaud G, Vijayaraghavan A, Batta K. (2016) Graphene Oxide promotes embryonic stem cell differentiation to haematopoietic lineage. ***Science Reports***, 6:25917.

Goode DK, Obier N, Vijayabaskar MS, Lie-A-Ling M, Lilly AJ, Hannah R, Lichtinger M, Batta K, Florkowska M, Patel R, Challinor M, Wallace K, Gilmour J, Assi SA, Cauchy P, Hoogenkamp M, Westhead DR, Lacaud G, Kouskoff V, Göttgens B, Bonifer C. (2016) Dynamic Gene Regulatory Networks Drive Hematopoietic Specification and Differentiation. ***Developmental Cell***, 36(5):572-87.

Largeot A, Perez-Campo FM, Marinopoulou E, Lie-a-Ling M, Kouskoff V, Lacaud G. (2016) Expression of the MOZ-TIF2 oncoprotein in mice represses senescence. ***Experimental Hematology***, 44(4):231-7.e4.

Draper JE, Sroczynska P, Tsoulaki O, Leong HS, Fadlullah MZ, Miller C, Kouskoff V, Lacaud G. (2016) RUNX1B Expression Is Highly Heterogeneous and Distinguishes Megakaryocytic and Erythroid Lineage Fate in Adult Mouse Hematopoiesis. ***PLoS Genetics***, 12(1):e1005814.

Other Publications

Lilly AJ, Lacaud G, Kouskoff V. (2016) SOXF transcription factors in cardiovascular development. ***Seminars in Cell & Developmental Biology***, S1084-9521(16) 30216-6.

Batta K, Menegatti S, Garcia-Alegria E, Florkowska M, Lacaud G, Kouskoff V. (2016) Concise Review: Recent Advances in the In Vitro Derivation of Blood Cell Populations. ***Stem Cells Translational Medicine***, 5(10):1330-1337.

Garcia-Alegria E, Menegatti S, Batta K, Cuvertino S, Florkowska M, Kouskoff V. (2016) Emerging concepts for the in vitro derivation of murine haematopoietic stem and progenitor cells. ***FEBS Letters***, 590(22):4116-4125.

Claus Jørgensen (page 42)

Systems Oncology

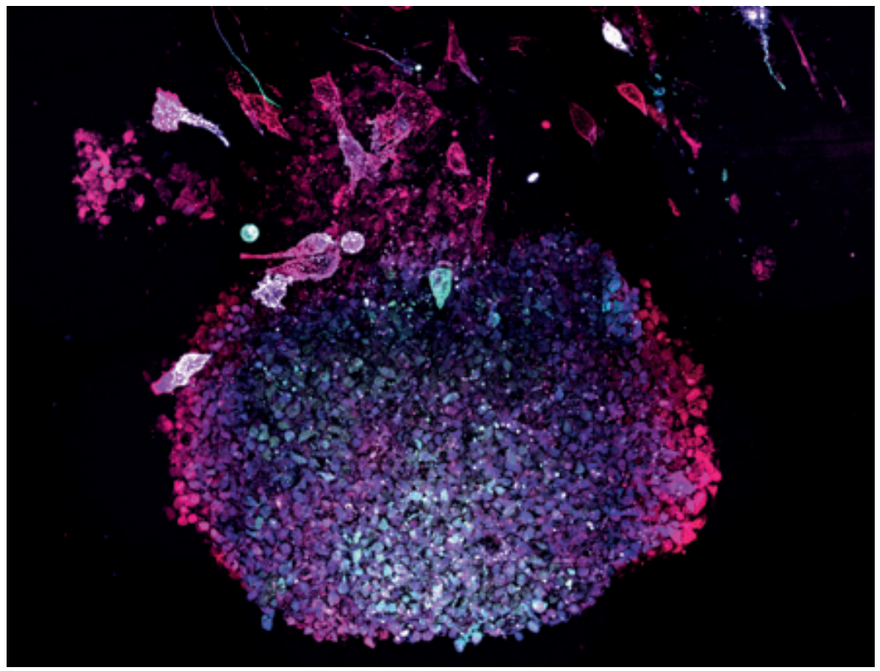
Refereed Research Papers

Mardakheh FK, Sailem HZ, Kümper S, Tape CJ, McCully RR, Paul A, Anjomani-Virmouni S, Jørgensen C, Poulogiannis G, Marshall CJ, Bakal C. (2016) Proteomics profiling of interactome dynamics by colocalisation analysis (COLA). ***Molecular Biosystems***, 13(1):92-105.

Tape CJ, Ling S, Dimitriadi M, McMahon KM, Worboys JD, Leong HS, Norrie IC, Miller CJ, Poulogiannis G, Lauffenburger DA, Jørgensen C. (2016) Oncogenic KRAS regulates tumor cell signaling via stromal reciprocation. ***Cell***, 165(4) 910-920.

Locard-Paulet M, Lim L, Veluscek G, McMahon K, Sinclair J, van Weverwijk A, Worboys JD, Yuan Y, Isacke CM, Jørgensen C. (2016) Phosphoproteomic analysis of tumour-endothelial signaling identifies EPHA2 as a negative regulator of transendothelial migration. ***Science Signaling***, 9(414):ra15.

Kümper S, Mardakheh FK, McCarthy A, Yeo M, Stamp GW, Paul A, Worboys J, Sadok A, Jørgensen C, Guichard S, Marshall CJ. (2016) Rho-associated kinase (ROCK) function is essential for cell cycle progression, senescence and tumorigenesis. ***Elife***, 5:e12994



Live image of small cell lung cancer cells labelled for actin, grown as a three-dimensional spheroid, leaving the main body of cells (bottom of the image) and invading into a layer of matrigel. Cell colours correspond to depth at which they were imaged, showing the cells moving in three dimensions.
Image supplied by Andrew Porter & Sophie Adlard (Cell Signalling)

Additional Publications

Gaignaux A, Ashton G, Coppola D, De Souza Y, De Wilde A, Eliason J, Grizzle W, Guadagni F, Gunter E, Koppandi I, Shea K, Shi T, Stein JA, Sobel ME, Tybring G, Van den Eynden G, Betsou F. (2016)
A Biospecimen Proficiency Testing Program for Biobank Accreditation: Four Years of Experience. ***Biopreservation & Biobanking***, 14(5):429-439.

Long J, Basu Roy R, Zhang YJ, Antrobus R, Du Y, Smith DL, Weekes MP, Javid B. (2016)
Plasma Membrane Profiling Reveals Upregulation of ABCA1 by Infected Macrophages Leading to Restriction of Mycobacterial Growth. ***Frontiers in Microbiology***, 7:1086.

Du W, Forte GM, Smith D, Petersen J. (2016)
Phosphorylation of the amino-terminus of the AGC kinase Gad8 prevents its interaction with TORC2. ***Open Biology***, 6(3). pii: 150189.

Mihic-Probst D, Shea C, Duncan L, de la Fouchardiere A, Landman G, Landsberg J, ven den Oord J, Lowe L, Cook MG, Yun SJ, Clarke L, Messina J, Elder DE, Barnhill RL. (2016)
Update on Thin Melanoma: Outcome of an International Workshop. ***Advances in Anatomic Pathology***, 23(1):24-9.

THESES

Kate Hogan
Molecular Oncology

The gene-gene and gene-environment interactions in melanoma

Dan Wiseman
Leukaemia Biology

Isocitrate dehydrogenase mutations as targets for monitoring and therapy in AML

Anna Woroniuk
Cell Signalling

The role of the Rac1 activator STEF/Tiam2 in front-rear polarity and migration

Rob Metcalf
Clinical and Experimental Pharmacology

The clinical utility and characterisation of circulating tumour cells in lung cancer

Alekh Thapa
Cell Regulation

The role of MEKK1-MKK4 signalling in tumour biology

Andrew Hudson
Signalling Networks in Cancer

Novel in-silico approaches to identify driver mutations in cancer genomics data

Ewelina Testoni
Signalling Networks in Cancer

Somatically mutated ABL1 is an actionable and essential lung cancer survival gene

Danielle Shaw
Clinical and Experimental Pharmacology

Magnetic resonance imaging biomarkers of angiogenesis and apoptosis in advanced cancer

EXTERNAL SEMINAR SPEAKERS 2016

The seminar series that we run is vital for the Institute, connecting world-class researchers across the broad spectrum of cancer research. We have enjoyed another successful year for scientific interactions with an excellent set of internationally renowned speakers visiting the Institute. The Breast Cancer Now Research Unit seminar series also continues to produce an outstanding range of speakers. The postdoctoral researchers at the Institute also give weekly seminars which are very well attended and help to integrate the entire cancer research efforts of the Institute.

Alberto Bardelli
University of Torino

Rachael Natrajan
The Institute of Cancer Research

Emmanuel Donnadieu
Institut Cochin

Xosé Bustelo
University of Salamanca

Norman Maitland
University of York

Linda Bauld
University of Stirling

Cédric Blanpain
Université Libre de Bruxelles

Simon McDade
Queen's University Belfast

Richard J. Gilbertson
Cambridge Cancer Centre

Sergio A. Quezada
UCL Cancer Institute

Richard Bayliss
University of Leeds

Dr Bissan Al-Lazikani
The Institute of Cancer Research

Sean Knight
Guy's and St Thomas' NHS Foundation Trust

Stefanie Jeffrey
Stanford University

Andrea Sottoriva
Institute of Cancer Research

Peter Campbell
Wellcome Trust Sanger Institute

Juan Carlos Acosta
The University of Edinburgh

Gabriel A. Rabinovich
Institute of Biology and Experimental Medicine (IBYME)

Mark O'Driscoll
University of Sussex

Nizar Batada
MRC Institute of Genetics and Molecular Medicine (IGMM)

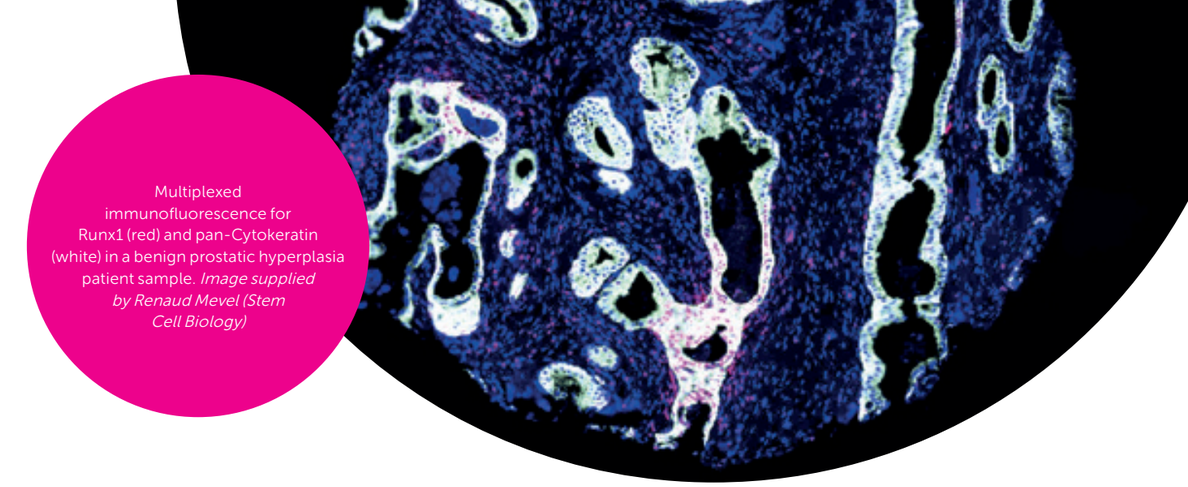
Arne Östman
Karolinska Institutet

Anne Ridley
King's College London

Roberto Buccione
EMBO Molecular Medicine

Carlos Garcia
The Francis Crick Institute

Katie Ridd
Nature Communications



Multiplexed immunofluorescence for Runx1 (red) and pan-Cytokeratin (white) in a benign prostatic hyperplasia patient sample. *Image supplied by Renaud Mevel (Stem Cell Biology)*

Kendle Maslowski
Riken

Lisa Belmont
Ideaya Biosciences

Bernd Pulverer
EMBO Press

Patricia Muller
MRC Toxicology Unit

Gerard Evan
University of Cambridge

Dirk Heerding
GlaxoSmithKline

Nicola Valeri
The Institute of Cancer Research

David Adams
The Wellcome Trust Sanger Institute

Ton Schumacher
Netherlands Cancer Institute

Julio Saez-Rodriguez
RWTH-Aachen University

Carla Martins
University of Cambridge

Jem Rashbass
Public Health England

David Carling
Imperial College London

Michael F. Olson
Cancer Research UK Beatson Institute

Bruno Silva-Santos
Institute of Molecular Medicine (iMM Lisboa)

Ewan Birney
The European Bioinformatics Institute (EMBL-EBI)

Karl Ekwall
Karolinska Institutet

William G. Kaelin
Dana-Farber Cancer Institute

Geert Kops
Hubrecht Institute

Leïla Perié
Institut Curie

Breast Cancer Now Seminars

Renée van Amerongen
University of Amsterdam

Simak Ali
Imperial College London

Jacco van Rheenen
Hubrecht Institute

Greg Hannon
Cancer Research UK Cambridge Institute

Fran Balkwill
Barts Cancer Institute

Anne-Lise Børresen-Dale
Oslo University Hospital

Clare Davies
Institute of Cancer and Genomic Sciences

Jack Cuzick
Wolfson Institute of Preventive Medicine

POSTGRADUATE EDUCATION

Postgraduate Education Manager

Julie Edwards

Postgraduate Tutor

Angeliki Malliri

Postgraduate Director and Chair of the Education Committee

Tim Somervaille

The Cancer Research UK Manchester Institute (CRUK MI) offers a range of graduate degrees for students interested in a career involving cancer research. The Institute considers education of both research and clinician scientists to be a major investment in the future of cancer research, and has an excellent track record of launching careers in basic, translational and clinical research.

As part of this commitment, we have an active postgraduate programme that provides students and clinical research fellows of outstanding potential the opportunity to study for a cancer-related PhD degree. This is achieved through a training programme that aims to improve effectiveness in research, provide professional and management skills and enhance career development. Our PhD students have exceptional employment prospects following graduation, with the great majority (>95%) continuing in academia, industry or healthcare, and securing positions in destinations across the UK, Europe and the USA.

In 2016, we welcomed ten graduate students and four clinical research fellows to our PhD programme, working in a variety of fields from RNA computational biology, clinical experimental pharmacology, leukaemia biology, molecular oncology through to stem cell biology. It was also particularly gratifying to see that, over the past twelve months, our students had published first author papers in a variety of journals including *Nature Medicine Leukemia*, *EMBO Molecular Medicine* and *Nature Communications*. During the course of this year, a total of four PhD students and three Clinical Research Fellows [CRF] were awarded their PhDs, a 100% success rate. From the seven graduates in 2016, two continued their careers in academia at the CRUK Manchester Institute in post-doctoral positions with one student moving to a scientific position in industry. Two CRFs returned to clinical training at The Christie NHS Foundation Trust and one successfully attained a highly prestigious Bloodwise Clinician Scientist award based at the CRUK Manchester Institute and The Christie NHS Foundation Trust.

The Cancer Research UK Manchester Graduate Programme

We aim for each student to receive high quality training in scientific research through an intellectually demanding but achievable research programme. Each project is peer-reviewed in advance and monitored throughout its course through a mixture of oral presentations, written reports, and progress meetings. These modes of assessment are designed not only to provide formal points at which progress (of both the student and the project) can be monitored, but also to help develop the presentation skills which are so fundamental to the majority of careers in science and elsewhere. Graduate training is monitored by the Education Committee, staffed by the Institute's Group Leaders and student representatives (see below). A main supervisor and a second or co-supervisor are nominated for each student, who are able to provide additional advice and consultation on both academic and non-academic matters. Each student is also assigned an advisor (similar to a personal tutor on an undergraduate programme) whose role is to provide impartial support and advice in a pastoral role. Further support is also available individually from the Director of Postgraduate Education, Postgraduate Tutor, Postgraduate Manager, or collectively as the Education Committee Administration Group.

The CRUK MI runs an external seminar series featuring talks from many of the key players in cancer research, and students are expected to attend. The speakers are internationally renowned scientists and we consider it essential that our students are exposed to outstanding research from leaders in different disciplines, which will give them a broad understanding of many aspects of cancer research and basic biology. In addition, we hold a series of weekly postdoctoral research seminars and attendance from PhD students is an integral part of the seminar programme. While students themselves are asked to give talks at key points during their PhD, they also have opportunities to present their work at lab meetings and during student forums within the Institute. STAy (short for Science TakeAway) is a group run by junior scientists in the CRUK Manchester Institute. Meetings are open to all early career scientists - PhD students, Postdocs and Scientific Officers from the Paterson Building and the Manchester Cancer Research Centre (MCRC) Building. The aim is to provide a forum for discussions and training related to research, communication of scientific engagement and development of social and networking opportunities. Topics in 2016 have included a careers panel and discussions around science communications.

The CRUK Manchester Institute Colloquium takes place annually in September, and is an excellent opportunity for our new intake of students to meet other established PhD students, members of the Institute, including Group Leaders, Postdoctoral Fellows, and Scientific Officers. This forum communicates up to date science in the form of oral presentations given by Group Leaders and second year PhD students, as well as poster presentations from a range of scientists across the Institute covering all aspects of cancer research. Poster prizes are awarded, including the Lizzy Hitchman Prize for the best poster presented by a PhD student or Clinical Fellow. In 2016 PhD student Emma Williams from the Leukaemia Biology group was the recipient of this prize. Emma's project has established that LSD1 inhibitors have the potential for treating other haematological disorders such as essential thrombocythemia.

Cancer Research UK contributes towards an annual International PhD Student Cancer Conference (IPSCC) allowing high calibre students (typically in 2nd & 3rd years) from top cancer research institutes across Europe to organise and present at their own scientific conference. Core participating Institutes include London Research Institute (LRI), Cambridge Institute (CI), Beatson Institute (BICR), Netherlands Cancer Institute (NKI), European School of Molecular Medicine, Milan (SEMM, IFOM & IFEO), and the German Cancer Research Centre (DKFZ).

In 2016, the 10th IPSCC was organised by PhD students from the Cambridge Institute and 10 students from the CRUK MI attended the conference in June. It is mandatory for participating student delegates to submit a poster to showcase their research either through a poster or oral presentation. Emma Williams from the Leukaemia Biology group, CRUK MI was selected to give an oral presentation and she was voted as the recipient for the prize of "best student oral presentation" during the conference.

PhD studentships

All of our CRUK core funded studentships are of four years' duration, and consist of an approved research project in one of our core funded research groups. Some students have joint supervisors in different groups, fostering important collaborations and providing exposure to different disciplines. Recruitment is highly competitive, with 300-500 applicants competing for around four-eight places each

year. Interviews are typically conducted annually over a two-day period in early January.

All of our students benefit from access to advanced state-of-the-art facilities, including advanced imaging, biological mass spectrometry, flow cytometry, histology and next generation sequencing. Our research groups offer PhD studentships and projects covering the entire breadth of research within the Institute.

Fellowships in Clinical Pharmacology Research

In order to help train the next generation of clinical pharmacologists with expertise in oncology, CRUK MI, in collaboration with the Manchester Cancer Research Centre (MCRC) and AstraZeneca, established in 2007 a fellowship scheme in clinical pharmacology research. The fellowships are open to applicants who have obtained, or are close to obtaining, their Certificate of Completion of Training (CCT) in Medical Oncology.

Each research fellow undertakes a four year PhD project, which provides training in biomarker discovery, method development/ validation, and clinical trial methodology. During their tenure at The Christie NHS Foundation Trust/CRUK MI, the post holders receive support from their clinical supervisor, and laboratory-based training from Clinical and Experimental Pharmacology (CEP) Group Leader Caroline Dive (in collaboration with MCRC colleagues). At AstraZeneca they receive training in clinical trials management, regulatory interaction, translational research through project management, and attend investigator meetings. Clinical training includes one research clinic per week, training in clinical trial design and methodology, ICH-GCP, EU Directives and research governance. Biomarker method development and application take place on both sites in all projects, with mutual benefit as each fellow brings newly acquired knowledge to each site. Regular meetings take place between the fellows, their supervisors, as well as other staff members involved in the project, ensuring effective collaboration and an integrated approach.

Education Committee 2016

The Education Committee (EC) acts for postgraduate students and consists of Group Leaders, the Chief Operating Officer, the Postgraduate Tutor and the Postgraduate Education Manager from the CRUK Manchester Institute.

Our goal is for every student to have a project that is both achievable and intellectually stimulating and demanding. Projects and students are monitored by the Education Committee which makes sure that the proposed plan of research is suitable, and that progress is made consistently throughout the course of the studentship. Various assessments throughout the studentship, including regular talks, progress meetings and written reports, are vital to ensuring successful completion and graduation for the PhD degree. Such assessments help not only to monitor progress, but also help to develop performance and presentations skills.

Education Committee Members

Tim Somervaille
Postgraduate Director & Chair, Education Committee
Angeliki Malliri
Postgraduate Tutor
Richard Marais
Ex-Officio Member
Wolfgang Breitwieser
John Brognard [until July]
Julie Edwards
Claus Jorgensen
Georges Lacaud [from November]
Donald Ogilvie
Jonathan Tugwood [from January]
Ian Waddell
Caroline Wilkinson

Student Representatives

Emma Williams [until October]
Amy McCarthy
Denys Holovanchuk [from October]

OPERATIONS

Chief Operating Officer
Caroline Wilkinson

Chief Laboratory Officer
Stuart Pepper

Head of Finance
Margaret Lowe

Head of Human Resources
Rachel Powell

The Operations Department provides the necessary infrastructure and services to facilitate the running of the Institute. Finance and purchasing, as well as Logistics, fall under the leadership of Margaret Lowe while Stuart Pepper oversees IT, Estates and Health and Safety; Rachel Powell is head of HR and Caroline Wilkinson is responsible for all aspects of Scientific Administration and acts as the primary point of operational contact within the Institute for both The University of Manchester and Cancer Research UK. This year, staff from across the Operations team have continued to help with arrangements to ensure that the Manchester Cancer Research Centre Building is fully operational and to assist with moving some of our research teams there. Other major projects included: the development of new online software tools for both our contribution review process and for PhD student applications, a major health and safety audit, and work towards our Athena SWAN application.

Director's Office and Administration Services

Ruth Perkins, Maria Belen Conti[1]

[1]joined in 2016

The office provides administrative support to the Director and to the Institute 's Group Leaders. In addition, the department has assisted with the organisation of several events over the course of the year, including the Institute Colloquium, the quinquennial review of the Director's research group and the first meeting of the Institute's Scientific Advisory Board. Administrative support is provided for the external seminar series, which has been a great success in 2016, hosting two seminars per week. The seminars serve to foster collaboration and encourage positive interaction within the wider scientific community. We aim to provide a varied programme of national and international speakers. Details can be found at www.cruk.manchester.ac.uk/seminars.

Estates

Steve Alcock, Graham Hooley, Steven Powell, Tony Woollam

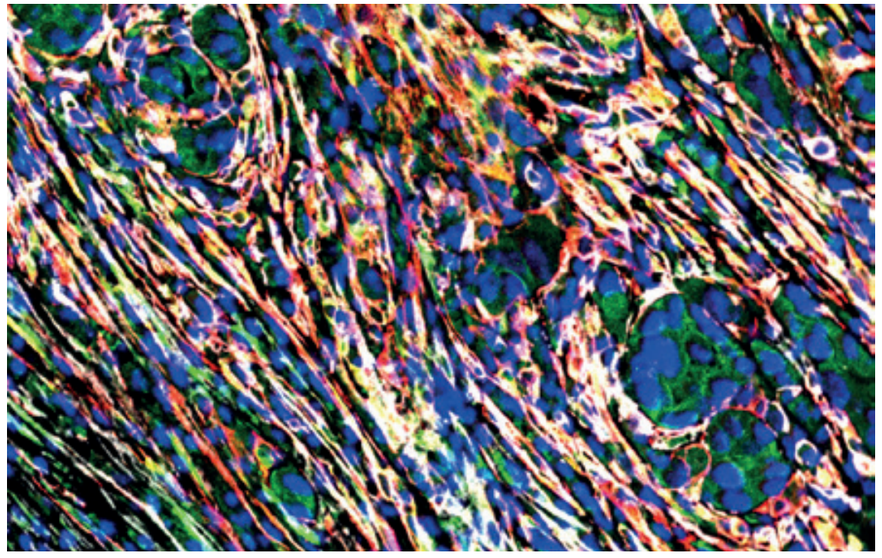
Our newest member Steve Powell has settled in well and is very enthusiastic about his role within the team. There have been many changes in the Paterson Building over the past year with groups moving around the Institute and some moving into the new MCRC Building across the road. These moves created a substantial amount of small works in the Paterson Building in order to prepare the areas for the new incumbents.

The Estates team have been pro-active with refurbishment of the main ventilation plants which were installed in 1992. Air Handling Unit 2 was refurbished earlier this year with AHUs 3, 4 and 6 being refurbished over the winter period. This will ensure their reliability for many years to come as well as being more sustainable, utilising more energy efficient fan motors and cooling equipment.

The reactive maintenance keeps the team busy as does responding to any fault reports from around the building. The team also adhere to many legislative requirements which include the monitoring of Legionella and its associated work to comply with regulations.

Estates also play a role in efforts to continually reduce the energy footprint of the Institute. There is a rolling program to replace traditional fluorescent strip-lights with more energy efficient LED lights. These newer lights use less energy but give better illumination to provide a brighter work environment.

Mouse pancreatic ductal adenocarcinoma stained with SMA (Red), LOX (Green), PDFR Beta (Grey) and DAPI (Blue). *Image supplied by Haoran Tang (Molecular Oncology)*



Steve Alcock, the Estates Manager, will be retiring in March 2017 after 20 years of service. Fortunately Steve's replacement will be in position prior to his departure to ensure a smooth transition.

Finance and Purchasing

Margaret Lowe, David Jenkins, Denise Owen, Muhammad Raja, Vikki Rosheski, Debbie Trunkfield

The Institute Finance team supports the Director with the management of the Institute's £25m budget, which is devolved to the various groups and service units. The team provides a comprehensive service to the Institute, which covers all areas of procurement and finance, ensuring we comply with the University financial regulations and procedures.

The Institute has been successful in securing several new external grant awards that were activated in 2016 or will be activated early 2017. We are also awaiting the outcome of several other applications. The team supports the research groups by providing effective and efficient professional advice when costing new research proposals. Group Leaders are encouraged to continue to apply for external funding to allow us to increase the breadth of research that we undertake.

The Institute receives funding from various different sources and we have the responsibility to ensure that funds are used for the purpose they are given. We monitor each individual award and provide financial information for the funders and principle investigators.

Health and Safety

Colin Gleeson

The Institute's health and safety management system was examined in 2015/2016 by means of an audit. The Health and Safety Management Audit Profile (HASMAP) system was implemented as it is designed specifically for the Higher Education sector. The audit was undertaken by people external to, and independent of, the Institute and assessed:

- Five themes relating to leadership and integration (stage 1). Evidence was gathered by email
- Nine themes (stage 2), focusing on personal protective equipment in the Drug Discovery Unit and the Cell Signalling group, and Manual Handling in the Logistics Department

The audit took several weeks and involved collating and supplying evidence of our

arrangements and staff attending interviews with the auditor. The Executive Report summary showed that we had performed very well in that we achieved nine "level 3" scores.

This was a pleasing outcome to the audit. A remedial action plan was developed and implemented to address the minor action points raised by this audit.

Compliance exercises including those of drug precursors, dangerous pathogens and toxins, Euratom materials and DEFRA materials were carried out. The building-wide data were fed back to the University Compliance Officer. Also, statutory checks were made of local exhaust ventilation and pressure vessels.

Work to facilitate the smooth running of the newly opened MCRC Building continued together with The University of Manchester's Technical Operations Manager for the Building, Peter Reid. The first inspections of the lab areas were also undertaken and reports generated. Likewise inspections were carried out in the Paterson Building, monitoring health and safety performance and taking remedial actions where appropriate. In 2017 we shall continue to embed our health and safety arrangements across both buildings.

Human Resources

Rachel Powell, Rachel Craven[1], Natalie Taylor[1], Laura Jones, Julie Jarratt, Emma Lloyd, David Stanier[2]

[1]joined in 2016 [2]joint with administration

Over the past year, the HR department has continued to deliver a high quality proactive service to the Institute and its staff. The department provides advice and guidance to managers and staff on all employment-related matters such as recruitment, policy guidance, legislation and best practice.

During 2016, 78 individuals were successfully appointed to enhance the work of the Institute. Throughout the year we have developed new advertising strategies for attracting candidates to the Institute; such as advertising on social media with the creation of short video clips. The current headcount of staff at the Institute has increased by 11.5% since 2015; from 280 to 312 staff at the end of 2016. This recruitment success highlights the continued growth and development of CRUK MI, which includes the new iDECIDE Clinical Trials Bioinformatics team.

The department administered the successful promotion of nine individuals and supported the retirement of four members of staff. We have continued our commitment to joint partnership, working with the union which has resulted in the revision of 17 HR policies and procedures, including the Staff Training and Development Policy, Recruitment and Selection Policy and the Career Path for Scientists Policy. During 2016, we also launched a new in house online performance review system which was a great success.

Currently we are working towards the Athena Swan accreditation. A self- assessment team has been established and is jointly chaired by Professor Caroline Dive and Dr Caroline Wilkinson. A staff survey will be sent out to staff and students in 2017 and we look forward to the outcomes that result from the work of this group and the benefits that it will bring to the Institute and its staff.

Next year, the focus will be on the recruitment of new research Group Leaders and their associated staff.

Information Technology

Steve Royle, **Matthew Young**, Hong Mach, Brian Poole

The CRUK Manchester Institute IT team provides a full catalogue of services including email, desktop support, file storage and mobile computing solutions. The CRUK MI network supports over five hundred staff across the Paterson and MCRC Buildings.

During 2016 the IT service underwent a detailed peer review process to look at how the service was performing. The report was very positive, commenting that user feedback was very good and that the services provided were appropriate for a modern research Institute.

2016 has been a year of consolidation. We have migrated and developed core services on our new server farm and network infrastructure. We shall continue to build and provide new services on this platform going forward, to provide our scientists with the high performance, high availability, fault tolerant IT services and resources needed to support their research.

Throughout 2016, the MCRC Building has seen a steady influx of new research groups. We have completed the desktop deployment and network connectivity to accommodate these new groups and the MCRC Building, which is now almost fully populated, is integrated into our support activities. The network link between the MCRC and the Paterson Buildings has been upgraded to dual, redundant, 10G fibre optic links. Also, the site internet connection via The University of Manchester campus network has also been upgraded to 10G.

The number of iOS devices used at the Institute has continued to rise, reflecting a broad trend in many institutes and businesses. To enhance our management and support provision for these products new enterprise class software has been purchased that will provide greater control and flexibility. This will provide us with a management tool we can use to deploy and manage all Apple devices to ensure they are all optimally configured and running supported software versions.

Looking ahead to 2017, planning has commenced for significant IT storage and security infrastructure upgrades to replace end of life equipment. This will include a refresh of the IT storage along with two major initiatives to strengthen our defence against cyber threats, including installation of a new next generation firewall and enhanced protection against email borne spam, viruses and malware.

Logistics

Andrew Lloyd, Michael Alcock, Edward Fitzroy, Sedia Fofana, Stephen Keane, Jonathan Lloyd, Robin Sherratt[1] and William Glover[1]

[1]joined in 2016

The past year has seen changes within the team's structure with the arrival of Robin Sherratt and William Glover. The team has expanded to cope with the increased demand on the service as it now also supports the MCRC Building. Since October there has been a member of the logistics staff based there offering goods in service and running of small stores.

The team has continued to deliver an efficient and effective service providing support for the research carried out in the Paterson Building and MCRC Building. This includes the receipting, checking, booking in and distribution of goods ordered by staff as well as the collection and removal of waste. We currently recycle all the Institute's waste cardboard, plastic bottles, tin cans, wooden crates and pallets, ink toners, and scrap metal which reduces the amount of waste going to landfill.

The Logistics team provides both buildings with liquid nitrogen collection and refill service three days a week, and a dry ice service with deliveries taking place twice a week. Gas cylinders are also monitored and replaced as necessary.

Researchers can order central stores stock items via the intranet, which can be collected or distributed by the Logistics team. We currently stock over 100 stores items from tissue culture essentials to cleaning products. Included in this system are the enzymes and media stored in the Institute freezers (Sigma, Invitrogen, Roche, Promega, New England Bio labs, Bio-Rad, Fisher kits and Qiagen). We now stock over 240 products, which are under continual review to meet the needs of users. We have been able to make savings by buying in bulk from suppliers. We have set up numerous "call off orders" for stores items, again making significant savings but also guaranteeing a stable stock.

The team also provides assistance with moving heavy equipment or furniture helping facilitate internal rearrangements and the arrival of new groups. The last few months have been especially busy as the team has helped with the relocation of multiple groups around the site. The team is also involved with the reconfiguration of meeting rooms for numerous events being held at the Institute. In addition, the team looks after the Institute's "Shred It" service and water coolers.

Scientific Operations and General Administration

Caroline Wilkinson, Tom Bolton[1], Gillian Campbell, Julie Edwards, Steve Morgan, David Stanier[2]

[1]joint with MCRC, [2]joint with HR

Scientific administration is overseen by the Chief Operating Officer, Caroline Wilkinson, who provides support to the Director in order to facilitate the day-to-day running of the Institute. The team is also responsible for producing a variety of scientific communications for the Institute including publications such as the Annual Scientific Report, the Institute's Newsletter, writing material for the intranet and external website and for the Institute's social media presence.

Gill Campbell is the Institute's Grants Advisor who helps our researchers also apply for external awards to extend the portfolio of research that we can undertake. This year, a Grants Committee has been established, chaired by Iain Hagan, to help our scientists prepare the strongest possible applications and ensure that there is a rigorous internal peer review process. This year we supported successful applications to a variety of organisations including the Wellcome Trust, the Rosetrees Trust and EMBO.

During the year, we produced our own software tools for our annual performance appraisal system and for our PhD student recruitment. Both were introduced successfully and have led to these processes being considerably streamlined. The team has worked on producing a new external website for the Institute which will be launched in 2017.

Animal Welfare

Simon Poucher, Regulatory Liaison and Training Officer, **Janet Watson** (AWERB Chair)

The welfare of mice that are used in experiments at the Institute is critical, not only for the animals, but also because using mice that are well cared for gives better quality of data. One of the driving forces supporting all staff on animal welfare is the Animal Welfare & Ethics Review Body (AWERB). This consists of experienced animal husbandry staff, a veterinary surgeon, Institute scientists, a statistician and lay members. We have an annual plan that covers areas of enhancement of animal facilities, care and husbandry of mice, encouraging implementation of the 3Rs (Replacement, Reduction, Refinement of animal use), reviewing animal ethics of collaborations and grant applications from Institute scientists, as well as management of compliance to the Animal Scientific Procedures Act and encouraging public engagement.

In 2016, our AWERB met formally on six occasions and reviewed four new project licence applications and seven amendments to the 21 project licences held at the Institute, before submission for review and granting by the Home Office. Additionally, we organise two annual meetings for project and personal licence holders where the success and best practice of working with mice was shared, along with the latest news on animal welfare practices and legislation. These meetings are also attended by our Home Office Inspector. We hold an annual 3Rs' Prize day and in 2016 received thirteen entries describing ways in which procedures undertaken on our mice can be replaced, reduced or refined.

A total of 25,252 mice were used and reported on regulated procedures in 2016, which included 8,800 on experimental procedures. 1,788 mice were used for the creation of new strains of mice and 905 were used for tissue in vitro studies. The remainder were used for the maintenance and breeding of mice used in procedures. We are proud of the quality of animal welfare at the Institute and members regularly talk to the public about our use of mice in cancer research. In December 2016, the Institute was recognised at a national event organised by Understanding Animal Research (UAR) on the Concordat on Openness with the "Award for Public Engagement".

Cancer Research Technology

Martyn Bottomley

Cancer Research Technology (CRT) is a specialist oncology-focused development and commercialisation company which has recently been integrated into Cancer Research UK's Research and Innovation Directorate. CRT aims to maximise patient benefit from CRUK-funded research worldwide by advancing research discoveries into development with pharmaceutical and biotechnology parties. We aim to bridge the gap between cutting edge academic research and industrial development of cancer therapeutics and diagnostics. We achieve this by working closely with prestigious international research institutes, such as the Cancer Research UK Manchester Institute and funding bodies to develop, protect and commercialise oncology-related discoveries. Core activities of business development and drug discovery are supported by specialists, integrated in the business with expertise in patents, legal, finance and marketing.

Our exclusive focus on oncology provides an unrivalled depth of knowledge and experience in cancer-specific translational development and commercialisation. We also have access to the CRT Pioneer Fund; this £70m fund has been established with Cancer Research Technology, the European Investment Fund (EIF) and Battle Against Cancer Investment Trust (BACIT) to bridge the investment gap between cancer drug discovery and early development. It will take potential cancer drugs, primarily discovered by Cancer Research UK, from discovery through to entry to Phase II clinical trials before partnering with pharmaceutical and biotechnology companies.

By arrangement with The University of Manchester, CRT owns and is responsible for the development and commercialisation of intellectual property arising from Cancer Research UK funded research at The University of Manchester (including the Cancer Research UK Manchester Institute). To effectively facilitate this, Martyn Bottomley, a CRT Business Manager is based on-site at the Cancer Research UK Manchester Institute and is dedicated to working closely with the staff at the Institute and also The University of Manchester. Martyn offers access to oncology-focused expertise in technology evaluations, patent applications and management, funding for development, commercialisation, drug discovery, market intelligence, and project management. He also works closely with UMIP, The University of Manchester technology transfer organisation.

CRT continues to work very closely with the Drug Discovery Unit based at the Cancer Research UK Manchester Institute to facilitate the development of small molecule drug therapies to satisfy the unmet clinical needs of cancer patients. This includes management of collaborations with Pharmaceutical partners such as Genentech, GSK, HitGen and AstraZeneca and also the filing and management of a number of patent applications for the Drug Discovery Unit to protect novel compounds resulting from their research. This year has seen the first pre-clinical candidate nomination from the DDU on the RET project and also transfer of the lead series from the collaboration with GSK to them for further development. CRT is also currently actively managing a broad portfolio of development programmes and exciting licensing opportunities originating from the Cancer Research UK Manchester Institute that continue to attract commercial partners. This includes negotiation and completion of a significant number of agreements between the Clinical Experimental Pharmacology group and commercial collaborators. We look forward to building on our successes and continuing to work closely with the Cancer Research UK Manchester Institute to advance discoveries to beat cancer in the years ahead.

Manchester City Council leader Sir Richard Leese getting hands on in the MBCF lab

CANCER RESEARCH UK'S RESEARCH ENGAGEMENT

Cancer Research UK's Research Engagement Manager

Sive Finlay

Public engagement featured largely across the Institute again in 2016. We've had a busy year of welcoming visitors to CRUK MI, bringing our science to life at events across the city and getting involved with our own fundraising.

Events at the Institute attracted over 1,500 people interested in getting a 'behind the scenes' insight into our work. Our regular programme of lab tours (over 50 this year) gave our donors, fundraisers, volunteers, ambassadors and corporate partners the opportunity to see the work that their support helps to fund. Tour highlights include the visit from the Sandbach and District Local Committee to celebrate their 60th year of fundraising for CRUK, engaging local councillors and MPs with our lung cancer research and a special visit from Loose Change Buskers after they received the Queen's Award for Voluntary Services having raised over £260,000 for the charity.

Our new research café series gives staff, patients and visitors an opportunity to find out more about our work through short, informal talks in the MCRC Building café (Café Vivo). Launched on World Cancer Day in February, the talks have covered a wide range of topics including basic research, pharmacy's role in clinical trials, insights into the role of a clinical scientist, patient involvement with research and an update on the forthcoming proton beam therapy centre at The Christie NHS Foundation Trust.

We held two successful open days in June and November which offered talks, tours and activities to engage both CRUK supporters and local members of the public with our work. Thanks to Café Vivo for their special opening arrangements and to all of our volunteers who helped to make the days so successful.

Outside the Institute, we took part in a range of activities and events to raise awareness of CRUK's important role within Manchester's vibrant research community.

Loose Change Buskers celebrating their Queen's Award for Voluntary Services

We celebrated International Clinical Trials Week in May with our Platform for Investigation day at the Museum of Science and Industry. This was our first solo event at the museum, taking over the ground floor with a range of hands-on activities that attracted just over 800 visitors. Highlights include new activities led by The Christie NHS Foundation Trust Radiotherapy Physics group, clinical trials activities with local research nurses and animal research activities which won the public engagement prize at the Understanding Animal Research Openness Awards in London towards the end of the year.

The European City of Science Festival in July celebrated Manchester's year as Europe's science capital. We had a busy week of activities which included a science lates evening at Manchester Museum, activities for the European Commission's "Science is a revolution" fair at the Museum of Science and Industry and taking part in soapbox science at Piccadilly Gardens. Our virtual reality lab tour also proved to be a highlight at The University of Manchester's stand at the festival's flagship Euroscience Open Forum conference.

Our main event for the festival was the week-long Fabric of Research Exhibition at Manchester Craft and Design Centre. Textile designers from The University of Manchester and Manchester Metropolitan University worked with researchers and patient volunteers to create unique artworks that showcase our latest research and tell the personal stories of people affected by cancer. Particular thanks to Steve Bagley for providing a wealth of images and expertise and to everyone who helped bring this project to life.

In September, we took part in lightning talks and science speed dating sessions at Manchester Museum's European Research Night showcase, a fun evening filled with hands-on activities where our virtual reality lab tour proved to be a hit. And we rounded off October with our Manchester Science Festival events including Your Choice (an interactive board game about the challenges of research) and our "Cell Explorers" stand at The University of Manchester's Science Spectacular.

As well as engaging people with our research, staff at the Institute continue to be generous fundraisers for the charity through a variety of events. We donned our unity bands in February to celebrate World Cancer Day and enjoyed a sumptuous spread from the lovely people at Warburtons for Britain's Biggest Breakfast in March. Our staff team for the Stockport Relay for Life held events throughout the year to raise a fantastic £2,757.86 to contribute to the

Patient representative Jo Taylor sharing her experience of getting involved with research at our research café series

Visitors learning about clinical trials at our June open day

impressive July event total of £84,617, all of which will go directly to fund work at CRUK MI. In October, a team from Drug Discovery took on the Bear Grylls survival race at Tatton Park to raise £1,780 for Stand Up to Cancer while our fundraising fete at the MCRC Building offered plenty of tasty treats and unusual prizes in aid of the national campaign. We rounded off the year with Institute fundraising for Movember including ever popular bake sales and raffles from David Jenkins, special bootcamp yoga sessions led by Lisa Waters and a host of other activities that raised over £1,400 and got everyone into the Mo-spirit!

It's been a great year with many more exciting plans to come. Thank you to everyone across the Institute who is so generous with their time, energy and enthusiasm for supporting our many activities!

Clockwise from top left: Elaine Mason and Nathalie Dhomen at the clinical trials and animal research activity stand for our June open day; The team behind our Understanding Animal Research public engagement prize; Research-inspired scarves at the Fabric of Research exhibition; Young visitors at Stockport Relay enjoying a virtual reality tour of our lab; Our inaugural pumpkin carving competition in aid of Stand Up to Cancer; David Jenkins – fundraiser extraordinaire!

ACKNOWLEDGEMENT FOR FUNDING FOR THE CANCER RESEARCH UK MANCHESTER INSTITUTE

The total funding of the CRUK Manchester Institute for 2016 was £25m. The major source of this funding was awarded by Cancer Research UK via a core grant of £12.2m plus additional strategic funding of £4.8m. This funding enables the various scientific groups and service units within the Institute to carry out their research.

The infrastructure of the CRUK Manchester Institute is funded by HEFCE generated income at a cost of £2.1m.

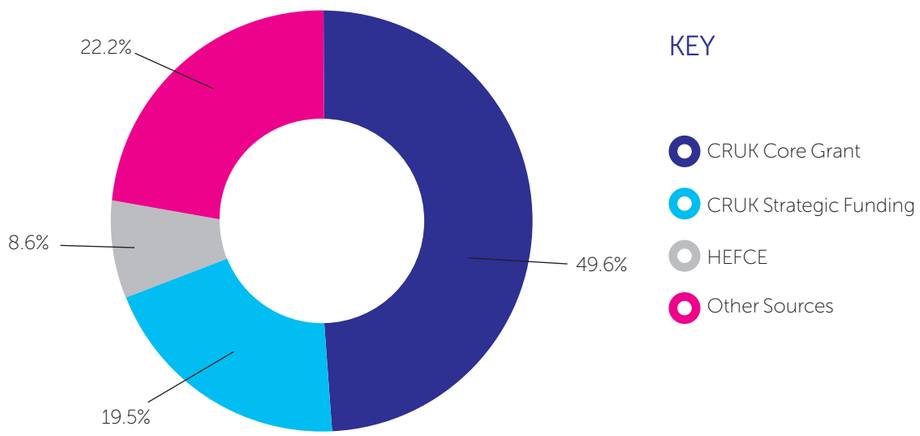
The balance of the Institute's funding is received from a number of additional sources. The research carried out through these additional projects enhances and supports the research undertaken by the core funding.

These sources are as follows:

- Angle Inc
- Astex Pharmaceuticals
- Astra Zeneca
- BBSRC
- Bloodwise
- British Lung Foundation
- Christie Hospital NHS Foundation Trust
- Clearbridge Biomedicals
- CRT Pioneer Fund
- European Commission
- European Research Council
- GlaxoSmithKline
- John Swallow Fellowship
- Kay Kendall Leukaemia Fund
- Leo Pharma Foundation
- Lung Cancer Research Foundation
- Medical Research Council
- Menarini Biomarkers Singapore
- Moulton Charitable Trust
- Pancreatic Cancer Research Fund
- Prostate Cancer UK
- Rosetrees Trust
- Roy Castle Lung Cancer Foundation
- The US Department of Health and Human Services
- Wellcome Trust
- Worldwide Cancer Research

We are immensely grateful to all our sponsors.

CRUK MANCHESTER INSTITUTE FUNDING 2016



CAREER OPPORTUNITIES AT THE CANCER RESEARCH UK MANCHESTER INSTITUTE

The Cancer Research UK Manchester Institute is located alongside The Christie NHS Foundation Trust, and has a strong programme of basic and translational research. There are close links with clinical and translational research groups throughout the Christie Hospital site.

The Institute offers excellent laboratory facilities and outstanding core facilities, including molecular biology services, next generation sequencing, real-time PCR, mass spectrometry, flow cytometry, histology, advanced imaging, and a biological resources unit. Details of all groups and facilities are given in this report, and can guide interested parties to the appropriate contacts.

Opportunities exist at a number of levels in the Institute. We have a well-established programme of degrees by research which is described in the section on Postgraduate Education. We encourage applications from suitably qualified graduates to apply to join either the PhD or MD programmes. Graduates with a first or 2.1 honours degree in a biological science can apply each year to train for a four-year PhD in one of our research laboratories. The University of Manchester offers a wide range of training for new and existing students which provides opportunities to acquire skills that will complement the research programme and help achieve personal and career development goals. At the Institute, we also ensure that postgraduate students are provided with high quality, relevant and appropriate training alongside development opportunities. The Institute also has a well-developed process for ensuring excellent pastoral care and mentoring for all students.

Postdoctoral applicants of high calibre are regularly sought. Although Postdoctoral Fellows will be encouraged to apply for their own fellowships, funded positions are available for outstanding candidates. Interested applicants should contact the Group Leaders directly, with details of their research interests and recent experience.

In addition to postgraduate and postdoctoral opportunities, the Institute is still seeking to recruit outstanding candidates to the positions of Junior and Senior Group Leaders. The packages provided are extremely attractive and commensurate with the experience of the applicant, with significant funding for personnel, recurrent expenditure and equipment. Junior Group Leaders are appointed for an initial six-year period with a review between five and six years for consideration of promotion to Senior Group Leader, with Senior Group Leaders appointed to non-time limited positions.

Specific vacancies can be found on our web pages (http://www.cruk.manchester.ac.uk/Jobs/), but suitably qualified and enthusiastic individuals should contact the Institute at any time to enquire about career possibilities.

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