

# SCIENTIFIC REPORT 2015

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**Cover image**

A high content screening investigation to examine co-culture of how tumour cells influence fibroblasts (a-SMA fibres stained red). Images captured using a x40 water objective lens on the PerkinElmer Opera Phenix.

*Image supplied by Amy McCarthy (Systems Oncology group).*

# SCIENTIFIC REPORT 2015

## MANCHESTER INSTITUTE

# CONTENTS



The Cancer Research UK Manchester Institute is located across The Paterson Building and The Manchester Cancer Research Centre Building



The Manchester Cancer Research Centre Building

## SECTION 1 DIRECTOR'S INTRODUCTION RESEARCH HIGHLIGHTS 2015

04  
07

### CANCER RESEARCH UK MANCHESTER INSTITUTE

#### RESEARCH GROUPS

Santiago Zelenay Cancer Inflammation and Immunity	12
Iain Hagan Cell Division	14
Nic Jones Cell Regulation	16
Angeliki Malliri Cell Signalling	18
Caroline Dive Clinical and Experimental Pharmacology	20
Donald Ogilvie Drug Discovery	24
Tim Somervaille Leukaemia Biology	26
Richard Marais Molecular Oncology	28
Esther Baena Prostate Oncobiology	30
Crispin Miller RNA Biology	32
John Brognard Signalling Networks in Cancer	34
Georges Lacaud Stem Cell Biology	36
Valerie Kouskoff Stem Cell and Haematopoiesis	38
Claus Jorgensen Systems Oncology	40
Michela Garofalo Transcriptional Networks in Lung Cancer	42

## SECTION 2 RESEARCH SERVICES

Steve Bagley Advanced Imaging and Flow Cytometry Facilities	46
Duncan Smith Biological Mass Spectrometry Facility	47
Kim Acton Biological Resources Unit Transgenic Breeding	48
Lisa Doar Biological Resources Unit Experimental Services	49
Natalia Moncaut Transgenic Production Facility	49
Garry Ashton Histology	49
Mark Craven Laboratory Services	50
Wolfgang Breitwieser Molecular Biology Core Facility	51
Wei Xing Scientific Computing	52

## SECTION 3

RESEARCH PUBLICATIONS	54
THESES	62
SEMINAR SERIES 2015	64
POSTGRADUATE EDUCATION	66
OPERATIONS	70
CANCER RESEARCH UK'S LOCAL ENGAGEMENT AND DEVELOPMENT	76
ACKNOWLEDGEMENT FOR FUNDING OF THE CANCER RESEARCH UK MANCHESTER INSTITUTE	79
CAREER OPPORTUNITIES AT THE CANCER RESEARCH UK MANCHESTER INSTITUTE	80
CONTACT DETAILS	80

## DIRECTOR'S INTRODUCTION



**Professor Richard Marais**  
Director of the Cancer  
Research UK Manchester  
Institute

The past year has been particularly successful for the Institute. Highlights in 2015 included the arrival of a new Junior Group Leader, Santiago Zelenay, the award of Cancer Research UK's Future Leaders' Prize to John Brognard, and the opening of the Manchester Cancer Research Centre Building.

Santiago Zelenay joined the Institute as a Junior Group Leader in August. He is establishing the Cancer Inflammation and Immunity group which will examine the balance between the tumour-promoting and tumour-suppressing properties of the immune system. Santiago joined the Institute after a very successful period of post-doctoral research at The Francis Crick Institute with Caetano Reis e Sousa where he discovered how cancer cells can dampen the immune response. We look forward to seeing him establish his own research group in this particularly exciting area of cancer research.

Our major research highlights in 2015 included the identification of a novel phosphatase relay controlling mitotic progression; uncovering a role for the mesenchymal transcription factor FOXC1 in the development of acute myeloid leukaemia; an analysis of protein kinase C mutations which revealed a surprising role for this kinase as a tumour suppressor; the development of panRAF inhibitors effective in drug-resistant BRAF mutant melanoma; and further technical developments in our efforts to profile patients' tumours from circulating tumour cells and circulating free DNA. Significant progress has also been made by our Drug Discovery Unit which has two projects in the lead optimisation phase including compounds against the product of the RET oncogene and the PARG enzyme.

During the summer, Angeliki Malliri and her Cell Signalling group underwent a successful quinquennial review of their research programme and have recently secured external funding from Worldwide Cancer Research to help progress one of their studies. Other successful external applications have been made to the European Research Council, the Prostate Cancer Research Fund, and the

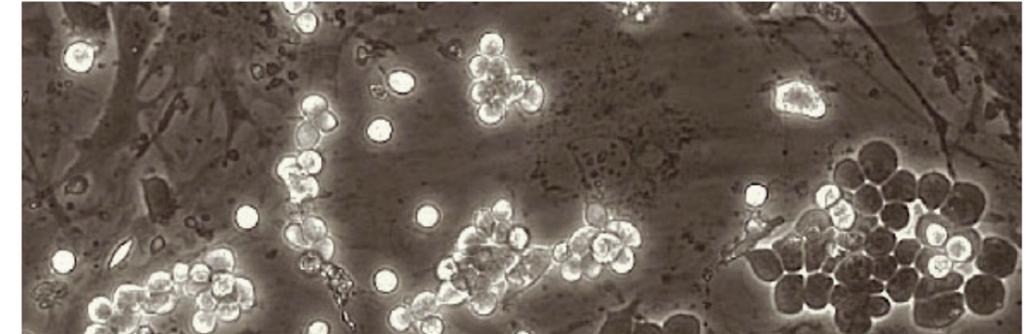
Lung Cancer Research Foundation, while two of our Clinician Scientists, Amaya Viros and Dan Wiseman, have been awarded fellowships from the Wellcome Trust and Bloodwise respectively. Overall, we are now supplementing our core Cancer Research UK funding by over £4.6m which allows us to significantly expand the breadth of research that we undertake.

It has been especially pleasing to see the success of our scientists recognised with a number of prestigious prizes and awards. Two of these were presented at The National Cancer Research Institute annual meeting in Liverpool. First, one of our Junior Group Leaders, John Brognard was awarded the Cancer Research UK Future Leaders' prize. This well-deserved honour reflects the significant progress that John and his Signalling Networks in Cancer team have achieved during their first five years at the Institute. The second award went to Romina Girotti from the Molecular Oncology group who was presented with the British Association for Cancer Research Translational Research Prize, which recognises the contributions made by young scientists to translational cancer research. Caroline Dive was elected Fellow of the Academy of Medical Sciences as well as being awarded a Breakthrough Prize from Manchester City Council to celebrate International Women's Day. Nic Jones was elected to honorary membership of the Royal College of Physicians and I was elected to the Academia Europaea. Such external recognition is highly rewarding and in total, 25 prizes and awards were received by members of the Institute during the year.

Our PhD students have also enjoyed a great deal of success. Marija Maric was awarded the Pontecorvo Prize for the best Cancer Research UK funded PhD thesis. Tim Somerville was

Generation of haematopoietic progenitors cells from reprogrammed fibroblasts.

*Image supplied by Kiran Batta (Stem Cell Biology)*



awarded The University of Manchester Doctoral College Best Outstanding Output Award for his work on acute myeloid leukaemia, which resulted in a first author publication in the prestigious journal *Cancer Cell*. He was also the recipient of the Institute's Dexter Prize for the best young scientist of the year. During the summer, our students organised and hosted the International PhD Student Cancer Conference which is aimed at promoting scientific discussion and networking amongst students across Europe. The event provided them with an excellent opportunity to develop additional skills and was a great success. It has also been exciting to see that the training and experience that our students receive at the Institute facilitates them securing post-doctoral positions at other prestigious institutions around the world, which this year has included the Cold Spring Harbor Laboratories, the Dana-Faber Cancer Institute and the Francis Crick Institute.

Another successful meeting that was held in Manchester was organised by the CRUK Lung Cancer Centre of Excellence. The Centre is based jointly at the University of Manchester and University College London and involves a number of the Institute's scientists. This first conference hosted by the Centre provided a tremendous opportunity for experts from around the world to discuss the latest advances in lung cancer research and develop new ideas and collaborations.

The Manchester Cancer Research Centre (MCRC) Building was opened in the summer and was marked by a number of events which provided an excellent opportunity to thank many of the donors and fundraisers who had

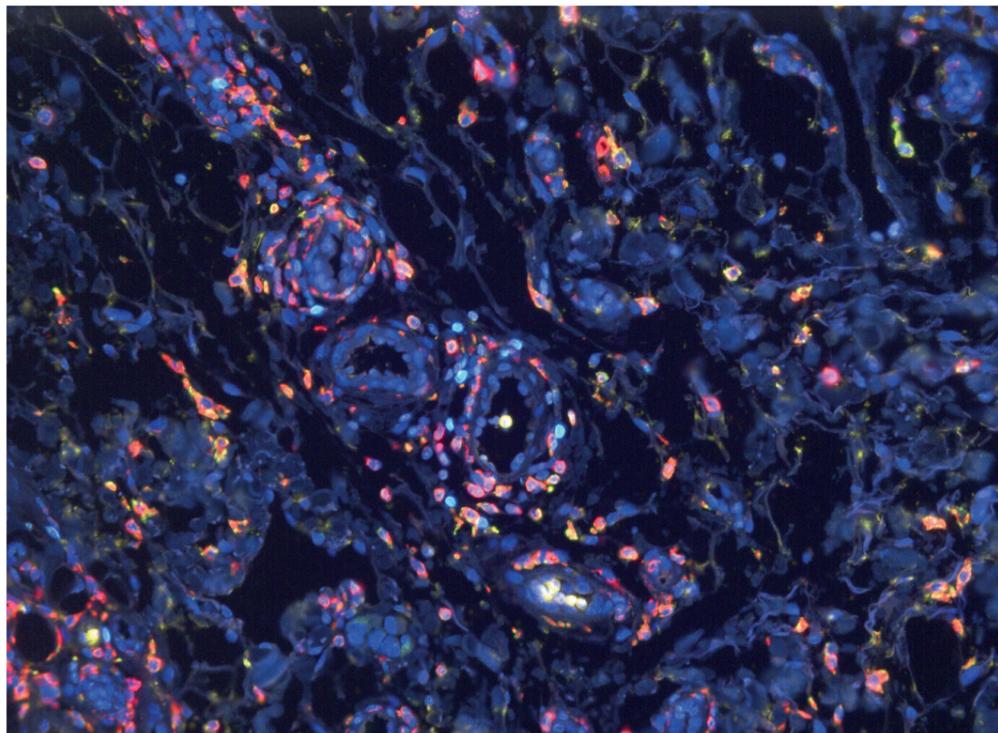
contributed to the project. We also welcomed members of the general public to look around this fantastic space and find out more about the research which will be carried out there. One of the first scientific meetings to be hosted in the building was the MCRC Autumn School. This event, which involved many of our scientists, invited young researchers from other CRUK centres to Manchester to learn more about how we are working towards implementing personalised medicine for cancer patients. Several of our research groups have moved across to the new building which is providing us with some much needed room for expansion. Many of our operational staff worked hard throughout the year to help ensure that the building was ready for occupation.

In order to support the Institute's expanding portfolio of research, we established two new core research services. The first is a transgenic production facility that will provide a complete service from experimental design to generation and cryogenic storage of transgenic lines and we welcomed Natalia Moncaut who is leading the team. The second facility will allow us to perform small molecule and RNAi screening which will aid our ambitions to accelerate the translation of our research. Ian Waddell from the Drug Discovery Unit is taking the lead on experimental design for this activity.

Engaging with the supporters who fund our research is incredibly important and the past year has seen us develop some new and innovative ways to communicate the progress that we are making. This year we welcomed Sive Finlay as our new Research Engagement Manager. Sive replaced Hannah Leaton who had

Tumour stroma with all cell nuclei blue and macrophages stained red/green.

Image supplied by Gabriela Gremel (Molecular Oncology)



spent a very successful 18 months in the role. Between them, Sive and Hannah helped us to host over 1,250 visitors to the Institute during 2015. Over 50 researchers were involved in our two open days and also supported a week-long programme of activities to celebrate the opening of the MCRC Building in June. The Institute hosted a very popular stand explaining our work on circulating tumour cells at the prestigious Royal Society Summer of Science exhibition. Combined with our participation in the Manchester Science Festival and support of CRUK events, we engaged with over 12,000 people at more than 50 external events in 2015. Our online and social media presence continues to grow with a popular video to accompany our Royal Society Summer of Science exhibition and a new virtual reality lab tour allowing even more supporters to engage with our work.

During 2016, we shall continue with further recruitment, particularly in our priority area of Molecular Pathology, while consolidating and continuing to develop our other key activities. This includes working with our partners in UCL and Belfast to develop our Lung and Prostate Centres of Excellence respectively. The European Association for Cancer Research (EACR) biannual meeting will take place in Manchester during the summer which coincides with my tenure as President of the EACR. The coming year also marks

Manchester's tenure as European City of Science. This festival offers some excellent opportunities for engaging the general public with our research and we look forward to getting involved in a variety of ways. Later on in the year, CRUK MI's Deputy Director Caroline Dive will chair the NCRI meeting in Liverpool. We shall continue to work with colleagues in the CRUK Manchester Centre and the MCRC over plans to develop the MCRC Building Phase 2 which will culminate in a new research facility that will house the National Centre for Biomarker Sciences, an initiative that will further develop the comprehensive range of cancer research and treatment available in Manchester. It promises to be an exciting year.

In this section we highlight some research publications from 2015 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.

Grallert A<sup>§</sup>, Boke E<sup>§</sup>, Hagting A, Hodgson B, Connolly Y, Griffiths JR, Smith DL, Pines J, Hagan IM. <sup>§</sup>equal contribution  
A PP1-PP2A phosphatase relay controls mitotic progression.  
*Nature*, 2015; 517:94-98.

Many regulatory pathways that go wrong in cancer involve cycles of phosphorylation in signal transduction cascades in which the phosphorylating activity of protein kinases is counteracted by specific phosphatases that remove the phosphate that has been put on substrates by kinases. Two phosphatases, protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A), account for over 90% of the phosphatase activity towards phosphorylated serine and threonine residues in human cells. As they have different partners and different substrates, their activities have been assumed to be independent of one another. We found an unanticipated link between PP1 and two isoforms of PP2A, PP2A-B55 and PP2A-B56. All three phosphatase activities are suppressed as cells enter the genome segregation phase of the cell division cycle, namely mitosis. PP1 repression is via direct phosphorylation from the mitosis-promoting Cdk1-Cyclin B complex. Once Cdk1-Cyclin B activity declines, PP1 auto-catalytically removes the phosphate Cdk1-Cyclin B placed on it. This newly activated PP1 binds to and re-activates PP2A-B55, which can then dephosphorylate a site within the PP1 docking site of PP2A-B56 to enable PP1 to bind and reactivate this second PP2A isoform. Thus, PP1 acts as a master controller in a phosphatase relay to control the de-phosphorylation events that are required to drive cells out of mitosis.

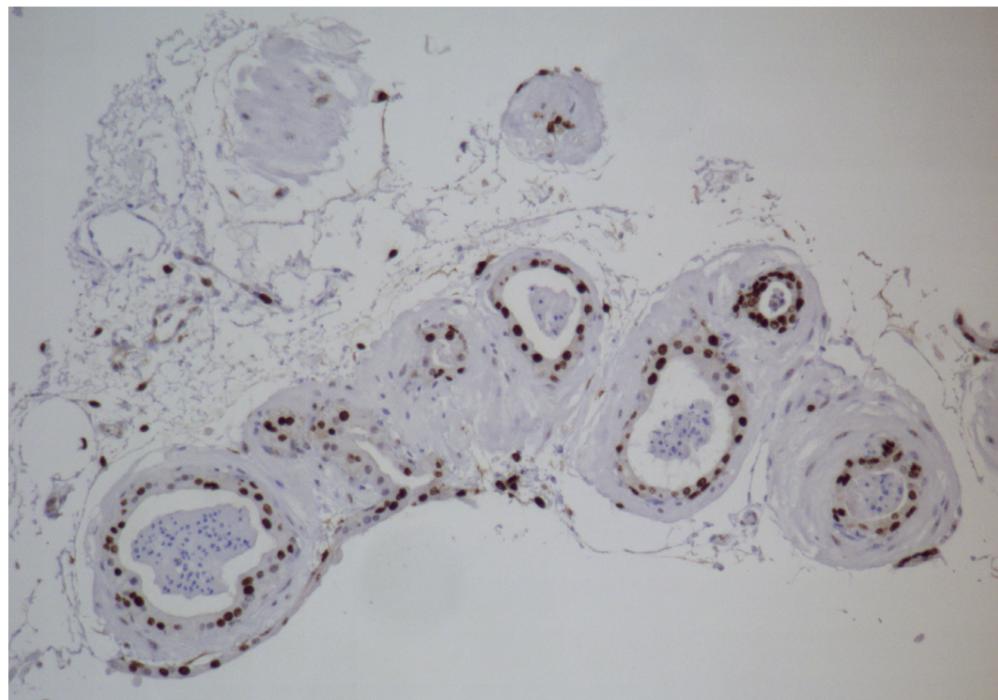
Somerville TD, Wiseman DH, Spencer GJ, Huang X, Lynch JT, Leong HS, Williams EL, Cheesman E, Somervaille TC.

Frequent derepression of the mesenchymal transcription factor gene *FOXC1* in acute myeloid leukemia.  
*Cancer Cell*, 2015; 28(3):329-42.

Acute myeloid leukaemia (AML) is a blood cancer characterised by accumulation in the blood and bone marrow of myeloid lineage blast cells that no longer undergo normal differentiation. Understanding the mechanisms that regulate leukaemia stem and progenitor cells by comparison with their normal cellular counterparts is a promising approach to developing targeted therapies for AML. Through bioinformatics analyses we identified the mesenchymal transcription factor gene *FOXC1* as among the most highly up-regulated transcriptional regulators in primary human AML stem and progenitor cells when compared to normal blood forming stem cells. Indeed, *FOXC1* is expressed at high level in approximately 20% of human AML patient samples, almost invariably in association with concomitant high level *HOXA/B* gene expression, but is not expressed in normal haematopoietic cells. Although *FOXC1* has previously been functionally implicated in solid malignancies, predominately due to its ability to promote epithelial-to-mesenchymal transition as well as invasion and metastasis, a pathogenic role in haematopoietic tissue had not been described. Surprisingly, functional experiments demonstrated that *FOXC1* contributes to the myeloid differentiation block and collaborates with *HOXA9* to accelerate significantly the onset of symptomatic leukaemia. Moreover, *FOXC1*<sup>high</sup> human AML cases exhibited reduced morphologic monocytic differentiation and inferior survival. Thus, our investigations highlight a hitherto unappreciated but frequent pathogenic mechanism in human AML: the tissue-inappropriate derepression of the mesenchymal transcription factor gene *FOXC1*

Ki67 staining to assess proliferation in mouse prostate tissue.

Image supplied by Valentina Ubertini (Prostate Oncobiology)



with unexpected functional consequences and prognostic significance.

Marusiak AA, Stephenson NL, Baik H, Trotter EW, Li Y, Blyth K, Mason S, Chapman P, Puto LA, Read JA, Brassington C, Pollard HK, Phillips C, Green I, Overman R, Collier M, Testoni E, Miller CJ, Hunter T, Sansom OJ, Brognard J. Recurrent MLK4 loss-of-function mutations suppress JNK signaling to promote colon tumorigenesis.

*Cancer Research*, 2016; 76(3):724-35.

Colorectal cancer remains one of the most commonly diagnosed cancers and leading causes of cancer mortality worldwide. The nature of colorectal cancer has been described as extremely heterogeneous where activation of different molecular pathways leads to different phenotypes. In our work we assessed the functional impact of mutations in MLK4 (Mixed-Lineage Kinase 4) in order to gain an insight into the role this kinase plays in colorectal tumorigenesis. MLK4 is an understudied protein kinase that is commonly mutated in cancer, specifically colon cancer at a frequency of 7%. We have characterised the panel of mutations in MLK4 as loss-of-function (LOF) and our biochemical and structural studies unequivocally indicated that these mutations abrogate the activity of the kinase. We also showed that MLK4 LOF mutants suppress the function of the WT allele leading to inactivation of signalling downstream of MLK4. Moreover, reconstitution of colorectal

cancer cell lines that harbour these LOF mutations in MLK4 with functional MLK4-WT slows the proliferation of the cancer cells, reduces colony formation and decreases tumour size 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 p21 and p15). In summary, we demonstrated that MLK4 is a novel colon cancer tumour suppressor harbouring frequent LOF mutations that promote cancer cell proliferation by suppressing signalling in the JNK pathway.

Antal CE, Hudson AM, Kang E, Zanca C, Wirth C, Stephenson NL, Trotter EW, Gallegos LL, Miller CJ, Furnari FB, Hunter T, Brognard J, Newton AC.

Cancer-associated protein kinase C mutations reveal kinase's role as tumor suppressor.

*Cell*, 2015; 160(3):489-502.

Protein kinase C (PKC) family kinases have been long thought to act in an oncogenic manner in many cancer subtypes. As a result there have been several unsuccessful efforts to develop efficacious cancer therapeutics by inhibiting PKC activity. The Signalling Networks in Cancer and Applied Computational Biology groups (in collaboration with Alexandra Newton's lab in San Diego) have reversed this dogma to show that PKCs act as tumour suppressors. Analysing a large number of PKC mutations in cancer, they demonstrated that most caused loss of function

and none were activating. Using CRISPR-mediated genome editing, they corrected a loss of function PKC-beta colon cancer mutation, observing reduced tumour growth in a xenograft model and confirming a tumour suppressive role for PKC-beta. Bioinformatic analysis revealed a wide spectrum of cancer subtypes with PKC mutations and demonstrated co-occurring mutations in other cancer drivers. Overall, the study provided compelling evidence that in PKC mutation positive cancer, therapeutics should focus on restoring, not inhibiting, PKC activity.

Thambyrajah R, Mazan M, Patel R, Moignard V, Stefanska M, Marinopoulou E, Li Y, Lancrin C, Clapes T, Möry T, Robin C, Miller C, Cowley S, Göttgens B, Kouskoff V, Lacaud G.

RUNX1B expression is highly heterogeneous and distinguishes megakaryocytic and erythroid lineage fate in adult mouse hematopoiesis.

*Nature Cell Biology*, Epub 2015 Nov 30.

Understanding how blood cells are generated has clear potential implications for the treatment of blood cancers and genetic diseases. Such knowledge could be critical for defining conditions to amplify haematopoietic stem cells (HSCs), or methods to produce HSCs from human embryonic stem (ES) cells or induced pluripotent stem (iPS) cells. We previously demonstrated that the first haematopoietic progenitors are generated by a subset of endothelial cells, a haemogenic endothelium (HE), and that the transcription factor RUNX1 is critical for this endothelial to haematopoietic transition (EHT). In this study, we establish that the transcriptional repressors GFI1 and GFI1B are critical targets of RUNX1 that control the down-regulation of the endothelial programme during the EHT leading to generation of HSCs. We also demonstrate that GFI1 proteins recruit the chromatin-modifying protein LSD1, a member of the CoREST repressive complex, to epigenetically silence the endothelial program in HE and allow the emergence of blood cells. Together, our study provides new mechanistic

insights into the EHT process and candidates for future studies of the molecular program governing the generation of HSCs. Deciphering this regulatory pathway is essential in our attempts to recapitulate this program during ESC differentiation or during reprogramming to ultimately generate patient tailored HSCs in vitro.

Whalley HJ, Porter AP, Diamantopoulou Z, White GR, Castañeda-Saucedo E, Malliri A.

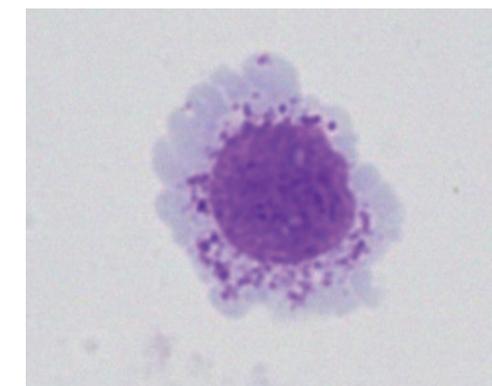
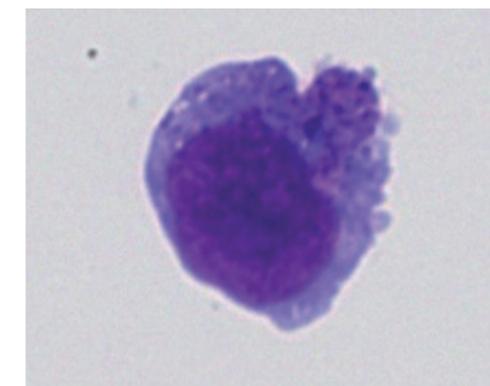
Cdk1 phosphorylates the Rac activator Tiam1 to activate centrosomal Pak and promote mitotic spindle formation.

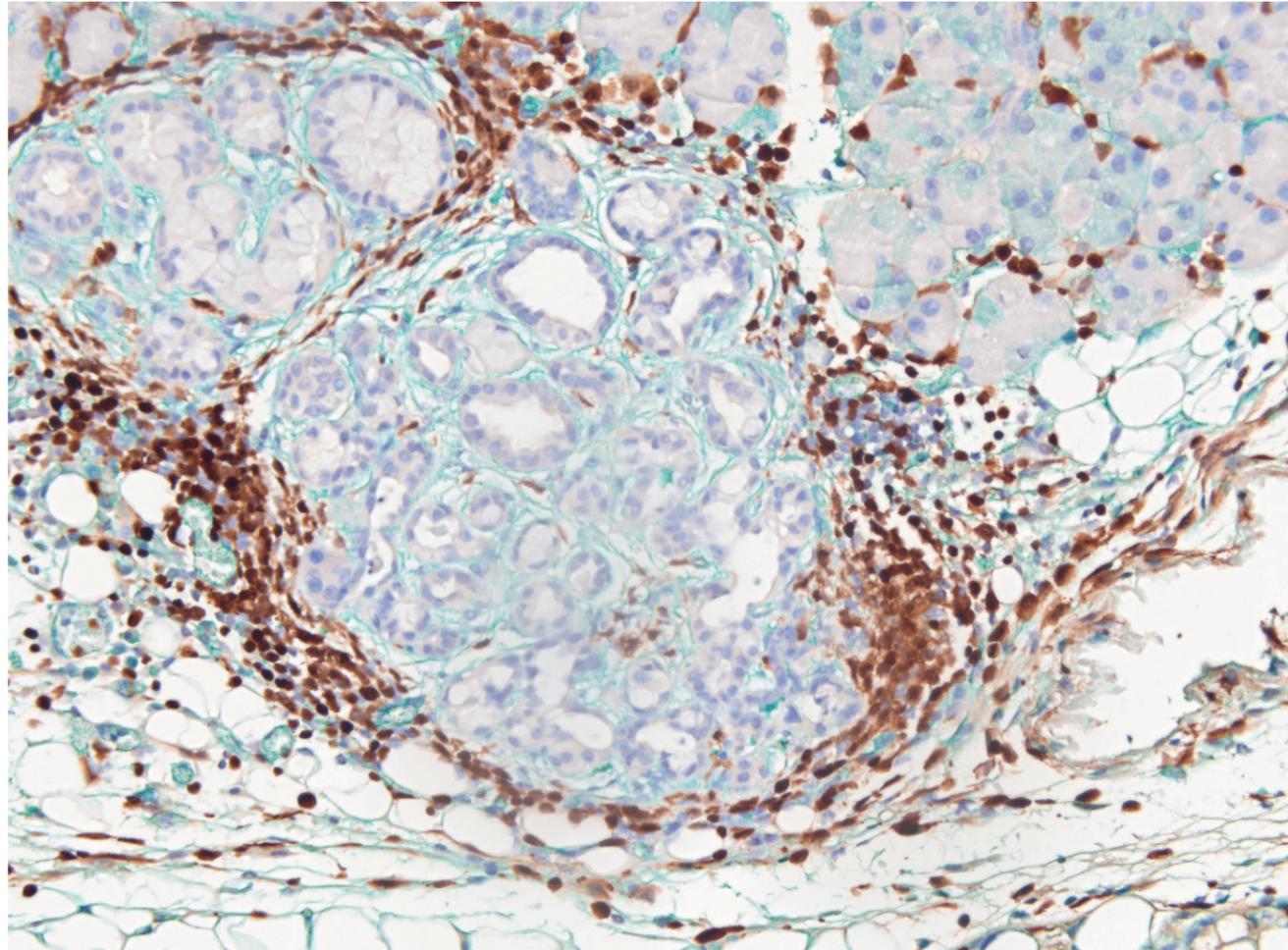
*Nature Communications*, 2015; 6:7437.

Centrosome separation is critical for bipolar spindle formation and accurate chromosome segregation during mitosis. The mechanisms regulating this process are still being elucidated, with the plus-end-directed kinesin Eg5 the most important player identified so far. Eg5 inhibitors induce monopolar spindles and mitotic arrest, which has led to much interest in these drugs as potential cancer therapies. It is clear that forces opposing separation are also important to achieve the correct balance for efficient centrosome separation and chromosome alignment. We had previously identified the guanine-nucleotide exchange factor Tiam1 and its substrate Rac as an important module that counteracts Eg5. In this study, we identified that for Tiam1 to antagonise Eg5 it needs to be phosphorylated by Cdk1, the master regulator of mitosis. Moreover, we showed that this phosphorylation is required for activation of Pak kinases, well-known Rac effectors, on centrosomes. Further, we demonstrated that both Pak1 and Pak2 counteract centrosome separation using their kinase activity. We also showed that depletion of Pak1/2, similarly to depletion of Tiam1 or inhibition of its phosphorylation, allows cells to escape cell cycle arrest by Eg5 inhibition, highlighting the importance of this signalling pathway for the development of Eg5 inhibitors as cancer therapeutics.

Two different blood cells: the left side shows a non-specialised 'blast' cell with FOXC1 switched on; the right side shows a cell with FOXC1 switched off and specialising into a macrophage.

Image supplied by Tim Somerville (Leukaemia Biology)





Co-staining for fibroblast markers in pancreatic cancer by immunohistochemistry. Two distinct populations of fibroblasts marked by a smooth muscle actin and fibroblast specific protein are localised to the pancreatic neoplasia.

*Image supplied by Claus Jorgensen (Systems Oncology) and Garry Ashton (Histology)*

# THE CANCER RESEARCH UK MANCHESTER INSTITUTE

RESEARCH GROUPS

# CANCER INFLAMMATION AND IMMUNITY

www.cruk.manchester.ac.uk/Research/CRUK-MI-Groups/  
Cancer-Inflammation-and-Immunity/Home



Group Leader

Santiago  
Zelenay<sup>1</sup>

Scientific Officer

Chikkanna-Gowda CP<sup>1</sup>

<sup>1</sup>joined in 2015

The extent to which the immune system acts as a natural barrier to cancer has been a subject of intense debate. This notion has recently gained support from the clinical success of therapies aimed at exploiting cells from the immune system. Yet, whether and how tumours simultaneously trigger and evade the immune system are longstanding questions in cancer biology.

The Cancer Inflammation and Immunity group investigates the mechanisms that control natural and therapy-induced tumour immunity with a particular emphasis on uncovering the cellular and molecular mediators that regulate the balance between tumour-suppressive versus tumour-promoting inflammation.

Although historically debated, compelling evidence demonstrates that the immune system can recognise and eliminate cancer cells playing a significant role in the control of tumour development and progression. This activity depends on multiple components from both innate and adaptive arms of the immune system. Lymphocytes, especially T cells, are major players of the anti-tumour response. Already in the 1950s they were postulated to constitute a fundamental component of a process termed 'cancer immune surveillance' that protects hosts against the development of primary tumours. Since then, accumulating evidence provided indisputable support for a role of the immune system as an extrinsic tumour-suppressor system. In many cancer

types, particular those considered immunogenic such as melanoma or renal cell cancer, T cells, some of which were demonstrated to be tumour-specific, can be detected in the circulation and infiltrating tumours. CD8<sup>+</sup> T cell infiltration, in particular, is associated with a favourable prognosis. Remarkably, over the last few years recent clinical trials, aimed at enhancing the effector function of tumour-specific cytotoxic T cells by blocking negative regulators of T cell activity, revealed significant tumour regression in a wide variety of malignancies. Clinical benefit has been observed even in treatment-refractory cancers such as metastatic non-small cell lung carcinoma, a tumour that so far has not been considered as responsive to immunotherapy. However, for all cancer types only a fraction of patients respond, with a very low proportion showing complete and durable responses, highlighting the need for mechanistic studies to understand why this is the case. Indeed, how tumours evade natural and therapy-induced immune responses is a very long-standing question in cancer biology.

## Dual role of inflammation in cancer

The concept that cancer induces inflammation and that inflammatory cells at the tumour site promote several key aspects of malignant tumours such as proliferation, invasion, angiogenesis or migration, is well established. Sub-types of macrophages, T cells, neutrophils, dendritic cells and other white blood cells, are commonly found infiltrating tumours. These cells produce mediators including cytokines, chemokines and growth factors that directly or indirectly support cancer growth and progression. On the other hand, effective anti-tumour immunity depends on inflammatory mediators that contribute to the activation of key immune cells such as dendritic

cells, a process essential for the induction of a robust and efficient T cell response. Moreover, some types of inflammation such as the one characterised by the presence of cytotoxic T cells, the Batf3-dependent family of dendritic cells, or immune factors such as IL-12 and type I and II interferons, are associated with a favourable prognosis and clinical benefit following therapy. Together, these observations indicate that cancer-associated inflammation can have both tumour-promoting and inhibitory effects. How different types of inflammation are established or manipulated by a growing tumour, and to what extent this constitutes an opportunity for therapeutic intervention, remains largely unexplored.

Melanoma, in particular, is a cancer type of special interest from an immunological perspective where immunotherapies such as those based on "immune checkpoint blockade" are showing remarkable and durable effects. Thus, melanoma is considered a highly immunogenic malignancy but at the same time is infamous for its potential for aggressive and malignant growth. These two attributes imply that melanoma cells do not pass unnoticed by the immune system but rather actively subvert natural tumour immunity. Investigating the underlying basis that accounts for the ability of melanoma to avoid immune detection and elimination, we recently uncovered a major role for tumour-derived prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) in cancer immune evasion (Zelenay et al., Cell 2015).

## Harnessing anti-tumour immunity manipulating cancer immuno-modulatory properties

Cyclooxygenase (COX)-2, one of the rate-limiting enzymes for production of prostanoids, is commonly upregulated in numerous cancers, and together with PGE<sub>2</sub>, has been implicated in several aspects of malignant growth including proliferation, angiogenesis and invasion. We found that COX-dependent production of PGE<sub>2</sub> by melanoma cells dominantly and primarily enables progressive tumour growth via immune escape. Notably, melanoma cells, unable to produce PGE<sub>2</sub> by means of genetic ablation of COX or prostaglandin E synthase using CRISPR-Cas9 technology, formed spontaneously regressing tumours in immune-competent mice but progressive tumours in immune-deficient hosts. By contrast, their PGE<sub>2</sub>-competent parental lines grew progressively, irrespective of the presence of a fully competent immune system, demonstrating an essential role for PGE<sub>2</sub> in immune escape. Importantly, PGE<sub>2</sub>-dependent immune evasion was crucial not only in melanoma but also in colorectal and breast cancer models, arguing that this is a general mechanism for subversion of anti-cancer immunity.

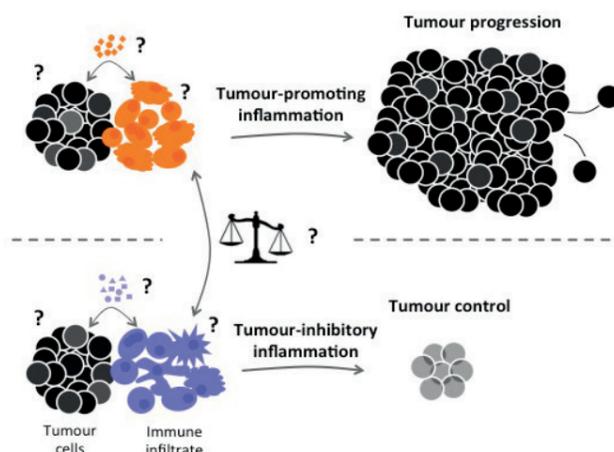
COX deficiency in cancer cells was associated with a marked shift in the inflammatory signature

at the tumour site, characterised by lower expression of cancer-promoting factors and concomitant increase in several mediators typically associated with anti-tumour immunity. These drastic changes in the inflammatory nature of the tumour microenvironment due to reduced PGE<sub>2</sub> levels suggested that COX inhibitors could be used to potentiate immune-dependent control. Indeed, we found that combining COX-inhibitors, such as aspirin, with anti-PD-1 blocking antibody synergistically promoted growth control of tumours poorly responsive to either monotherapy. Finally, analysis of publically available human cutaneous melanoma datasets revealed a remarkable conservation of the COX-driven mouse inflammatory signature, suggesting that COX activity might be a driver of immune suppression across species.

This recent proof-of-principle work underscoring a critical role for cancer-derived cyclooxygenase activity on tumour immune evasion has several important implications. First, it suggests that manipulation of the type of inflammation and subversion of immune surveillance by cancer cells is essential for their ability for progressive growth. Second, it indicates that relevant tumour rejection antigens can be kept cryptic within cancer cells thanks to the activity of immunomodulatory factors such as PGE<sub>2</sub>. Third, it implies that the expression of specific inflammatory signatures in cancer patient biopsies might help predict treatment outcome. In particular, COX-2 expression and/or PGE<sub>2</sub> levels might constitute useful biomarkers of unresponsiveness to immunotherapy. And fourth, it indicates that COX inhibitors, such as aspirin, could represent a useful addition to both conventional and immune-based therapies such as those based on immune checkpoint blockade.

All these implications are testable and constitute the basis for the initial research plans of the Cancer Inflammation and Immunity group. A main focus is to identify factors that regulate the balance between tumour-promoting versus tumour-inhibitory inflammation (Figure 1). Combining the use of genetically engineered cancer models with the analysis of samples from cancer patients, we aim to identify the underlying mechanisms that allow evasion of immune control and enable progressive tumour growth distinguishing mediators that favour tumour progression. This distinction should allow us to stratify subgroups of cancer patients with an immune-promoting tumour environment likely to benefit from existing immunotherapy from those with inhibitory pathways resulting in local immune suppression. Ultimately, with this knowledge, we hope to develop novel targeted interventions to disrupt immune suppression, promote tumour immunity and enhance the efficacy of cancer therapy.

Figure 1  
Schematic representation to illustrate the main research questions of the Cancer Inflammation and Immunity group focused on uncovering the cellular and molecular mediators that regulate the balance between the tumour-promoting and tumour-inhibitory properties of the immune system.



# CELL DIVISION

www.cruk.manchester.ac.uk/Research/CRUK-MI-Groups/Cell-Division/Home



The inappropriate proliferation of cancer cells can arise from unchecked cell division, a failure to engage cell death pathways, or a simultaneous defect in both. Understanding how the diverse external and internal cues are integrated to co-ordinate cell division and death therefore sits at the heart of our need to understand the basic biology of cancer.

**Group Leader**  
Iain Hagan

**Associate Scientist**  
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**Postdoctoral Fellows**  
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Jennifer Winstanley<sup>1,2</sup>  
Lenka Halova<sup>2</sup>

**Senior Scientific Officers**  
Ben Hodgson  
Kuan Yoow Chan

**Graduate Student**  
María-José Villalobos Quesada<sup>1,3</sup>

<sup>1</sup>left in 2015

<sup>2</sup>joined in 2015

<sup>3</sup>joint with RNA Biology

Because the regulatory networks that control cell division are highly conserved, understanding how the relatively simple unicellular yeasts control cell division greatly accelerates the analysis of the more complex issue of cell division controls in humans. We study cell division in the fission yeast *Schizosaccharomyces pombe* because it is a simple, unicellular organism with excellent genetics, biochemistry and cell biology that is cheap to grow and divides rapidly.

The duplication of the genome in S phase of the cell division cycle is separated from genome segregation in mitosis by a gap phase called G2. Passage from G2 into mitosis is driven by the activation of Cdk1-Cyclin B protein kinase complex. Cdk1-Cyclin B promotes the activity of downstream kinases towards a range of targets to drive the disassembly of interphase architecture and promote the formation of the mitotic spindle. As the spindle forms, the chromosomes condense and a specialised chromosome region on each of the two sister chromatids of each chromosome attach to the microtubules that emanate from either pole of the bipolar spindle (Figure 1). Once all chromosomes have established bipolar attachment, Cdk1-Cyclin B activity is repressed through the destruction of Cyclin B and the bonds that hold the two sister chromatids together are split to enable the newly released sister chromatids to migrate to opposing spindle poles. Reversal of mitotic phosphorylation events by specific phosphatases then drives mitotic exit.

DNA integrity checkpoints (DICs) ensure that cells do not activate Cdk1-Cyclin B when DNA is damaged or replication is incomplete. The

spindle assembly checkpoint (SAC) ensures that Cyclin B is not degraded until every kinetochore has established a functional interaction with microtubules. Two features of tumour cells mean that chemotherapeutic strain on either checkpoint is clinically successful. First, transformation imposes "oncogenic stress" to generate elevated levels of DNA damage. Second, tumour cells invariably have an abnormal complement of chromosomes (aneuploidy) that alters the stoichiometry of DNA replication and repair components to accentuate stress upon DICs. Aneuploidy also enhances the load on the mitotic spindle to impose a greater reliance upon SAC function during division. As continued activation of either checkpoint triggers cell death, chemotherapeutic stress on either checkpoint pathway initiates death in tumours at doses that have minimal impact on normal tissue.

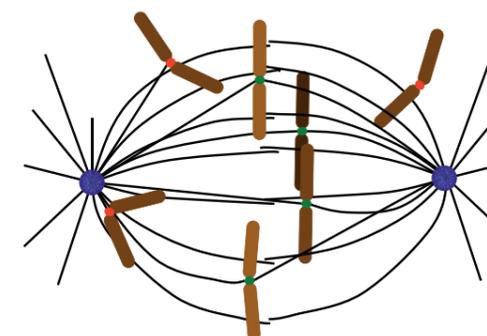
We pursue two goals: to decipher the decision to enter mitosis (the target for DICs) and to understand how to manipulate phosphatases to trap cells in mitosis independently of SAC activation.

### Mitotic commitment and the spindle pole

Interphase Cdk1-Cyclin B is repressed through phosphorylation by Wee1 kinase before Cdc25 phosphatase reverses this phosphorylation. Active Cdk1-Cyclin B then triggers polo kinase activity to further boost Cdc25 and repress Wee1 activities in feedback loops that ensure that mitotic commitment is a rapid, bi-stable, switch. We have found a key role for the spindle pole in this switch. Blocking the recruitment of protein phosphatase 1 (PP1) to the spindle pole component Cut12 enhances polo activity and

**Figure 1**  
The mitotic spindle and SAC signalling

A schematic of the mitotic spindle with representation of the centrosomes (purple), chromosomes (brown) and microtubules (black). Kinetochores are shown as either red or green ovals depending upon their SAC checkpoint status. Unattached kinetochores that generate APC inhibitory SAC signals are shown in red while the attached kinetochores that emit no signal are green.



● Both kinetochores attached: no SAC activating signal generated  
● At least one kinetochore unattached: SAC activating signal generated

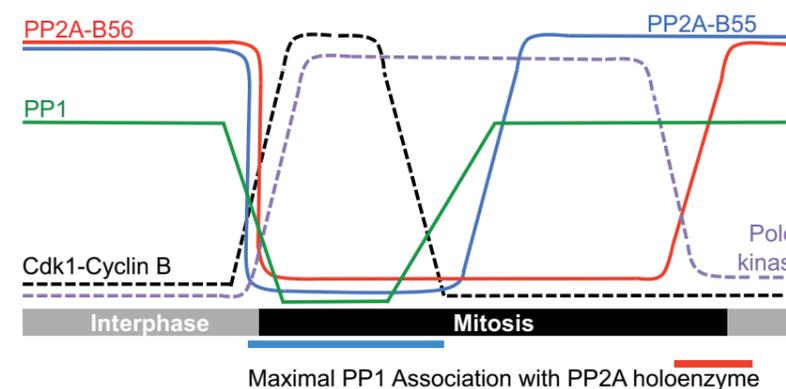
**Figure 2**  
The mitotic PP1-PP2A phosphatase relay

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 itself 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.

affinity for the pole to abolish the requirement for Cdc25. Similar modulation of polo behaviour is seen when environmental cues alter polo's affinity for the spindle pole to adjust the timing of division to suit the demands of the new environment. Modification of a second spindle pole component, Sid4, enhances Cut12 function in mitotic control. Such dialogue between structural components at the pole is suggestive of integration of inputs from diverse signalling pathways to generate a single, coherent, decision to divide.

### A self-organising mitotic protein phosphatase relay

PP1 and protein phosphatase 2A (PP2A) account for over 95% of serine/threonine directed phosphatase activities in human cells. Although PP1 is monomeric, PP2A is trimeric with catalytic, structural and regulatory subunits. Of four types of regulatory subunit, PP2A holoenzymes containing the B55 and B56 isoforms have been linked to mitotic exit. PP1 and each PP2A phosphatase had been assumed to play a distinct and specific role in mitotic exit until we uncovered unanticipated dialogue between PP1 and PP2A. We found that mitotic reactivation of PP2A-B55 and PP2A-B56 relies upon the physical recruitment of PP1 to the B55 and B56 regulatory subunits. Because PP1 activity is repressed by Cdk1-Cyclin B phosphorylation, PP2A activities cannot



recover until Cyclin B is destroyed. Importantly, the enzyme that removes the inhibitory phosphate from PP1 is PP1 itself. Thus, Cyclin B destruction automatically supports the reactivation of PP1 and any PP2A enzyme to which PP1 can be recruited.

Although PP1 can bind and re-activate the B55 regulatory subunit when Cyclin B is destroyed, PP1 binding to the B56 regulatory subunit is blocked by phosphorylation within the PP1 docking site by the mitotic protein kinase polo. This phosphate must be removed before PP1 can be recruited to restore PP2A-B56 activity. The enzyme responsible for removing this phosphate is PP2A-B55. Thus, an ordered sequence of events follows Cyclin B destruction with the first being the auto- reactivation of PP1. As PP1 can bind PP2A-B55 at this time, PP2A-B55 reactivation is coincident with PP1 reactivation. This newly reactivated PP2A-B55 then tries to dephosphorylate B56, however polo phosphorylation of B56 is more efficient than PP2A-B55 de-phosphorylation. PP1 is therefore unable to bind and reactivate PP2A-B56 until polo activity declines at the end of mitosis (Figure 2).

We believe that the sequential nature of this phosphatase relay will only be true in in vitro biochemical reactions. We anticipate that the specific concentration of each component of this relay at any given location will determine which activity predominates at this site in vivo. Thus, full PP2A-B56 activity may be supported at one discrete location, while the remaining enzyme throughout the rest of the cell is repressed.

A collaboration with Prof. Jon Pines' laboratory (Institute of Cancer Research) demonstrated recruitment of PP1 to PP2A-B56 in human cells. This potential for PP1 control of PP2A-B56 reactivation in humans is likely to impact upon cancer research beyond mitotic control. Although the PP1 docking site of fungal B56 molecules contains a site for phosphorylation by polo, the equivalent site in two of the five human B56 isoforms is SP/TP (Cdk/MAPK consensus) with SQ/TQ (consensus for the DNA damage kinases ATM, ATR, DNA PK) being found in the remaining three. A preceding lysine in all five isoforms could direct phosphorylation by AGC family kinases while a second threonine could enable further kinases to regulate PP1 recruitment. Thus, while the fungi have refined the relationship between PP1 and PP2A-B56 for mitotic control, human cells may well use PP1 reactivation of PP2A-B56 in a broader range of signal transduction contexts.

**Publications listed on page 54**

# CELL REGULATION

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The stress-activated kinase JNK plays an essential role in the cellular response to many different extracellular and intracellular signals and as a result regulates many key biological processes, such as cell proliferation and cell death. Not surprisingly, JNK has been implicated in cancer development and progression, supported by the observations that inactivating mutations in several kinases lying upstream of JNK can be found in human cancer.

Extensive analysis in a number of different models reveals a very complex picture where JNK signalling can either promote or inhibit tumour growth depending upon cellular context. Our goal is to better understand the role of JNK signalling and that of specific downstream effectors of JNK in particular human cancer types.

We have focused on a particular downstream target of JNK namely the transcription factor ATF2. Our studies have shown that ATF2 inhibits Ras-dependent tumour formation in an orthotopic mouse model of liver cancer. Furthermore, in this model we find that ATF2 acts as an effector of JNK-dependent tumour suppression; ATF2 is dependent upon JNK for mediating suppression and equally tumour suppression by JNK is lost in the absence of ATF2. This is consistent with the observation that JNK is essential for phosphorylation of ATF2 on two key residues in its activation domain – the main step in engaging ATF2's transcription factor function. Our studies have led us to identify a novel ATF2-driven transcriptional programme that is activated in response to stress stimuli. To assess the status of ATF2 activity in human tumours, we interrogated transcriptomal datasets, using our newly identified panel of ATF2 target genes as a proxy reporter of ATF2 activity. We observe a striking down-regulation of ATF2 target genes in several tumour types (including breast, lung and ovarian) compared to normal tissues, strongly suggesting that ATF2 can play a tumour suppressive role in humans, as has been found in a number of mouse models.

## Stress-dependent transcription in prostate cancer

In order to gain greater insight into tumour suppression by ATF2 and its evasion in tumours, we have conducted an in-depth analysis of prostate tumours using molecular profiling resources available to the research community. Our interest in prostate cancer stems from the fact that in mouse models, signalling via the JNK pathway impairs prostate tumourigenesis. Although the JNK targets responsible for this effect remain unidentified, given our observation that ATF2 is responsible for JNK-dependent tumour suppression in the liver, we hypothesise that ATF2 has a similar function in the prostate.

Our analysis demonstrates that loss of ATF2 target gene expression is strongly correlated with features typical of advanced or aggressive disease. For instance, ATF2 targets are down-regulated in metastatic versus primary tumours, and there is a clear trend towards loss of expression with increasing Gleason score. We derived a novel prognostic gene signature based on ATF2 target genes and, using two independent datasets, showed that a positive clinical outcome was strongly correlated with high expression (greater than median), of these genes (Figure 1). These exciting findings may have translational relevance – a key challenge in management of patients with prostate cancer stems from the current inability to accurately predict the course of the disease, making it difficult to determine which newly diagnosed prostate tumours are relatively benign requiring minimal intervention versus those that are more

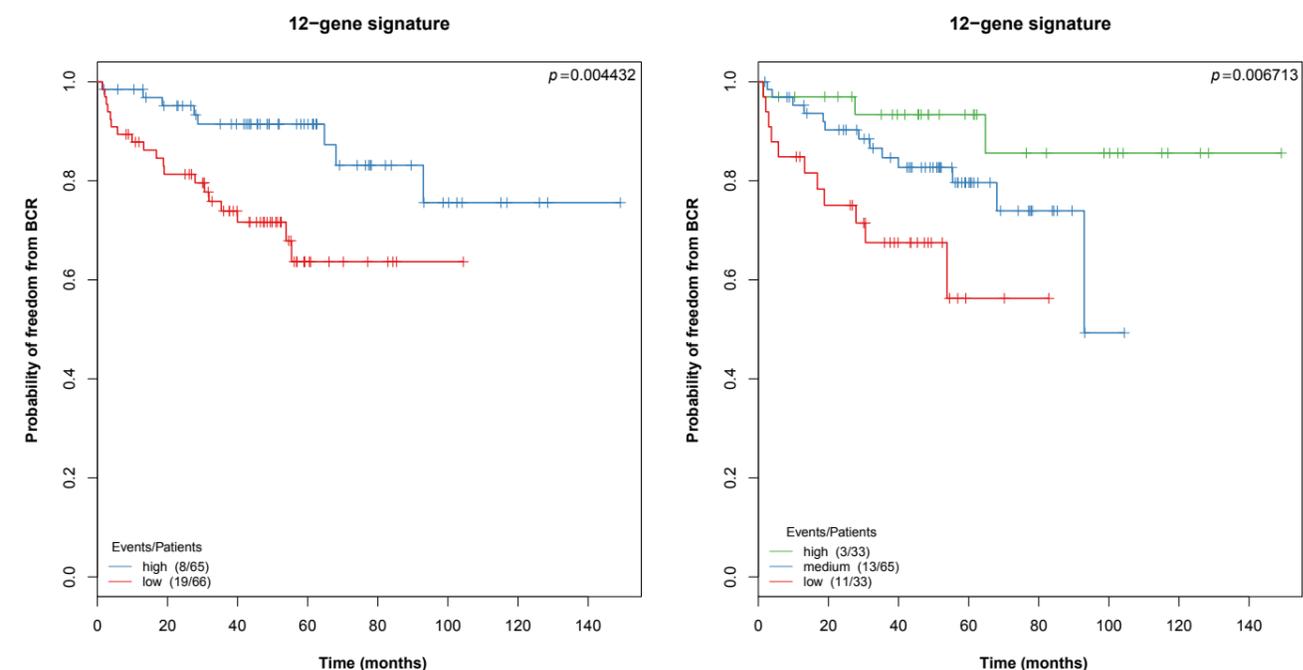


Figure 1  
Reduced expression of ATF2 target genes is associated with a poor clinical outcome in prostate cancer. We derived a gene signature comprising 12 ATF2 target genes that are under-expressed in metastatic prostate tumours. An analysis of gene expression data from 131 patient biopsies (Taylor et al. Cancer Cell, 2010) shows that reduced expression of the signature was associated with early biochemical recurrence (BCR) following radical prostatectomy. Left panel: a comparison of patients with greater (blue line), or lower (red line) than median expression of the signature. Right panel: patients with high (upper quartile), medium or low (lower quartile), expression of the signature are compared.

aggressive requiring complex treatment. There is a clear need therefore for biomarkers that are capable of stratifying patients into treatment versus non-treatment cohorts. We are also conducting research aimed at understanding the mechanisms driving loss of ATF2 target gene expression in prostate cancer. Since ATF2 requires stress-dependent phosphorylation for its activity, we are exploring the possibility that MAPK signalling is altered during progression of the disease. In addition, we have preliminary data indicating that ATF2 targets are subject to epigenetic regulation, and can be activated by HDAC inhibitors. Therefore, aberrant epigenetic activity may contribute to down-regulation of ATF2 targets in prostate cancer. Finally, we are conducting genetic experiments which will determine whether ATF2 can function as a tumour suppressor in a mouse model of prostate cancer.

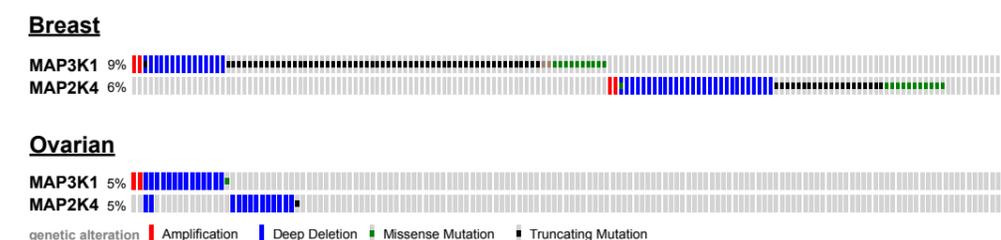
## Characterisation of tumour suppression by MEKK1-MKK4 signalling

Comprehensive characterisation of cancer genomes has provided another important line of evidence suggesting that the status of

stress-dependent signalling pathways is relevant to tumourigenesis. Although neither JNK nor p38 are recurrently mutated in tumours, it is clear that several kinases upstream of these proteins 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 (Figure 2). These data strongly suggest that MEKK1 and MKK4 function together to suppress tumour formation in breast and ovarian tissues, however the mechanisms responsible for this effect are yet to be identified. We are establishing transgenic mouse models of both breast and ovarian cancer in which floxed alleles of either MEKK1 or MKK4 will be deleted by tissue-specific expression of Cre recombinase. This strategy should allow us to determine the role played by these kinases in the aetiology of breast and ovarian cancers.

Publications listed on page 54

Figure 2  
Genetic alteration of MEKK1 (MAP3K1) and MKK4 (MAP2K4), in breast and ovarian tumours. TCGA sequencing data was analysed using the online www.cbioportal tool. Mutations in these kinases show a strong trend towards mutual exclusivity.



# CELL SIGNALLING

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Neoplasia is driven by deregulated intracellular signalling. Mutations and overexpression in human tumours and cell lines implicate the small GTPase RAC and its activators, the Guanine nucleotide Exchange Factors (GEFs), in the formation and dissemination of a range of cancers. Furthermore, the effects of ablating genes encoding RAC proteins or RAC GEFs in mouse, or of pharmacologically inhibiting RAC-GEF/RAC interactions, strongly suggest that targeting RAC signalling could constitute an effective treatment.

However, owing to multiple physiological roles of RAC and moreover RAC functions that antagonise tumour dissemination, sustained suppression of RAC signalling may well be detrimental. Given this challenge, the research of the Cell Signalling group aims to distinguish RAC-dependent effects that promote tumour growth and progression from those that antagonise tumour progression or that are essential for normal physiology, so that RAC signalling might ultimately be targeted more effectively.

RAC cycles between a GDP- and a GTP-bound state. When GTP-bound, it interacts with various effector molecules that elicit downstream responses including, notably, actin cytoskeletal reorganisation. Multiple mechanisms control RAC activity including control of nucleotide binding and hydrolysis by Guanine nucleotide Exchange Factors (GEFs) and GTPase Activating Proteins (GAPs) respectively; regulation of subcellular localisation; modulation of RAC protein levels; 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).

GEFs are typically large proteins harbouring multiple protein-protein interaction domains. Besides stimulating guanine nucleotide exchange, GEFs act as molecular scaffolds targeting active RAC to particular subcellular locations and potentially increasing the local concentration of selective effector molecules, thereby influencing downstream processes.

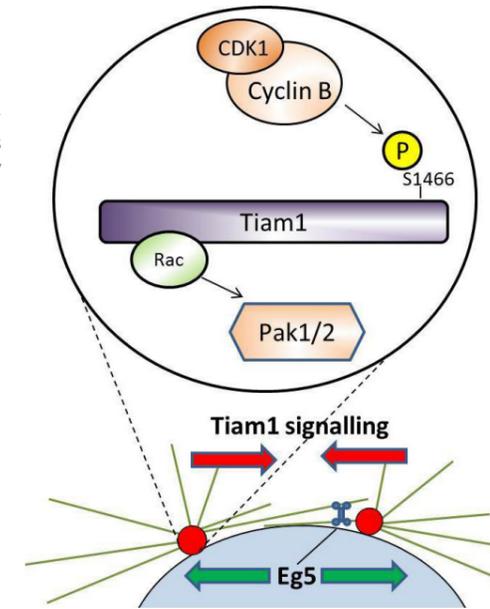
Through influencing selectivity in RAC signalling, GEFs could perform non-redundant signalling roles which can confer a significant involvement in tumorigenesis. Indeed, mice deficient for the GEF Tiam1 are resistant to the formation of skin tumours induced by chemical carcinogens which target H-Ras 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, which the Cell Signalling group is currently elucidating.

## Differential RAC signalling by GEFs implicates FLII in regulating RAC-driven cell migration

Activation of RAC can lead to opposing migratory phenotypes— cell-cell adhesion versus motility— raising the possibility that targeting RAC in a clinical setting could exacerbate 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.* in press), we demonstrate 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 known to be important for the formation and maintenance of cell-cell adhesions, we demonstrate that P-REX1 stimulates migration through enhancing 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 provide

Figure 1

For a mitotic spindle to form, centrosomes must migrate to opposite poles of the cell. This is a balancing act of pushing and pulling forces. The kinesin motor protein Eg5 pushes centrosomes apart. This is counterbalanced by a force generated downstream of the Tiam1 and RAC localised on centrosomes. Recently, we showed that activation of Tiam1 on centrosomes entails phosphorylation by Cdk1/CyclinB. This phosphorylation event is coupled to activation of PAK by RAC. PAK is also required to impede centrosome separation.



direct evidence that RAC GEFs are critical determinants of selectivity in signalling events downstream of RAC and identify FLII as a novel mediator of RAC-driven migration and invasion, which now needs evaluation as a factor promoting tumour metastasis.

## TIAM1-RAC signalling regulates bipolar spindle assembly, chromosome congression and mitotic progression dependent on phosphorylation of TIAM1 by Cyclin B/CDK1

To better understand the role of Tiam1 in promoting tumour growth we have examined its role in the cell cycle. Previously, we revealed that Tiam1 and RAC localise to centrosomes during prophase and prometaphase, and Tiam1, acting through RAC, ordinarily retards centrosome separation. Tiam1-depleted cells transit more slowly through mitosis and display increased chromosome congression errors. Significantly, suppression of the microtubule motor Kinesin-5/Eg5 in Tiam1-depleted cells rectifies not only their increased centrosome separation but also their chromosome congression errors and mitotic delay. These findings identified Tiam1-RAC signalling as an antagonist of centrosome separation during mitosis and demonstrated its requirement in balancing Eg5-induced forces during bipolar spindle assembly (Woodcock et al. *Curr Biol.* 2010). In a follow-on study (Whalley et al. *Nature Comms.* 2015), we find that Tiam1 is phosphorylated by Cyclin B/CDK1 at the onset of mitosis. We map the dominant phosphorylation site on Tiam1 and by creating a non-phosphorylatable mutant demonstrate that while not required for Tiam1 localisation to centrosomes, phosphorylation is essential for its role in regulating centrosome separation. Furthermore, we find that this phosphorylation of Tiam1 is required for the activation of group I

p21-activated kinases (PAKs) on centrosomes in prophase. Additionally, we show that both PAK1 and PAK2 counteract centrosome separation in a kinase-dependent manner and demonstrate that they act downstream of Tiam1. We also show that depletion of PAK1/2 allows cells to escape monopolar arrest by Eg5 inhibition, highlighting the potential importance of this signalling pathway for the development of Eg5 inhibitors as cancer therapeutics.

## TIAM1 antagonises malignant progression

Despite their slower growth, tumours arising in Tiam1-deficient mice progressed more frequently to malignancy (Malliri et al. *Nature* 2002). One mechanism by which Tiam1 and RAC suppress malignant progression is through promoting cell-cell adhesion. We recently identified  $\beta$ 2-syntrophin, a component of the dystroglycan adhesion complex, as a Tiam1 binding partner. Our study (Mack et al. *Nat Cell Biol.* 2012) unearthed a novel role for this complex in promoting tight junction formation and the establishment of apicobasal polarity through the generation of a RAC activity gradient in the membrane region encompassing these junctions.

Malignant progression can entail the loss of cell-cell adhesion. The oncoprotein Src, a non-receptor tyrosine kinase, targets adherens junctions (AJ) for disassembly. Previously, we showed that Src phosphorylates Tiam1 inducing its cleavage by Calpain and its depletion from AJs. Abrogating Tiam1 phosphorylation by Src suppressed AJ disassembly (Woodcock et al. *Mol Cell* 2009). In a recently published study (Vaughan et al. *Cell Reports*, 2014), we have now found that Tiam1 is ubiquitylated and degraded upon treatment of cells with hepatocyte growth factor (HGF), a cytokine that is abundant in cancer and believed to promote invasion of cancer cells. We have mapped the ubiquitylation site on Tiam1 and also identified the responsible E3 ligase as being the HECT family member HUWE1. Moreover, we show that interfering with Tiam1 ubiquitylation by depleting HUWE1 or mutating the ubiquitylation site retards the scattering and invasion of cells by delaying AJ disassembly. HGF and HUWE1 are abundant in lung cancer; we show that HUWE1 and Tiam1 expression are inversely correlated in lung cancer specimens. Significantly, we demonstrate that HUWE1 promotes lung cancer invasion by degrading Tiam1. Potentially, pharmacological agents capable of disrupting the HUWE1-Tiam1 interaction could also antagonise the invasion of lung and other cancer cells, reducing the risk of metastasis.

## Publications listed on page 54

# CLINICAL AND EXPERIMENTAL PHARMACOLOGY

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The CEP group places emphasis on the discovery, development, validation, followed by implementation of biomarkers to facilitate drug development and to aid cancer patient treatment decision making in early clinical trials. This year we began to expand our activities as we develop a Manchester Centre for Cancer Biomarker Sciences. CEP is now comprised of six teams: the Biomarker Portfolio team which includes a Quality Assurance, the Nucleic Acids Biomarkers (NAB) team, the Circulating Tumour Cell (CTC) team and the Preclinical Pharmacology and Tumour Cell Biology team; this year we added a new team to address the pivotal area of Biomarker Bioinformatics and Statistics.

We currently provide biomarker science in 54 clinical trials and studies that are underway or set up in collaborations with Pharmaceutical companies and academic clinical investigators. We also work closely with biomarker technology providers. We are now routinely assessing circulating tumour DNA (ctDNA) in our flagship biomarker study TARGET, performed in close collaboration with our clinical colleagues in the Early Clinical Trials Unit of The Christie NHS Foundation Trust. With Lung Cancer our major focus, and within the CRUK Lung Cancer Centre of Excellence with University College London, we work in close partnership with thoracic oncologist Dr Fiona Blackhall. We are exploiting our expanding panel of small cell lung cancer (SCLC) CTC derived patient explant (CDX) models to test new therapies alongside parallel biomarker development with notable success this year leading to a drug combination trial in 2016. We also use our CDX models to explore the relevance of vascular mimicry (the ability of tumour cells to adopt endothelial cell behaviour) in the progression and drug responses of SCLC. Several exciting international collaborations have been developed this year, notably with the recent award of a joint NIH R01 grant with Charles Rudin at Memorial Sloane Kettering to study the epigenetics of SCLC.

#### SCLC CDX and drug development

This year we expanded our panel of SCLC patient derived explant models (CDX) of SCLC from 4 to 17 models, including two matched pairs representing disease at both baseline and progression after treatment with standard of care therapy. These models recapitulate the spectrum of patient response to cisplatin and etoposide. In collaboration with Pharma partners, our strategy is to test new therapies in CDX with parallel biomarker development in CDX and CTCs in order to rapidly translate promising treatments to early phase trials at The Christie. 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 tested 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 of up to a year after final drug treatment. These exciting data contributed to AstraZeneca's decision to take this drug combination to clinical trial in SCLC in 2016 with The Christie as one of the clinical trial sites. We have also generated and validated

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short term ex vivo cultures derived from CDX which maintain the salient features of both the patient sample and the parent CDX (Figure 1). CDX cultures are currently being used as a platform for both faster drug screening and functional analysis of drug resistance mechanisms. During new therapy efficacy testing in CDX/PDX models, we develop and validate Proof of Mechanism (POM) and Proof of Concept (POC) Pharmacodynamic (PD) Biomarkers (Figure 2A). This year we have focused on components of the DNA-Damage Response/Repair pathway and have a panel of ~15 biomarker assays in varying stages of validation. For SCLC where biopsies are scarce, these PD biomarker assays are then refined for application in CTCs using liquid-based staining methods which are compatible with our multiple CTC enrichment and isolation platforms in readiness for clinical trial deployment (Figure 2B).

#### Vasculogenic Mimicry in SCLC

Vasculogenic Mimicry (VM) describes the ability of aggressive tumour cells with 'stem-like' plasticity to adopt endothelial characteristics and form fluid conducting channel-like structures independent of host vasculature. We have shown that VM occurs in SCLC and correlates with worse patient overall survival. The paucity and low quality of SCLC biopsies makes the study of VM challenging and we turned to our CDX models to investigate VM further (Figure 2C). It was critical to confirm that cells forming channels and exhibiting the phenotype of VM in vivo (CD31 negative, periodic acid shift (PAS) positive) were indeed of tumour origin. Using laser capture

microdissection (LCM) of regions of CDX enriched for phenotypic VM channels, we determined that VM channel cells are human (and not mouse host vasculature) and exhibit copy number alterations consistent with SCLC. Interestingly, VM channel regions have clonal architecture distinct from the bulk 'VM low' CDX regions indicating that this subpopulation of SCLC cells which have acquired the ability to undergo VM may represent distinct previously undescribed subclones of SCLC. CDX regions of high VM co-localise with vascular endothelial (VE)-Cadherin expression, a proposed molecular regulator of VM which we have also found to be expressed by a sub-population of CTCs from SCLC patients. This suggests that SCLC cells which undergo VM may have an increased ability to disseminate, a hypothesis currently under investigation. The impact of VM on tumour growth and cisplatin delivery was assessed in xenografts using a SCLC cell line with high and knocked down levels of VE-Cadherin; reduction of VE-cadherin reduced VM, increased the latency period before logarithmic tumour growth and decreased cisplatin delivery into tumours, indicating the complex impacts of this manifestation of tumour plasticity. Ben Abbott, a Manchester University Masters student studied VM with our team during his research placement and won the 2015 Student of the Year prize.

#### Analysis of tumour heterogeneity and evolution using liquid biopsies

This year the Nucleic Acids Biomarkers (NAB) team led by Ged Brady continued their development and application of molecular profiling methods suitable for monitoring

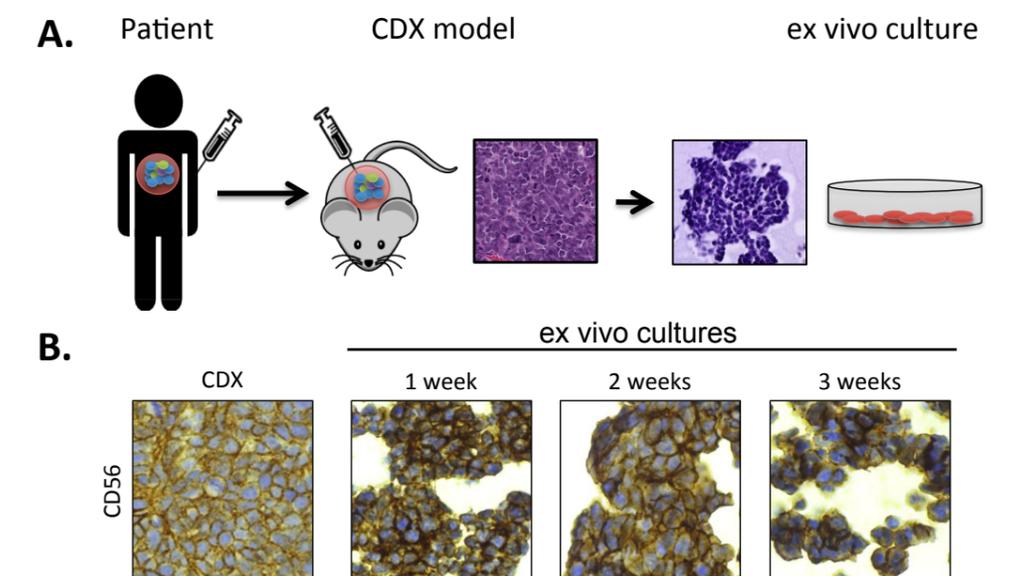
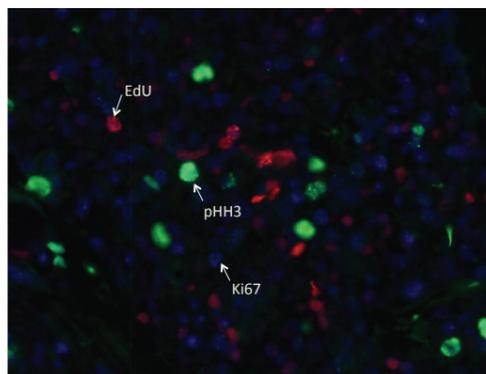


Figure 1 SCLC CDX ex vivo culture characterisation. A) Schematic demonstrating the generation of short term cultures. Patients' circulating tumour cells can be implanted into immune-deficient mice to generate CDX tumours. These tumours can be dissociated and cultured ex vivo to interrogate various aspects of SCLC biology. B) Ex vivo culture validation. Expression of the neuroendocrine lineage marker CD56 is stable over the course of the ex vivo culture period.

Figure 2A. Cell cycle markers in SCLC CDX tumours. Immunofluorescence staining of EdU (Red) pHH3 (green) and Ki67 (Blue) in a SCLC CDX tumour.



tumour heterogeneity and evolution from a simple blood sample. Use of circulating biomarkers (liquid biopsies) to determine the molecular status of the patient's tumour also reduces reliance on tumour specimens. In addition to our routinely used workflow for whole genome amplification of single patient derived CTCs and subsequent next generation sequencing (NGS) based copy number analysis (CNA), the approaches established by the NAB team in 2015 include:

a) Marker dependent and independent enrichment and isolation of single CTCs to enable molecular analysis (see Chudziak et al. Analyst, 2015); b) Processing of a single blood sample to deliver both CTC gDNA and cfDNA up to 4 days post blood sample draw (see Rothwell et al. Molecular Oncology, 2015); and c) Bioinformatic pipelines for NGS analysis of liquid biopsies in collaboration with Crispin Miller (RNA Biology, Cancer Research UK Manchester Institute) and within the recently awarded MRC Single Cell Centre of Excellence where Caroline Dive leads the oncology hub. The following workflows are in optimisation phase: (i) application of targeted and whole exome sequencing (WES) NGS to identify potential clinically addressable mutations in patient CTCs; (ii) tailored NGS library generation to maximise sensitivity and reproducibility of molecular analysis of cfDNA (as applied to the TARGET protocol described above); (iii) targeted pull-down and PCR based NGS panels for mutational analysis of over 600 cancer associated genes from patient cfDNA (see below); and (iv) single cell mRNA profiling by Fluidigm, Nanostring and RNA-Seq.

**Real time analysis of patient ctDNA and stratification of Phase I clinical trial patients**

As part of a Precision Medicine initiative led by Richard Marais (Molecular Oncology, Cancer Research UK Manchester Institute) and Salvador Moncada (The University of Manchester), and in collaboration 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, CEP was part of the team that set up the TARGET protocol and this year completed an initial feasibility stage. The CEP Nucleic Acids Biomarkers (NAB) team led by Ged Brady developed a circulating tumour DNA (ctDNA) workflow based on a sensitive next generation sequencing (NGS) approach that enables parallel sequencing of over 600 cancer associated genes selected to cover a wide range of current drug targets in patient plasma samples. A dedicated QA scientist is currently working with the NAB team to ensure the developing blood-borne NGS assays become GCP compliant to allow clinical decision making. The 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 newly established TARGET Tumour Board. A comparison of the cfDNA NGS data to the patient's archival tumour biopsies profiled using a standard Oncocarta gene panel showed a clear overlap in detected mutations (Figure 3). Ongoing analysis is now aimed at increasing ctDNA assay sensitivity and classification of cfDNA mutations not covered or identified by tumour analysis. CEP are also increasingly involved in the generation of patient derived explant models (PDX) derived from research biopsies from patients enrolled within the TARGET clinical protocol that will allow investigation of predictors of therapy response and resistance.

**ctDNA monitoring of disease response**

The development of ctDNA analysis within the NAB team was put to good use in 2015 within a collaborative study led by Richard Marias investigating the feasibility of using ctDNA to monitor patient responses to treatment and to determine mechanisms of resistance to therapy in melanoma patients. BRAF specific quantitative PCR assays and targeted NGS approaches allowed accurate and sensitive monitoring of disease responses from longitudinal plasma samples collected from melanoma patients during treatment. We have also established WES of ctDNA in retrospective patient samples that identified a potential mechanism of resistance (NRASQ61K mutation) that appeared at the time of disease progression (Girotti et al. Cancer Discovery, 2015).

**The CEP Clinical Trials Circulating Biomarker Portfolio**

In 2015, 2,187 blood samples were processed for the enrichment and/or enumeration of CTCs within clinical trials (1010 samples) or

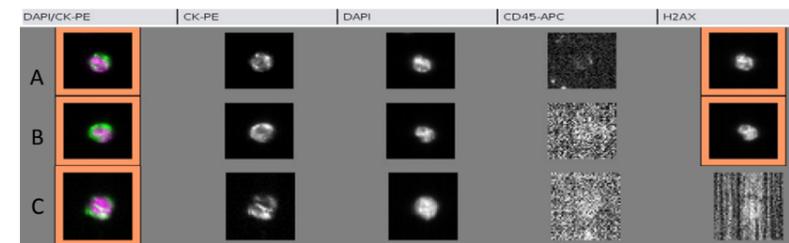


Figure 2B. Baseline SCLC patient sample (Chemores) showing CellSearch 4th channel yH2AX staining. A and B are examples of CTCs staining positive for DNA damage marker yH2AX; C is an example CTC with negative staining for yH2AX.

Figure 2C. Vasculogenic mimicry in SCLC CDX tumours. PAS+/CD31+ endothelial channels (black arrow) and PAS+/CD31- VM networks (red arrow) in a SCLC CDX tumour.

Figure 3. Archival FFPE Tumour and ctDNA Analysis. A panel of 237 mutations across 19 oncogenes were evaluated by both SEQUENOM OncoCarta and NGS for 19 patients. In the 13 cases where sufficient archival FFPE tumour DNA was available, there was a 100% concordance between SEQUENOM OncoCarta and the Clinical & Experimental Pharmacology NGS pipeline. NGS analysis of ctDNA revealed a clear overlap between the detected mutations in archival FFPE tumour samples.

experimental medicine projects to assist with biomarker development and qualification. The EpCam capture based CellSearch system was used for the majority of clinical trials samples but we are increasingly deploying the more recently acquired 'marker-independent' platforms that are under evaluation (Parsortix and Clearbridge Spiral Chip). Six new clinical trials and 5 new experimental medicine projects joined the CEP biomarker project portfolio in 2015. To augment our CTC technology capability, we purchased the RareCyte AccuCyte/CyteFinder and ALS CellSelector systems to assist with CTC enrichment and analysis and in particular to facilitate the analysis of circulating tumour microemboli (cell clusters). This year we also installed an Aushon CiraPlex system for multiplex ELISA analysis

Figure 2C

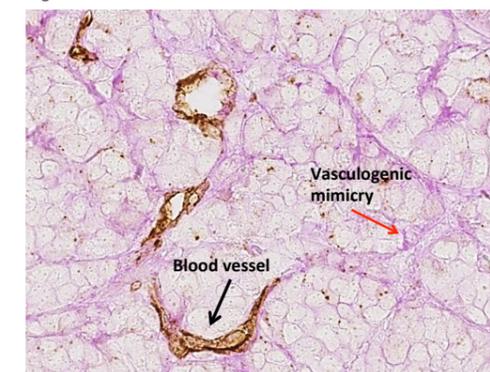


Figure 3

Patient	OncoCarta Readout	Clinical & Experimental Pharmacology NGS pipeline		
	FFPE Tumour	FFPE Tumour	ctDNA Mutation	ctDNA Mutation Frequency (%)
TAR-0001	ND	Insufficient DNA provided	ND	ND
TAR-0002	ND	ND	ND	ND
TAR-0003	ND	ND	ND	ND
TAR-0004	EGFR (G1795 S768I)	Insufficient DNA provided	ND	ND
TAR-0005	KRAS (G12V)	KRAS (G12V)	ND	ND
TAR-0006	EGFR deletion (EGFR c2236_2250del15 p.(Glu746_Ala750del))	EGFR deletion (EGFR c2236_2250del15 p.(Glu746_Ala750del))	ND	ND
TAR-0007	PIK3CA (R88Q)	Insufficient DNA provided	ND	ND
TAR-0008	ND	ND	ND	ND
TAR-0009	ND	ND	ND	ND
TAR-0010	ND	ND	ND	ND
TAR-0011	AKT1 (E17K)	Insufficient DNA provided	AKT1 (E17K)	21
TAR-0012	NRAS (Q61K)	NRAS (Q61K)	ND	ND
TAR-0013	ND	ND	ND	ND
TAR-0014	FGFR3 (Y373C) and PIK3CA (E542K)	FGFR3 (Y373C) and PIK3CA (E542K)	ND	ND
TAR-0015	AKT1 (E17K)	Insufficient DNA provided	AKT1 (E17K)	6
TAR-0016	ND	ND	ND	ND
TAR-0017	ND	ND	ND	ND
TAR-0018	KRAS (G12A)	KRAS (G12A)	KRAS (G12A)	10
TAR-0019	ND	Insufficient DNA provided	ND	ND
		ND	No Oncocarta Mutation Detected	

and qualified this system for use with clinical trials samples.

Highlights of the team this year include the biomarker science we contributed to two Phase II trials published in Lancet Oncology in collaboration with Manchester Cancer Research Centre colleagues Catharine West and Juan Valle (Symonds et al. Lancet Oncology, 2015; Valle et al. Lancet Oncology, 2015). In the CIRCCA randomised, double-blind, placebo-controlled Phase II trial, Cediranib was combined with carboplatin and paclitaxel in patients with metastatic or recurrent cervical cancer and reductions in circulating VEGFR2 support its use as a biomarker of the biological activity of cediranib. In the ABC03 randomised Phase II trial Cediranib or placebo were assessed in combination with cisplatin and gemcitabine chemotherapy for patients with advanced biliary tract cancer. Analysis of CTCs in ABC03 showed for the first time their prognostic significance in this disease.

**Biomarker science to the regulatory standards of Good Clinical Practice**

Essential for biomarker driven clinical trials is the ability to use biomarker data to make clinical treatment decisions. In the European Union, this requires biomarker science to be conducted to Good Clinical Practice (GCP) standards. Much of the activity of the CEP Quality Assurance team in 2015 centred on the revision of the CEP Quality Management System, in addition to supporting on-going GCP related activities. Validation of Q-Pulse compliance management software was completed in 2015. As a consequence of introducing the Documents module, revision and migration of 300 plus documents from a manual paper-based system into an electronic format was required. Plans for implementation of a harmonised audit and non-conformance process are well advanced within the QA team. Two external audits were hosted in 2015 to provide assurance to our sponsors of compliance with GCP. There were also 12 internal data audits conducted to assure the accuracy of reported data and compliance with GCP and local procedures. In addition, CEP was also involved in the Human Tissue Authority (HTA) inspection of The Christie campus. The QA team also maintained external links, and have collaborated with The Christie Hospital QA team and the ECMC QA network, sharing knowledge, experience and practices. Tony Price, our QA lead, is a member of the QA sub-committee of the ECMC QA network. Awareness of current regulatory issues and hot topics within the GCP arena was also maintained by attendance at the Annual Research Quality Association (RQA) Conference in November 2015.

**Publications listed on page 54**

## DRUG DISCOVERY

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Graeme Walker<sup>1</sup>  
Mandy Watson

The past 12 months have seen significant changes in the Drug Discovery Unit as we strengthen both our team and infrastructure to position ourselves for future success. This has involved an increase in the size of the group by around 20% and the use of new technologies.

In particular, the establishment of a dedicated target validation team will help ensure a steady flow of new, clinically relevant, drug targets for the group to prosecute. We have also strengthened our informatics capabilities to help our project decision-making and seen delivery of considerable success in X-ray crystallography, which will directly influence our chemistry strategy over the coming months.

### Progressing our project portfolio

During 2015, five of the projects in our drug discovery portfolio underwent phase transitions to more advanced stages.

After an extensive medicinal chemistry campaign in the PARG project, we have identified a structurally diverse shortlist of compounds with pharmacokinetic properties suitable for pharmacodynamic assessment and these studies are presently underway. We disclosed the underlying pharmacology of these first-in-class compounds for the first time in an oral presentation at the 2015 AACR annual meeting in Philadelphia and have recently completed filings of two chemical patent applications for our lead series in PARG; these will be published in May 2016. This planned structural disclosure has provided us with opportunities to share our proprietary in vitro tool compounds with several key collaborators so that we can expand, and subsequently publish, additional mechanistic understanding around this important DNA repair drug discovery target.

The RET project has benefited greatly from top-up funding from Sixth Element Capital which was used to access additional chemistry, crystallography, pharmacology and drug metabolism resources. This has allowed the

identification of potent, selective RET inhibitors with pharmacokinetic properties suitable for investigation of efficacy. Intriguingly, high resolution co-crystals with compounds from this series reveal a hitherto unprecedented binding mode to the RET protein, and provides a clear rationale for their exquisite selectivity. We have also made significant progress on the targeting of anticipated RET inhibitor resistance mutants. Using a combination of computational modelling and virtual screening we have identified novel potent and mutant RET-selective hit matter with submicromolar activity in cells. Representatives of four distinct series have also been successfully characterised at high resolution in co-crystals by our collaborator, Dr Richard Bayliss at the University of Leicester. These data reveal the precise binding mode of inhibitors to the enzyme protein and provide further medicinal chemistry opportunities.

During the last year, our collaboration with the GlaxoSmithKline (GSK) Cancer Epigenetics Discovery Performance Unit based in Philadelphia has progressed rapidly and in April, the project transitioned into Lead Identification phase. Since then, progress on the project accelerated further and less than six months later is now in the Lead Optimisation phase. One of the attractions of the DDU for our external partners is our proximity to expert scientists in the Institute and we have provided early proprietary epigenetic tool compounds from this project to Dr Tim Somerville's team for investigation of their preclinical pharmacology in Acute Myeloid Leukaemia models. We have continued to develop an excellent working relationship with our partners in GSK and are currently exploring the possibility of collaboration on additional epigenetic targets.

### Graduate Students

Elizabeth Hogg<sup>1</sup>  
Daniel Mould

### Undergraduate Students

Kelly Ayton<sup>2</sup>  
Shaun Johns<sup>1</sup>

<sup>1</sup>joined in 2015

<sup>2</sup>left in 2015

SMARCA2, our first target to have been identified by bioinformatics, has now been fully validated internally and has progressed into Hit Identification. This target and our approach to it attracted a lot of interest when we presented a poster on it at the AACR annual meeting. This resulted in discussions with several Biotechnology and Pharma companies who expressed an interest in collaborating with us on this exciting target. We are currently progressing the Pharma collaboration that gives us the most potential to take this challenging project forward.

Lastly, through a screening arrangement with AstraZeneca, we successfully validated a fascinating chemical series as inhibitors of the oncometabolite enzyme mutant IDH1. This is a target that has long been of interest to the team but has so far proved challenging to find credible hit matter. Collaboration with Dr Colin Levy at The University of Manchester delivered high-resolution crystal structures of these early ligands bound to the target protein and these provided inspiration for an aggressive medicinal chemistry campaign. However, acceptable pharmacokinetic properties proved difficult to obtain and, given the significant competition, the difficult decision to stop this project was taken in order to allow us to focus upon our more competitive and advanced projects.

With three resource-intensive, Lead Optimisation projects progressing at the same time, it has been difficult to invest as much effort

as we would like in new projects to refresh the portfolio. This problem was recognised by CRUK and from April 2015 we were awarded additional ongoing funding to allow expansion of our target identification and validation capabilities. After an intensive recruitment campaign we have appointed six new drug discovery scientists and now have a "ring fenced" target validation team, including our first biology PhD student. We are particularly focusing our efforts and reaching out to potential collaborators around DNA Damage Response/Synthetic Lethality, Epigenetic Mechanisms and Lung Cancer as priority areas. With our RET and PARG inhibitors, and working with internal and external Principal Investigators, we are also establishing an interest in additional disease areas including Prostate and Pancreatic Cancers. These efforts will bear fruit in 2016 and the years to come. Closer to home, we continue to engage with our local PIs to discuss and progress novel drug discovery targets.

### The Future

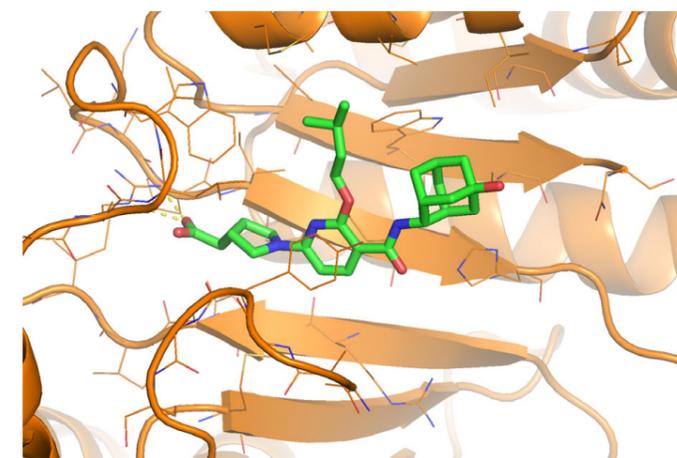
The primary aim of the DDU in this quinquennium is to deliver our first candidate drug into pre-clinical development through our own efforts alone or in partnership with a collaborator. During 2015, we have made significant progress towards this objective and have also taken steps to ensure the long term sustainability of our project portfolio.

### Publications listed on page 55

**Figure 1**  
The enhanced DDU team.



**Figure 2**  
Crystal structure of a DDU inhibitor (in green) bound to mutant IDH1-1.



# LEUKAEMIA BIOLOGY

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Visiting Worker

John Chadwick<sup>2</sup>

<sup>1</sup>left in 2015

<sup>2</sup>joined in 2015

## Understanding how acute myeloid leukaemia (AML) cells differ from their normal bone marrow (BM) cell counterparts is an important strategy for uncovering new targets for therapy.

We have recently identified several transcription factor genes selectively expressed in human AML cells but not normal BM cells, and have demonstrated experimentally that their tissue-inappropriate mis-expression makes an important contribution to the differentiation block characteristic of the disease. Of course, the presence of multiple genetic mutations is another essential way in which AML cells differ from their normal counterparts. Using material from the Manchester Cancer Research Centre's Tissue Biobank, we have investigated mutation acquisition in isocitrate dehydrogenase 1 or 2 (IDH1 or IDH2)-mutated AML samples. We discovered that, following chemotherapy, reconstitution of IDH-mutant clonal haematopoiesis is frequent, even in long term clinical remission, providing compelling evidence for the pre-leukaemic or leukaemia-initiating nature of these mutations.

### Frequent derepression of the mesenchymal transcription factor gene *FOXC1* in acute myeloid leukemia

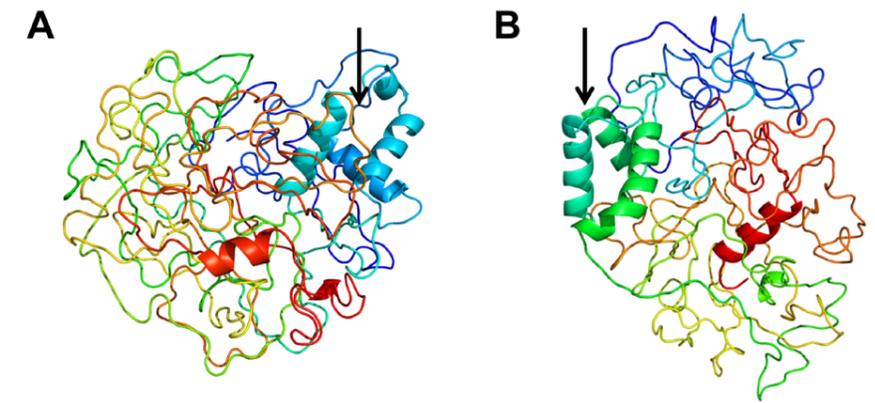
Transcription factors are important regulators of cellular fate and function. We were surprised to discover through bioinformatics analyses that the Forkhead transcription factor gene *FOXC1* (Figure 1A) was among the most highly up regulated transcription factor genes in primary human acute myeloid leukemia (AML) stem and progenitor cells when compared with normal haematopoietic stem and progenitor cells (HSPCs), almost invariably in association with concomitant high level HOXA/B (Homeobox gene clusters A and B) expression. Indeed, fully 30% of human HOXA/B-expressing AML cases exhibited derepressed *FOXC1* gene expression (and 20% of cases overall). These observations raised the question as to why a transcription factor which is required for normal mesenchymal and mesodermal differentiation during embryogenesis should be mis-expressed in leukaemic haematopoietic tissue.

Using genetic knockdown and over expression strategies we demonstrated that mis-expression of *FOXC1* contributes to the differentiation block, which is characteristic of AML, through reducing both B-lineage and monocyte/macrophage lineage differentiation. We also observed that it collaborates with the homeodomain transcription factor HOXA9 to accelerate the onset of symptomatic leukaemia in an in vivo model. Remarkably, patient samples with high *FOXC1* expression exhibit reduced morphologic monocytic differentiation and patients with *FOXC1*<sup>high</sup> AML exhibit inferior survival. *FOXC1* achieves this differentiation block through repression of Kruppel-like factor 4 (*KLFA4*), a critical regulator of monocyte differentiation. To explore the mechanism of *FOXC1* derepression in AML, we focused on the Polycomb repressive complex (PRC) which is known to repress expression of lineage-specific transcription factors. Treatment of human CD34+ HSPCs with PRC2 inhibitors resulted in substantial up regulation of *FOXC1*, indicating that continued repression of *FOXC1* in the haematopoietic system is mediated by PRC2 and that loss of PRC2 activity at the *FOXC1* locus contributes to its derepression in AML. These studies, led by Tim Somerville and published in Cancer Cell in September 2015, will lead to improved risk stratification and personalisation of therapy in AML, in addition to providing valuable mechanistic insights.

### Aberrant expression of *IRX3* contributes to leukaemic transformation

We also observed that the Iroquois homeodomain transcription factor gene *IRX3* (Figure 1B) which has essential developmental roles in mesodermal tissues such as heart, fat and bone, is also minimally expressed in normal BM cells but extensively mis-expressed in AML. In fact high level expression of *IRX3* was found in approximately 35% of primary human AML samples, again in close association with expression of HOXA/B expression. Interestingly,

Figure 1  
Structural representations of DNA binding domains of *FOXC1* and *IRX3*. These two transcription factors are mis-expressed in human AML leading to enhanced differentiation block. Phyre<sup>2</sup> predicted structure of (A) *FOXC1* and (B) *IRX3*. The triple helix *FOXC1* winged helix DNA-binding domain and *IRX3* DNA-binding homeodomain are indicated by arrows.



unlike *FOXC1* expression, there was also high level expression of *IRX3* in 50% of cases of human T-acute lymphoblastic leukaemia (ALL) and 20% of cases of B-ALL, once more in association with high levels of HOXA/B expression. Functionally, forced expression of *IRX3* in murine BM HSPCs resulted in enhanced serial replating in vitro with significantly impaired morphologic differentiation. In transplantation experiments, recipients of *IRX3*-expressing BM HSPCs exhibited a significant expansion of LY6C+ T-cells and later developed donor-derived T-cell leukaemias with incomplete penetrance. In co-expression experiments, *Hoxa9/IRX3* double transduced BM HSPCs exhibited significantly greater clonogenic cell frequencies and morphologic differentiation block by comparison with control cells. In vivo, in the post-transplant period *Hoxa9/IRX3* BM HSPCs exhibited multilineage differentiation but with skewing away from the myeloid lineage and towards the B-lineage by comparison with control *Hoxa9/MTV* cells. Nevertheless, donor-derived AML subsequently developed in all mice in both cohorts. Unexpectedly, recipients of control *Hoxa9/MTV* cells developed AML significantly more rapidly than recipients of *Hoxa9/IRX3* cells (median 125 days versus 270 days). However, *Hoxa9/IRX3* recipients developed leukaemias with a much more pronounced differentiation block by comparison with *Hoxa9/MTV* recipients, as evidenced by immunophenotype, morphology and transcriptome. *IRX3* knockdown experiments in both human and murine AML cells led to induction of differentiation and loss of clonogenic potential, whereas similar experiments in primary normal human CD34+ cells did not affect clonogenic potential. Our data indicate that derepression of the mesodermal transcription factor gene *IRX3* in human acute leukaemia is both frequent and functional, contributing to leukaemic transformation through skewing differentiation, enhancing self-renewal and modulating the cellular consequences of HOX gene expression. These data are shortly to be submitted for publication.

### Direct comparison of quantitative digital PCR and 2-hydroxyglutarate enantiomeric ratio for IDH mutant allele frequency assessment in myeloid malignancy

Mutations in the metabolic enzymes isocitrate dehydrogenase 1 (IDH1) or isocitrate dehydrogenase 2 (IDH2) occur in approximately 15-20% of patients with acute myeloid leukemia (AML). A consequence is the aberrant accumulation of the (D)-enantiomer of 2-hydroxyglutarate (D-2-HG), a putative oncometabolite which competitively inhibits  $\alpha$ -ketoglutarate-dependent epigenetic regulators such as TET2 and Jumoni domain histone demethylases. To determine how best to monitor the response to treatment of patients with IDH mutations, we developed an allele-specific digital PCR method for detecting recurring IDH1 and IDH2 mutations in AML, and made a direct comparison of this novel approach to the better established technique of determining the D:L enantiomeric ratio of 2-HG in concomitantly collected plasma samples. We discovered that digital PCR delivers superior sensitivity to the 2-HG enantiomeric ratio at lower mutant allele frequencies, while for monitoring of mutant IDH allele burdens of >1% the enantiomeric ratio was equivalent. This work was published in Leukemia in December 2015, with Dan Wiseman as lead author.

Finally, we welcome to the lab Isabel Romero-Camarero who has recently completed her doctoral studies at the University of Salamanca, Spain; Mark Williams, a Kay Kendall Leukaemia Fund Clinical Training Fellow; and Fabrizio Simeoni, who joins the Institute's graduate studies programme following his Masters degree at the Sapienza University of Rome. We congratulate Tim Somerville who was awarded the Institute's Dexter Prize for the most promising young scientist; and Dan Wiseman who was awarded a highly prestigious four-year Bloodwise Clinician Scientist Fellowship.

### Publications listed on page 56



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Our laboratory focuses primarily on malignant melanoma, a disease that affects over 12,000 people in the UK each year and is therefore our fifth most common cancer. A key aim of our studies is to translate our basic research findings into patient benefit, and in the past year we have developed a range of technologies to allow us to personalise medicine for cancer patients.

Recent years have seen extraordinary advances in the treatment of malignant melanoma, the most deadly form of skin cancer. The two most common driver oncogenes in melanoma are *NRAS* (about 20% of cases) and *BRAF* (about 45% of cases), and the proteins produced by these mutant genes activate a conserved signalling pathway that controls cell growth and survival and drives melanoma development. Therapies that target this signalling pathway can achieve impressive responses in patients, particularly when *BRAF* is mutated. Additionally, immunotherapy approaches, whereby the patient's own immune system is harnessed to fight the melanoma, have also shown remarkable success in this disease. However, not all patients respond to these therapies, and those that do often eventually become resistant. Our studies over the past year have focused on monitoring patient response to therapy, identifying mechanisms of resistance, and crucially, determining how resistance can be overcome for individual patients to allow us to implement personalised medicine protocols.

A key aspect of our studies is the collection and analysis of tumours from melanoma patients being treated at the Christie NHS Foundation Trust. Working closely with Dr Paul Lorigan and the hospital's melanoma team, our researchers have now collected over 135 tumours from stage IIIb, IIIc and IV melanoma patients, and we have developed patient derived xenografts (PDXs) from an impressive 72% of these patients. This allows us to study each patient's tumour in great detail and in particular, allows us to explore how these tumours respond to treatment. We can mirror a patient's course of treatment in the PDXs, discover when, why and how they become resistant to therapy, and test alternative

therapeutic strategies for individual patients, based on next generation sequencing (NGS) of the patient's tumour and subsequent validation in the patient's own PDX.

Despite our success at developing PDX models, it is not always possible to obtain samples from patients because their tumours may be inaccessible. To overcome this challenge, we have also been developing xenografts from circulating tumour cells (so-called CDxs) that are present in patient blood. This approach represents an enormous technological advance, because it allows us to study the tumours of patients for whom we are otherwise unable to acquire appropriate samples. We demonstrated the potential impact of these approaches in a recent publication in *Cancer Discovery* (Girotti et al, in press) in which we describe not only the methodologies, but also how our platform of technologies can assist clinical decisions in personalised medicine protocols.

In a complementary approach, we have collaborated with the Clinical and Experimental Pharmacology group to establish a pipeline to analyse these liquid biopsies. In addition to facilitating the generation of the CDx models mentioned above, this collaboration has allowed us to study circulating tumour DNA (ctDNA) by targeted next generation sequencing or droplet digital PCR. Critically, this means that we can identify and quantify ctDNA mutations from the tumours of patients on treatment, and for targeted therapies we have been able to provide evidence of disease progression up to 8 weeks before this was confirmed by radiological scans; we have established protocols that can reveal mechanisms of resistance in individual

<sup>1</sup> joined in 2015  
<sup>2</sup> left in 2015  
<sup>3</sup> summer student

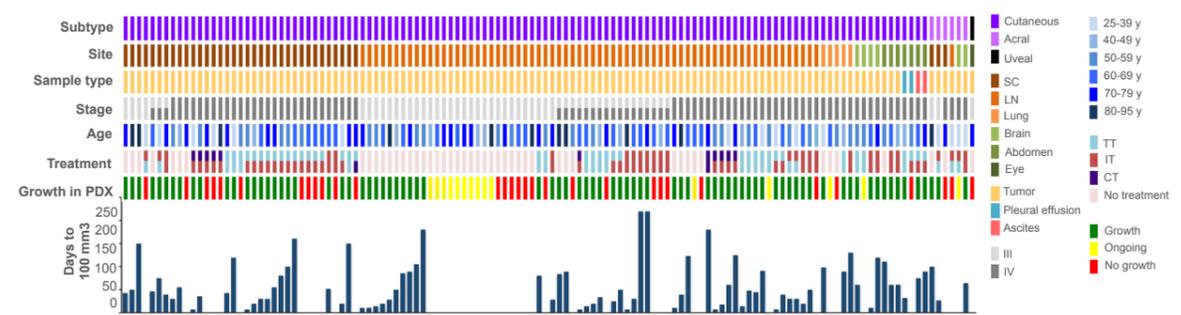


Figure 1  
Developing PDX to validate new therapies for melanoma patients. The figure shows an overview of 126 stage III and IV cutaneous, mucosal, acral and uveal melanoma patient samples implanted into NSG mice to establish patient-derived xenografts (PDXs). Our current success rate for tumour growth is 72%.

patients. We also find that this approach is useful in immunotherapy patients, and our initial data suggest that this approach can reveal responses to immuno-therapies significantly earlier than radiological scans. The potential impact of these approaches on patient care is shown in our recent paper in *Cancer Discovery* (Girotti et al, in press).

An exciting development in 2015 was the initiation of a clinical trial assessing the safety and efficacy of a panRAF/SRC inhibitor developed by our lab in collaboration with Professor Caroline Springer at the Institute of Cancer Research in London. In 2015 we published a study in *Cancer Cell* in which we showed that these inhibitors are active in PDXs from patients resistant to BRAF and BRAF/MEK inhibitor combinations (Girotti et al, *Cancer Cell*, 2015). These agents were licensed to Basilea Pharmaceutica Ltd in April 2015 and are currently undergoing clinical trials at the Christie and Royal Marsden NHS Foundation Trusts in Manchester and London respectively (NCT02437227).

We have also continued to investigate mechanisms of resistance to targeted therapies in melanoma and during 2015 we discovered a resistance mechanism driven by cells switching from glucose to glutamine as a carbon source (Baenke et al, *Molecular Oncology*, Epub 2015). This reveals not only an intriguing cellular response, but also identifies potential new therapeutic strategies for these patients.

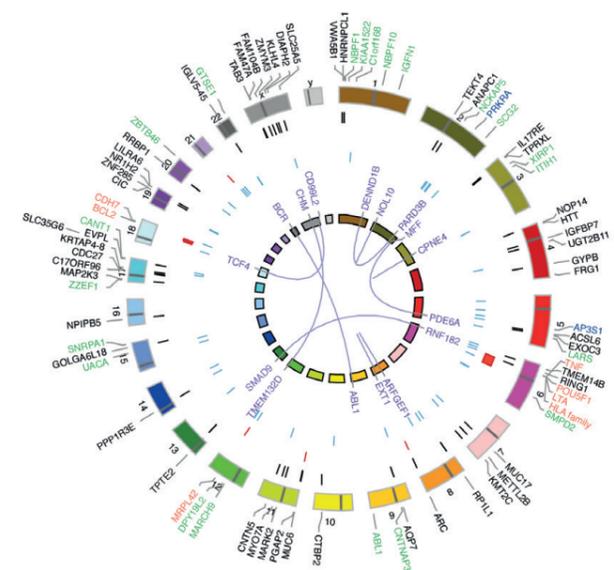
Moreover, we collaborated with Dr Manel Esteller in Barcelona to reveal epigenetic control of EGFR as a resistance mechanism (Vizoso et al, *Nature Medicine*, 2015), and we collaborated with Dr Erik Sahai to investigate how the tumour microenvironment can drive resistance (Hirata et al, *Cancer Cell*, 2015). We also collaborated with Dr Caetano Reis e Souza's group to understand how we may be able to improve response to immunotherapies (Zelenay et al, *Cell*, 2015).

Finally, we have examined how to use these types of approaches in chronic myeloid leukaemia (CML). CML is a disease that is largely driven by the BCR-ABL1 fusion protein and tyrosine kinase inhibitors (TKI) that target the ABL kinase domain have transformed the clinical management of this disease. However, many patients develop resistance to these agents through the acquisition of mutations in ABL that block TKIs binding to its kinase domain. Second and third generation inhibitors have been developed to overcome this type of resistance, but patients still develop resistance through the acquisition of multiple mutations within the same kinase domain (so-called compound mutations). We performed whole genome sequencing of a CML patient who developed resistance to the 3<sup>rd</sup> generation ABL inhibitor ponatinib. This revealed that the tumour had developed compound mutations that blocked ponatinib binding, but also that the patient had amplification of *BCL2*, which encodes an anti-apoptosis protein. We demonstrated that a combination of the BCL2 inhibitor navitoclax (ABT-263) with ponatinib inhibited growth of this patient's leukaemic cells, providing a potential hypothesis-driven treatment for this patient and demonstrating the feasibility of personalised medicine in CML patients.

Our studies of the last year have enabled us to test the feasibility of using personalised medicine approaches in cancer patients and have revealed that for some patients these methods can inform treatment strategies. Our aim now is to learn how to use these approaches to improve patient outcomes.

Publications listed on page 56

Figure 2  
Genomic characterisation of patient with chronic myeloid leukemia. Circos plot illustrating predicted somatic variants including SNVs (green gene names), LOH (black gene names), in-frame fusions (purple gene names), amplifications (red gene names), deletions (blue gene names), and inter- and intrachromosomal rearrangements (purple lines). Locations of LOH SNVs (black lines), amplifications (red line) and deletion (blue line) are indicated. Grey lines mark the position of centromeres in each chromosome.





For the past 70 years, androgen deprivation therapy has remained the core treatment in prostate cancer (PCa), the second cause of cancer-related mortality in men worldwide. Although most prostate cancers initially respond to androgen deprivation, many will ultimately progress to lethal castration-resistant disease (CRPC).

The extensive usage of next generation anti-androgen agents, such as Abiraterone (CYP17A1 inhibitor) and Enzalutamide (AR-antagonist), continues to highlight the clinical significance of the emergence of treatment-resistant disease. Thus, understanding the molecular mechanisms that promote tumour propagation during the progression of prostate cancer to castration-resistance is of fundamental importance for the development of reliable biomarkers and effective treatments. The main question we aim to address is what causes lethal prostate cancer. To tackle this challenge, we focus on the molecular and cellular regulation response to androgen axis therapies.

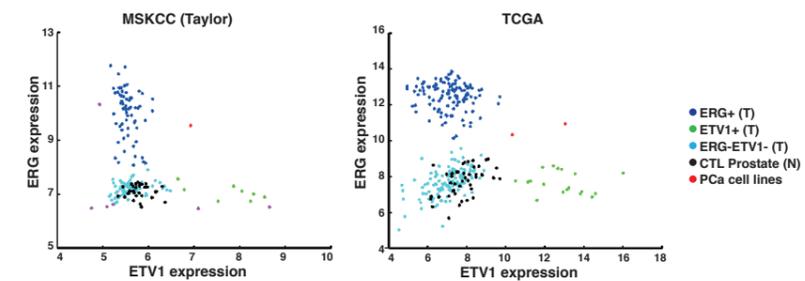
### Molecular heterogeneity of prostate tumours

Next generation sequencing has revealed the presence of recurrent mutations in primary and metastatic samples, however functional characterisation is required to elucidate the driving forces for lethal PCa. Indeed, the interaction of prostate growth signals with signalling through the androgen receptor (AR), a hormone-activated transcription factor in the nuclear receptor superfamily, is critical for normal prostate development and prostate cancer (PCa) progression.

One of the key PCa pathway alterations is found in the ETS transcription factor gene fusions which occurs in ~50% of human prostate cancer cases, where several members of the ETS family are rearranged under androgen response elements. Although these fusions occur early in the disease spectrum, the most frequently occurring ETS fusions, *TMPRSS2-ETV1* and *TMPRSS2-ERG*, cannot alone promote the development of adenocarcinoma in transgenic mouse models of prostate cancer. In fact, cooperation of *ETV1* with *PTEN* deletion is

needed for aggressive cancer to develop, and this is important to note when designing combination trials. Specifically, *ETV1* directs androgen synthesis as well as a pro-inflammatory gene signature, providing new insights into how this oncogene acts to promote hormone unresponsive lethal disease.

Altered cellular metabolism is a hallmark of cancer, contributing to malignant transformation and tumour progression. Our current data show that forced expression of *ETV1* in normal prostate epithelial cells increases expression of genes involved in metabolism and inflammation. More specifically, although *ETV1* expression is insufficient to transform prostate epithelial cells, our expression and chromatin-binding studies show that the metabolic program of *ETV1*-expressing prostate cells resembles those of cancer cells, as indicated by upregulation of genes involved in glycolysis and steroid/lipid biosynthesis, supported by mass spectrometry-based metabolome analysis on human prostate cancer cells with different levels of *ETV1*. In addition, this group of metabolic genes significantly contributes to the enrichment of the upregulated signature in the human lethality signature and in *ETV1*-expressing tumours. Interestingly, analysis of *ETV1*-expressing PCa patients revealed a strong inflammatory signature. We are currently evaluating which specific downstream targets of *ETV1* are critical drivers of metabolic reprogramming and its relationship with the co-expressed inflammatory signature as responsible for prostate epithelial cell transformation. We are currently characterising the selected *ETV1* direct targets involved in lipid metabolism and inflammation showing altered expression in *ETV1*-expressing patients in TCGA and MSKCC cohort patients (Figure 1). In



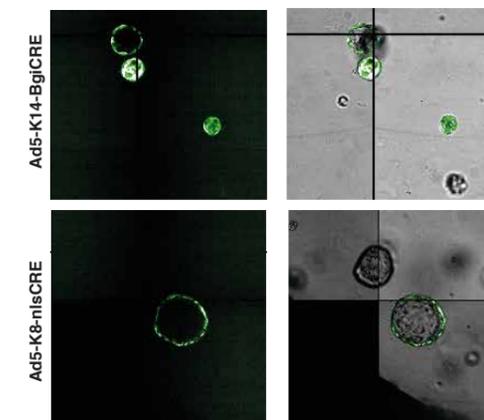
**Figure 1**  
Expression of ERG and ETV1 in prostate cancer patient TCGA and MSKCC cohorts (Cancer Genome Atlas Research Network, 2015; Taylor et al., 2010). ERG and ETV1 expression are mutually exclusive and constitute different disease subtypes.

addition, we will perform a drug screen against metabolic and inflammatory pathways, specifically in the context of androgen deprivation therapies. To determine if the genes targeted are acting downstream of *ETV1*, we will perform reciprocal rescue experiments. Then, we will test the requirements for selected *ETV1* metabolism and/or inflammation-associated targets for tumour growth and immune cell recruitment in vitro using human PCa primary cell organoid co-culture, and in vivo using our *Tmprss2-ETV1* and preclinical *ETV1*-xenograft mouse models. We anticipate that *ETV1* metabolic targets and its inflammatory counterpart will be potential tumour targets to pursue for improving current therapeutics.

### Cellular heterogeneity of prostate tumours

Tumours contain genetically heterogeneous cellular clones which constantly evolve during disease progression and clinical treatment. Clonal evolution, driven by genetic instability of cancer cells, generates cellular heterogeneity and promotes tumour progression. On the other hand, growing evidence proposes that tumour initiation from distinct cell types in the lineage hierarchy gives rise to tumour subtypes with different prognoses and/or treatment responses. The occurrence of CRPC following androgen-deprivation therapy is a strong indicator that within the prostate tumour there are subpopulations of castration resistant progenitor cells capable of driving tumour progression towards more aggressive disease. Recently, several groups have used elegant lineage-tracing approaches to demonstrate that

**Figure 2**  
Generation of prostate epithelial organoids from lineage-marked luminal cells. Views of YFP-infected organoids that are filled or hollow.



basal and luminal lineages within the adult prostate are largely maintained separately by their unipotent progenitor cells under physiological homeostatic conditions. In particular, these studies have supported the existence of luminal stem/progenitor cells that repopulate the luminal lineage by functioning as castration-resistant (CR) luminal stem/progenitor cells with luminal epithelial regeneration capacity and serve as effective cell-of-origin of PCa.

Understanding the cell distribution in a given population under castration (androgen-deprived) conditions may facilitate further understanding of tumour initiation, and tumour evolution towards the castration-resistant stage. Indeed, the identification of inherently CR tumour-initiating cells would be key to the development of novel therapeutic approaches or combinatorial treatments. Our long-term research goals are directed toward identifying cells of origin of CRPC, and the pathways responsible for the resistance to current therapeutics.

By coupling single-cell gene expression with a lineage-tracing approach, we have uncovered remarkable heterogeneity within the prostate luminal lineage and are currently validating novel markers of prostate stem/progenitor cells and CR luminal cells which can directly contribute to more advanced PCa. This approach has identified novel cell surface markers enriched in CR prostate subpopulations carrying specific genetic/epigenetic expression profile. We are isolating the diverse novel subpopulations and testing their differentiation potential and lineage specifications in vitro (organoid 3D culture) and in vivo (hormone-naïve versus castrated mice). Next, we will evaluate whether the CR luminal subpopulations defined in our single-cell screening might represent tumour-initiating or tumour-propagating cells of CRPC, using a conditional allele of the *Pten* tumour suppressor for inducible inactivation in mice (viral CRE recombinase). Indeed, the cell distribution in a given population under androgen-deprived conditions may facilitate further understanding of tumour initiation, and tumour evolution towards the castration-resistant stage. In parallel, these novel cell-surface markers are under evaluation in human prostate cells, and we will move towards the isolation of human tumour subpopulations to assay directly for tumour-initiation/propagation capacity.

Understanding and elucidating the roles of and the interrelationship between the molecular and cellular heterogeneity will offer fresh insight in designing novel therapeutics to target lethal CRPC and metastasis.

## RNA BIOLOGY

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RNA-Biology-Computational-Biology/Home



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The RNA Biology group is interested in how changes in gene expression drive the changes that lead to tumour growth, development and maintenance. The group is particularly focused on the role played by noncoding RNAs (ncRNAs) in modulating these processes. Noncoding RNAs are transcripts that never get translated into proteins.

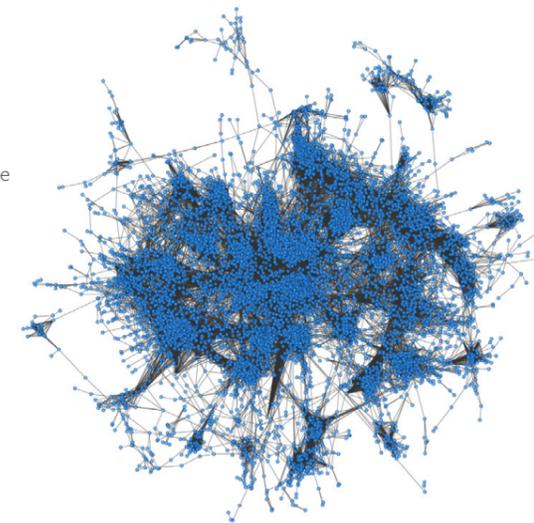
Increasing amounts of evidence indicates that ncRNAs can be functional in their own right, and that they often serve to regulate the expression of other, often protein-coding, genes. The RNA Biology group is a highly interdisciplinary group that uses computational biology and bioinformatics to identify candidate noncoding RNAs of relevance in cancer, which it then follows up experimentally at the bench.

One of the surprises following the initial draft sequencing of the human genome in 2000 was the relatively small number of protein coding genes in humans in comparison with other organisms. It is now known that less than 2% of the genome encodes proteins, raising the question as to what, if anything, the remaining 98% of the genome does. Advances in microarray technology and, more recently, deep sequencing, have revealed substantial numbers of transcripts expressed from these noncoding regions. It is now known that as much as 90% of the genome is transcribed and as more samples are sequenced in different contexts, this number continues to grow. While some may encode novel proteins (Bitton et al. PLoS ONE 2010; Bitton et al. Genetics 2011), the majority do not. Although the vast majority of these noncoding transcripts have yet to be characterised, those that have been studied exhibit a variety of different functions driven by their interactions with DNA, with other RNA molecules and with proteins. Often these appear to be regulatory. Since noncoding genes are just as susceptible to mutations as protein-coding loci, the noncoding genome may provide a large repository of novel tumour suppressors and oncogenes. The primary goal of the RNA Biology group is to find these genes and study them.

### Data analysis pipelines

RNA sequencing analysis typically involves aligning the billions of short reads produced by a deep sequencer to the reference genome, asking which of these aligned reads overlap a known gene and then counting the number that hit each locus. This is then used to provide an estimate of the amount of that transcript in the original sample. The process however, is confounded by a number of factors. Firstly, splicing, in which the cell removes different parts of the nascent transcript (introns) before joining the remaining fragments (exons) together to yield the mature transcript; this means that most sequenced RNA molecules don't align perfectly to the genome, and techniques are required to manage reads that start at the end of one exon and finish at the beginning of the next. Secondly, counting only reads that hit known genes makes it hard to identify novel noncoding transcripts emerging from previously uncharacterised loci. The RNA Biology group has been developing sample specific de novo annotation strategies to deal with these issues. These strategies aim to incorporate reads that do not fit current database models of the human genome alongside those that do. In this way novel dynamic changes in transcription can be modelled. A third issue, and central to many aspects of cancer biology, is the question of how mutations in the genome affect function (see Hudson et al. Pharmacogenomics, 2015 for a review). We continue to collaborate with other groups in the Institute to call mutations, and are increasingly interested in how these affect noncoding loci. A major output of the group has been the development of novel strategies for handling deep sequencing data.

**Figure 1**  
Co-expression networks  
built from human tumours.  
Each node in the network  
represents a gene, with links  
joining genes with similar  
expression profiles across the  
tumour dataset.



### High Performance Computing (HPC)

The commissioning of a large HPC facility on site has been a particularly exciting development for the group since this offers a step change in the volume and complexity of the data we are able to analyse. Three postdocs in the group are developing novel algorithms for analysing the 'big data' that is emerging from the cancer genomics community (see for example, Figure 1). In addition, we are collaborating increasingly with the Scientific Computing Team to make these algorithms run effectively on our HPC systems leading to substantial speed-ups over initial more naive implementations.

### Single cell genomics

In collaboration with the Clinical and Experimental Pharmacology group and the Faculty of Life Sciences within The University of Manchester, we have established a Single Cell Research Centre Funded by the MRC. Chang Kim, a postdoc within the group, is now developing novel analysis pipelines that exploit the parallelism available from HPC to deal with the high volumes of data that can arise from single cell experiments. In addition we have continued to collaborate with CEP on the analysis of single cell data.

### Noncoding RNA analysis

A major aspect of the group's work is the downstream characterisation of noncoding RNAs of relevance in cancer. We use bioinformatics models to predict possible functions for these transcripts and pursue them at the bench. In many cases, model organisms such as Fission Yeast (*Schizosaccharomyces pombe*) offer experimentally tractable systems with which to explore basic mechanism and function. In the context of noncoding RNAs, *S. pombe* is particularly useful because the key protein-coding genes known to be involved in noncoding RNA-driven mechanisms, including kinase signalling, chromatin interacting enzymes and siRNA pathways, are conserved

with humans, and we have continued to collaborate with the Cell Division group to investigate basic mechanism.

Increasingly, new technologies such as CRISPR are making it possible to perform mechanistic characterisations in human cell lines. We are now exploiting these new approaches to investigate noncoding RNA function directly in human cells. A major aspect of this year's work has been establishing these protocols within the lab, and we now have a dedicated team of postdocs and students working on human long noncoding RNAs (lncRNAs) with a particular emphasis on their role in lung cancer.

### Computational Biology Support

Hui Sun Leong, Christopher Wirth,  
Sudhakar Sahoo<sup>1</sup>

<sup>1</sup>joined in 2015

The Computational Biology Support Team was established in March 2015 to deliver bioinformatics support and collaborative data analysis solutions to all CRUK core funded groups within the Institute. The group is committed to providing a strong infrastructure that enables the application of advanced bioinformatics analyses to cutting-edge scientific research.

In the last year, the group has worked closely with individual research groups as well as other service units in the Institute (in particular the Scientific Computing and Molecular Biology Core Facilities) to develop pipelines and statistical approaches for analysing high-throughput datasets generated from next generation sequencing (NGS) platforms, including RNA-seq, smallRNA-seq, ChIP-seq, exome, targeted re-sequencing and whole-genome and DNA sequencing, among many others. These collaborative projects have contributed to a number of high-impact publications (e.g. Rothwell et al. Mol Oncol. 2015; Antal et al. Cell 2015; Somerville et al. Cancer Cell 2015; and Hudson et al. Pharmacogenomics 2015).

In addition to the ongoing development of analysis pipelines for NGS-based tasks, the group has also been exploring new methods and tools for more customised project needs (e.g. genome-wide CRISPR/Cas9 knockout screens; integrative analysis of public and private multi-'omics' datasets). In 2016, the group will be working with the Biological Mass Spectrometry Facility and Systems Oncology group to develop dedicated workflows for analysing high-throughput proteomics datasets generated by research programmes within the Institute.

**Publications listed on page 57**

# 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 programmes 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.

## Overview

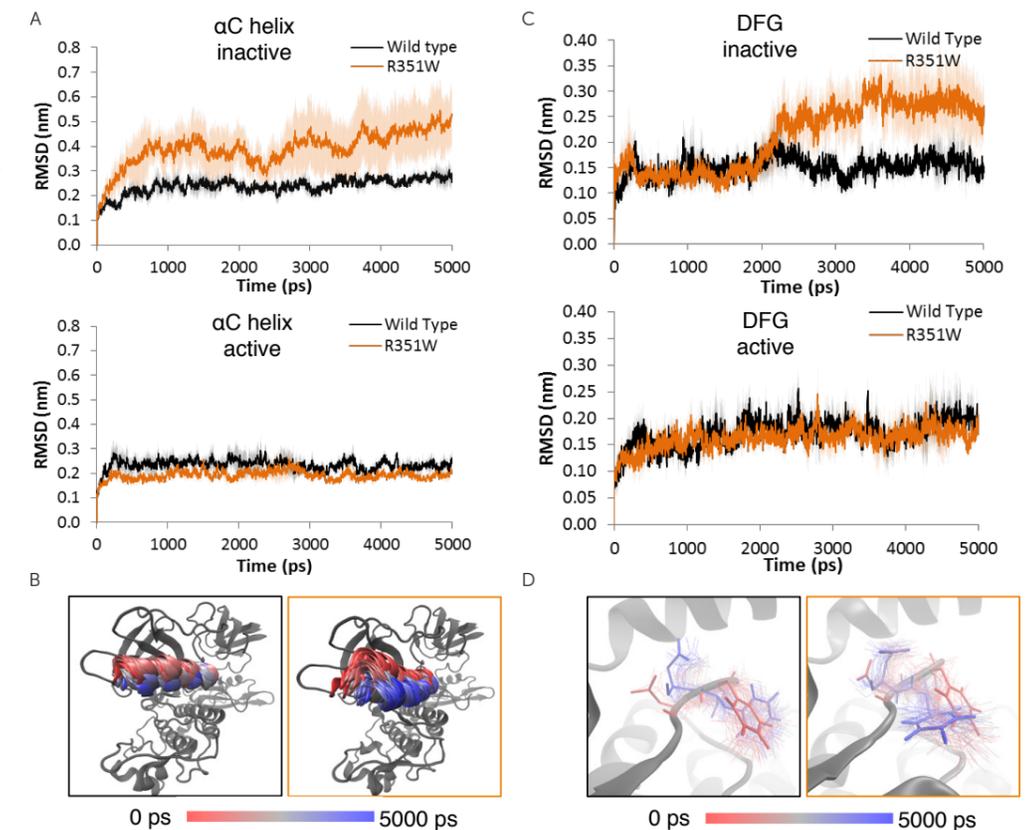
The lab utilises a multitude of strategies to identify critical pathways required to promote tumorigenesis. These include high-throughput bioinformatics and structural modelling, siRNA screening, and precision genome editing to establish various functional genomic approaches to identify novel drivers. 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 whether the mutations are likely to 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. in press, see also Figure 1), 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.

## Discrepancies in cancer genomic sequencing highlight opportunities for driver mutation discovery

In an additional approach, the lab focuses on uncovering novel mutations that reside in unsequenced regions of the exome (cold-spots) that may have been missed by large cancer genomic consortiums. To identify these cold-spots, we performed a comparison of two of the most prominent cancer genome sequencing databases from different institutes (CCLE and COSMIC), which revealed marked discrepancies in the detection of missense mutations in identical cell lines. We demonstrated, using a newly identified PAK4

Figure 1  
ABL1 mutations lead to an intermediate increase in kinase activity.  
A–D (A, C) Root-mean-square deviation (RMSD) analysis of the movement of (A) aC-helix and (C) DFG motif in the active and inactive conformation of ABL1 kinase with R351W mutation (orange) compared to the wild-type (WT) kinase (black). (B, D) Time-lapse images show movement within the aC-helix (B) and DFG motif (D) of the inactive structure of the WT (black outline) and R351W (orange outline) constructs.



mutation as proof of principle, that specific sequencing of these GC-rich cold-spot regions can lead to the identification of novel driver mutations in known tumour suppressors and oncogenes.

## Cancer-associated protein kinase C mutations reveal kinase's role as tumour suppressor

We also focus on studying the PKC family of kinases, which have been intensely investigated for over 25 years in the context of cancer. Historically, this arises from the discovery of PKC as the receptor for the tumour-promoting phorbol esters, which suggested that activation of PKC by phorbol esters promoted tumorigenesis induced by carcinogens. However, this interpretation is now open to question, since long-term treatment with phorbol esters is known to initiate degradation of PKC, thus down-regulating its activity. In collaboration with Dr. Alexandra Newton's lab at UCSD, we performed a bioinformatics analysis to assess the frequency of PKC mutations present in cancer genomic sequencing studies and to determine the functional impact of these mutations. A majority of mutations were identified to be loss-of-function (LOF) and for heterozygous mutations they could act in a dominant negative manner to suppress the activity of other PKC isoforms. Consistent with PKCs being tumour suppressors, we identified

germline LOF mutations in PKC delta, associated with increased survival and proliferation of B cells, and the mutations were causal in juvenile lupus. In summary, our data provide compelling evidence that PKCs in general are tumour suppressors and PKC inhibitors should be used to treat cancer patients with mutations in this family of kinases.

## The role of the Mixed Lineage Kinases in cancer

Lastly, the lab investigates the role of a novel family of kinases, the Mixed Lineage Kinases (MLKs), in various forms of cancer including lung and colon cancer, melanoma, and head and neck squamous cell carcinoma. We recently demonstrated that the MLK1-4 promotes resistance to RAF inhibitors in melanoma by directly phosphorylating MEK to reactivate the MEK/ERK pathway. These kinases play a complex role and can act as both tumour suppressors and oncogenes depending on the genetic make-up and origin of the cancer. The lab will continue to investigate the importance of this family of kinases and the signalling pathways they regulate in various forms of cancer and assess whether inhibition of the MLKs can be exploited to suppress tumorigenesis for specific types of cancer, including lung cancer.

**Publications listed on page 58**



# STEM CELL HAEMATOPOIESIS

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A highly-held therapeutic goal of the stem cell field aims at generating in vitro cell populations usable in the clinic for regenerative medicine purposes. For example, the in vitro generation of haematopoietic stem cells would be of great interest for therapeutic applications such as the replacement of the complete blood system following chemotherapy or the treatment of haematopoietic malignancies. However, these therapeutic aims can only be achieved with a full understanding of the differentiation processes leading to the production of clinically useful cell populations. To date, this remains one of the principal limitations and most challenging tasks of the regenerative medicine field.

## Development of the haematopoietic system

In the mouse embryo, one of the best studied model systems, the haematopoietic system develops in successive waves, each characterised by specific location, time and progenitors produced. The first wave occurs around E7.5 in the yolk-sac and gives rise to primitive haematopoiesis including primitive erythrocytes, macrophages and megakaryocytes. This is shortly followed at E8.25 by a second wave, also initiated within the yolk-sac and generating definitive erythromyeloid progenitors. A third wave of haematopoiesis occurs from E10.5 onward in the dorsal aorta, the vitelline and umbilical arteries. This intra-embryonic wave of blood development leads to the generation of haematopoietic stem cells (HSCs). Shortly after their formation, HSCs seed the foetal liver where they actively proliferate; later these cells migrate to the bone marrow where they reside throughout adult life producing a constant supply of blood cells. At the cellular level, all blood cells are derived from mesoderm precursors which differentiate into a transient and specialised population of endothelial cells termed haemogenic endothelium (HE). Through an endothelial to haematopoietic transition, HE cells give rise to blood progenitors. This transition has been documented in most species studied to date and was shown to occur during both extra-

embryonic and intra-embryonic haematopoiesis. As such, the HE plays a central role in the establishment of the haematopoietic system. However, despite its fundamental importance, we know very little about the origin and potential of this cell population.

## Haemogenic competence of endothelial progenitors

A large body of work has emerged in recent years regarding the role of the ETS transcription factor ETV2 at the onset of blood and endothelium specification. The expression of ETV2 is restricted to a narrow window of embryonic development between E7.0 and E9.5. In the absence of ETV2, mesodermal progenitors differentiate toward the cardiomyocyte fate concomitantly with a complete defect in haematopoiesis and vasculogenesis. It is now clear that the expression of ETV2 in mesoderm is essential for the progression towards haematopoiesis and endothelium. However, the molecular mechanisms that confer haemogenic competence to ETV2-expressing progenitors are poorly understood. We therefore set out to investigate the haemogenic potential of ETV2-expressing progenitor populations at the early stages of embryogenesis using a well characterised ETV2::GFP reporter mouse. We found that both the E7.5 extra-embryonic and E8.5 intra-embryonic ETV2::GFP<sup>+</sup> cells share

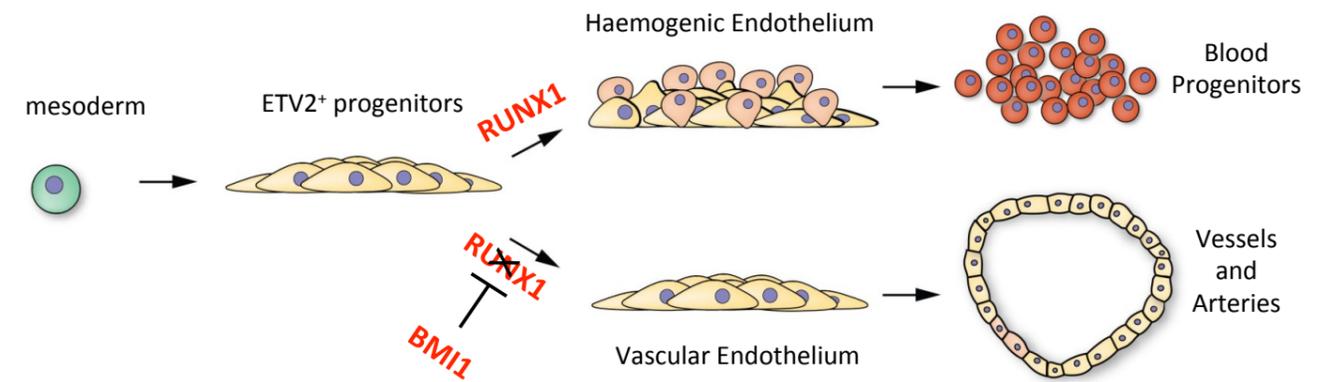


Figure 1  
During embryonic development, the cell fate of ETV2-expressing progenitors is controlled by RUNX1 expression. Silencing of Runx1 expression by BMI1 leads to specification toward vascular endothelium while expression of Runx1 leads to specification toward blood progenitors

similar expression of FLK1, TIE2 and cKIT markers of HE, while lacking expression of CD41, marking the first emerging blood cells. When plated in conditions that promote HE maintenance and growth, the E8.5 population maintained an endothelial identity retaining high expression of FLK1 and TIE2 while the E7.5 population downregulated the expression of these endothelial markers in CD41<sup>+</sup> emerging cells. Accordingly, E8.5 cells gave rise to very few haematopoietic colonies when plated in clonogenic assays. Global gene expression analysis identified *Runx1* as one of the few haematopoietic transcription factors highly differentially regulated between the two ETV2::GFP<sup>+</sup> populations, a finding further validated by in situ immunofluorescence and single cell expression analysis. To examine the functional role of *Runx1* in ETV2::GFP<sup>+</sup> cells we ectopically expressed *Runx1* in this population isolated from E8.5 embryos. Interestingly, *Runx1* expression dramatically increased the haemogenic potential of these cells, demonstrating that the expression of *Runx1* on its own is sufficient to confer haemogenic competence in ETV2<sup>+</sup> progenitors.

The role of *Runx1* in the emergence of HSCs and haematopoietic progenitors has been well characterised both in vitro and in vivo. *Runx1*-deficient mice fail to generate haematopoietic stem and progenitor cells. Our results demonstrate that the ectopic expression of *Runx1* alone is sufficient to confer haemogenic competence to E8.5 ETV2<sup>+</sup> endothelial progenitors which lack *Runx1* expression and the potential to generate blood progenitors. These findings reveal that the entire molecular framework required for blood specification is present in these endothelial progenitors with the notable exception of RUNX1. To uncover the molecular mechanism involved in the silencing of *Runx1* expression, we interrogated the global transcriptomic data of E7.5 and E8.5 endothelial progenitors. This analysis revealed that BMI1, a subunit of the Polycomb repressive complex was highly expressed in E8.5 ETV2::GFP<sup>+</sup> but not detectable in E7.5 ETV2::GFP<sup>+</sup> cells. To assess

the role of BMI1 in *Runx1* silencing, we performed loss-of-function experiments. BMI1 inhibition in the E8.5 population resulted in an increase in *Runx1* expression along with other haematopoietic genes, but also in an increase in the haemogenic potential of this population. While the role and requirement of BMI1 at later stages of haematopoiesis is well established, its role in early endothelial progenitors has not previously been addressed. Our data identify a novel role for BMI1 in maintaining an endothelial fate in E8.5 ETV2-expressing progenitors. This finding suggests that in E8.5 ETV2<sup>+</sup> progenitors the haematopoietic programme is silenced via a Polycomb-mediated repression mechanism. The ectopic re-expression of *Runx1* or its de-repressed expression via BMI1 inhibition results in the acquisition of haemogenic fate, suggesting that the haematopoietic programme needs to be repressed for further progression of the endothelial fate as embryonic development progresses.

Our study suggests that during early embryonic development blood and endothelium cell fates are differentially established through the active repression of RUNX1 in common progenitors to allow endothelial fate to proceed (see Figure 1). Altogether, our results raise questions about the plasticity of early mesoderm progenitors and the window of opportunity for differential cell fates that might help us devise more efficient protocols for the derivation of repopulating blood progenitors in vitro.

Publications listed on page 59



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<sup>1</sup>left in 2015  
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In the Systems Oncology group we focus on understanding 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 "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. The general appreciation is that interactions between tumour cells and "normal" cells in the tumour stroma modulate key processes such as tumour progression, metastasis and response to therapy. As such, it is key to determine how tumour cells exchange signals with cells in the stroma and how specific signals from the tumour cells can co-opt the stromal cells to promote malignant progression.

**Integration of protein kinase interaction maps with phenotypic analysis to estimate information flow in signalling networks**

Protein kinases are key modulators of cellular signal processes and regulate a multitude of cellular functions such as protein localisation, protein-protein interactions, protein half-life and enzymatic activity. However, how information flows through individual pathways is notoriously difficult to assign on a global scale. As such, generating models of cellular signal networks and their response to treatment is challenging. Recently, together with collaborators at the Lunenfeld-Tanenbaum Research Institute in Toronto, we conducted a global analysis of protein kinase signalling networks integrating protein-interaction analysis with RNA interference screening and quantitative phosphoproteomics to develop a framework to assign kinases that may influence response to treatment (So et al., Sci Signal 2015). Specifically, we conducted a global proteomics analysis of protein interactions of 107 protein kinases following stimulation with the pro-apoptotic ligand TRAIL. In parallel we conducted a phenotypic analysis to assign a pro- or anti-apoptotic role of the kinases using a combination of RNA interference screening and overexpression analysis. Integrating quantitative phosphoproteomics analysis and

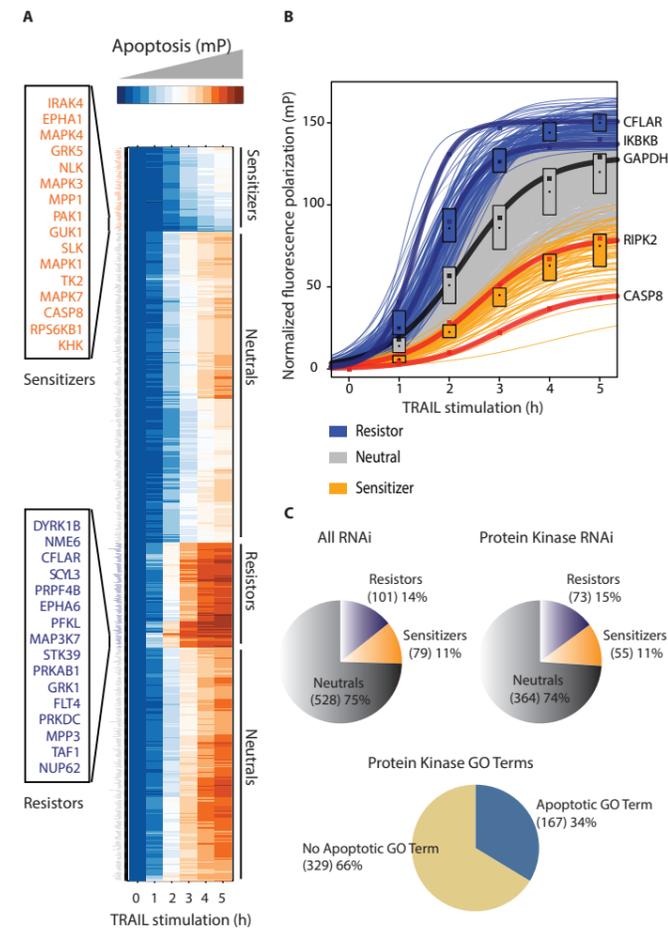
computational prediction of kinase-substrate relationships, together with the interaction analysis and phenotypic screening, we assembled protein interaction maps. Using these maps, we modelled information flow through the networks and identified apoptosis-modifying kinases. Together we present a resource of potential targets that modify TRAIL-induced apoptosis.

**Development of a mass spectrometry assay to quantify kinases**

In addition to protein kinase activity, levels of expression and stability are also frequently altered in tumour cells. However, robust quantification of all protein kinases is not easily obtained due to a low level of expression and the lack of reagents (antibodies). To address this we have developed a mass spectrometric assay to accurately quantify human kinases (Worboys et al., Nat Methods 2014). In order to quantify most proteins by mass spectrometry they are enzymatically cleaved into peptides, which then are measured as proxies for the protein level. Importantly, the underlying assumption is that peptides unique to the protein can be used to determine their level of expression. However, for proteins that are subjected to extensive post-translational modifications, such as protein kinases, this may not always hold true. In fact, we observed that indeed it is critical to assess how well each peptide represents the level of the intact protein in order to avoid bias in the measurement. Using this assay, we have quantified the levels of protein kinases across human pancreatic cancer cells to identify putative targets with altered levels of expression.

**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

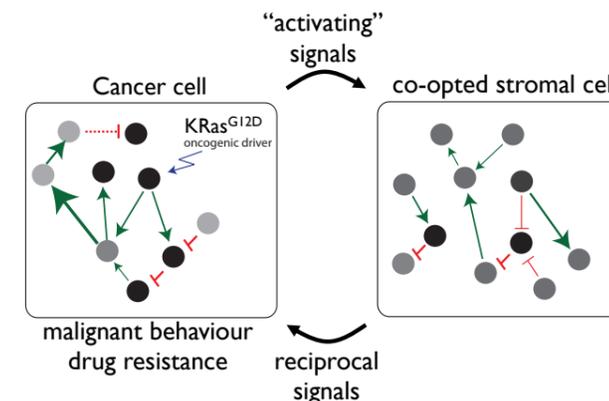


**Figure 1** Identification of apoptosis modulating kinases by RNA interference screening. A) Hierarchical clustering of siRNA screening data. Kinases were assigned as 'sensitizers', 'resistors' or 'neutrals' depending on their modulatory role of a caspase 3 reporter. B) Median reporter readout of all siRNA targets. C) Summary of screen results of all siRNA targets and GO term distribution.

**Figure 2** Working model of reciprocal tumour-stroma signalling. Expression of mutant KRas in tumour cells leads to changes in their cellular signalling network alongside increased secretion of paracrine acting growth factors, cytokines and morphogens. Neighbouring genetically normal stromal cells respond to these changes and consequently adapt an 'activated' phenotype, which contribute to the desmoplastic reaction by increased expression of extracellular matrix proteins and growth factors. These changes to the extracellular environment are hypothesised to elicit a subsequent change to the signalling in the tumour cells, leading to the increased aggressive behaviour and resistance to therapy.

diagnosis. The most frequent 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 TP53 and SMAD4 function occurring in 50-60% of all cases. Moreover a high number of low-frequency mutations have been identified.

The clinical benefit of chemotherapy is very limited and the abundant stroma is known to confer therapeutic resistance. Critically, PDAC is characterised by an extensive activated stroma and desmoplasia, which also affects tumour growth and metastasis. However, the mechanisms whereby tumour cells recruit and co-opt "normal" stromal cells and conversely how the stromal cells support tumour cell growth and impair drug sensitivity is not well



known. Delineating these mechanisms is therefore important and may lead to the identification of novel therapeutic targets in both the tumour and the genetically stable stromal cells.

**Analysis of tumour-stroma signalling through cell-specific labelling and quantitative proteomic analysis**

Critical to our understanding of cellular communication is our ability to quantitatively determine how signal transduction cascades are regulated, in a multicellular environment. Although solid tumours contain multiple cell types, most studies of cellular signalling are conducted in isolated, homogeneous cell populations. To define how tumour and stromal cells exchange signals we utilise state of the art mass spectrometry, which allows us to interrogate the cellular proteome and post translational processes in a robust and quantitative manner. To analyse reciprocal cell communication, we have developed methods for labelling heterotypic cellular populations, which allows the assignment of identified signalling molecules to their cell of origin (Jorgensen et al., Science 2009). As such, we perform direct co-cultures between tumour and stromal cells and analyse how signalling is specifically regulated in the individual cell types. The major advantage of this approach is that all exchanged signals are included in the analysis and as such, heterotypic cell signalling is interrogated as a multivariate system. More recently, we have developed an improved methodology based on cell-specific labelling using precursors of amino acids, which has allowed us to study direct co-cultures for up to 10 days (Tape et al., Mol Cell Proteomics 2014). Because extended co-cultures permit signals to evolve, for example, a signal from cell type A accumulates sufficiently to activate cell type B, which then elicits a reciprocal signal to cell type A, individual signals can be perturbed and the consequences can be determined. In essence, this approach has dramatically improved our ability to interrogate how reciprocal cellular signalling networks develop between tumour and stromal cells.

Publications listed on page 60

# TRANSCRIPTIONAL NETWORKS IN LUNG CANCER

[www.cruk.manchester.ac.uk/Research/CRUK-MI-Groups/Transcriptional-Networks-in-Lung-Cancer/Home](http://www.cruk.manchester.ac.uk/Research/CRUK-MI-Groups/Transcriptional-Networks-in-Lung-Cancer/Home)



**Group Leader**  
Michela Garofalo

**Postdoctoral Fellows**  
Srivatsava Naidu  
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**Scientific Officer**  
Peter Magee

**Graduate Student**  
Athanasios Paliouras<sup>†</sup>

<sup>†</sup>joined in 2015

MicroRNAs (miRNAs), single stranded RNAs, 19–25 nucleotides in length that negatively regulate gene expression by translational inhibition or degradation of messenger RNA targets, are key players in cancer onset and progression, functioning as tumour promoters (TP) or tumour suppressors (TS). Resistance to cancer chemotherapeutic treatment is a common phenomenon, which ultimately represents the main cause of cancer-related deaths. Emerging evidence shows that microRNAs regulate drug sensitivity of tumour cells.

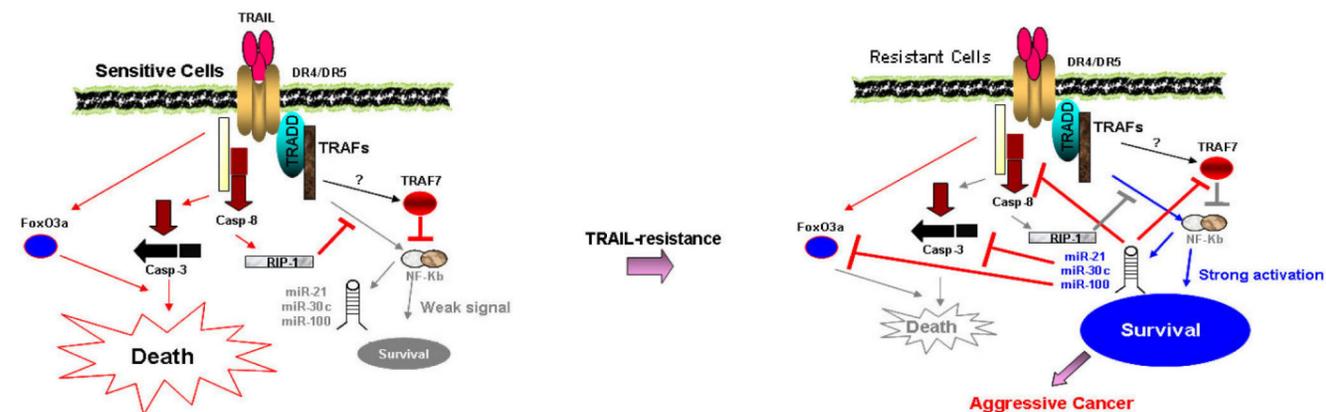
We found that different miRNAs play a fundamental role in the response to TRAIL-induced apoptosis by targeting important tumour suppressor genes. Moreover, we showed the therapeutic efficacy of anti-miR-31 in vivo in esophageal squamous cell carcinoma (ESCC), a major cause of cancer death worldwide. We discovered that miR-31 by targeting STK40, an NF-κB inhibitor, induced NF-κB activation and upregulation of inflammatory genes that led to cell proliferation and ESCC. Importantly, downregulation of miR-31 in vivo by using anti-miRNA oligonucleotides was able to reduce inflammation and the incidence of ESCC.

## MicroRNAs and acquired TRAIL resistance

TRAIL is a member of the TNF superfamily and is considered a potential anti-cancer agent, as it shows selective high cytotoxicity towards tumour cells and little or no toxicity against normal cells (Ashkenazi et al., 1998). The anticancer activity of TRAIL is ascribable to its ability to induce apoptosis through the binding of DR4 and DR5 and the recruitment of intracellular apoptosis-initiating caspase-8 through Fas-associated death domain (FADD) for the assembly of the death-inducing signalling complex (DISC). The cleavage of caspase-8 in the DISC is a critical upstream event in TNF family ligand-induced apoptosis. Cross-linking of TRAIL to its death receptors leads to the recruitment of Fas-associated death domain, procaspase-8, and procaspase-10 to form DISC (Bodmer et al., 2000; Kischkel et al., 1995; Muzio et al., 1998). TRAIL is a promising

anticancer agent that can be potentially utilised as an alternative or complementary therapy because of its specific anti-tumour activity. However, TRAIL can also stimulate the proliferation of cancer cells through the activation of NF-κB, but the exact mechanism is mostly unknown. To understand the mechanisms involved in acquired TRAIL resistance we established TRAIL-resistant human isogenic cell lines (H460R and H292R) from the parental TRAIL-sensitive (H460S and H292S) cells. Both cell lines H460R and H292R had similar TRAIL receptor expression levels compared to the corresponding sensitive cells and did not show any mutation in their sequences, suggesting that the resistance may be linked to the alteration of downstream pathways.

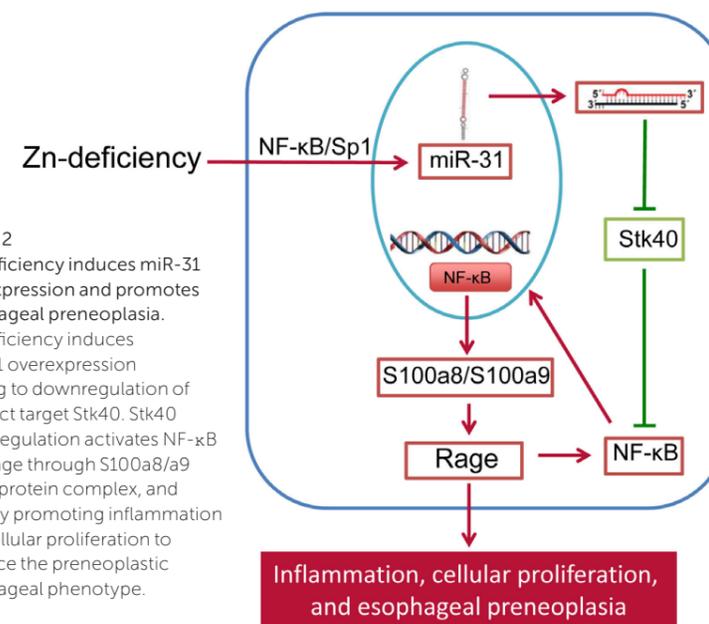
Therefore, we analysed the miRNA expression profile in H460R versus H460S, using nanoString technology. Several miRNAs were found dysregulated and we focused on three microRNAs with the highest fold change in expression: miR-21, miR-30c and miR-100. Enforced expression of these three miRNAs was sufficient to induce resistance to the drug by directly targeting fundamental tumour suppressor genes and effectors of the TRAIL pathway, such as *caspase-8*, *caspase-3*, *FoxO3a* and *TRAF-7*, an NF-κB inhibitor. Of note, downregulation of caspase-8 by miR-21 blocked RIP1 cleavage and induced the activation of NF-κB, which transcriptionally activates these miRNAs (Figure 1). Thus, TRAIL activates a positive feedback loop that sustains



**Figure 1** NF-κB induces acquired TRAIL resistance and EMT through miR-21, miR-30c and miR-100 activation. In TRAIL-sensitive cells, the binding of TRAIL to its receptors induces cell death through the canonical caspase-cascade and FoxO3a up-regulation. In cells with acquired TRAIL-resistance subtoxic concentrations of TRAIL are not sufficient to induce apoptosis and lead to NF-κB activation, which in turn transcriptionally regulates miR-21, miR-30c and miR-100. Downregulation of caspase-8, caspase-3, FoxO3a and TRAF-7 by these microRNAs causes acquired resistance to TRAIL and epithelial-mesenchymal transition (EMT).

the acquired resistance and causes an aggressive phenotype (Figure 1). Finally, we proved that combinatory treatment of NF-κB inhibitors and TRAIL is able to reverse resistance and reduce tumour growth with important consequences for clinical practice (Jeon et al., 2015).

Conversely, miR-148a was the most downregulated microRNA in NSCLC cells with both primary and acquired TRAIL resistance observed, when compared to TRAIL-sensitive cells. MMP15 and ROCK1 have been identified as miR-148a direct targets. Rho-associated coiled protein (Ser/Thr) kinase-1 (ROCK1) is an essential effector kinase downstream of Rho GTPases that has a central role in many motile responses that involve the actin cytoskeleton and/or microtubule network, from neurite extension to phagocytosis and cancer-cell invasion (Riento et al., 2003). ROCK1 has been implicated in anchorage-independent growth and metastasis in several cancers (Sahai et al., 1999; Zohrabian et al., 2009). MMP15 is a membrane-type metalloproteinase that has also been identified as an anti-apoptotic marker in cancer cells and its inhibition increases



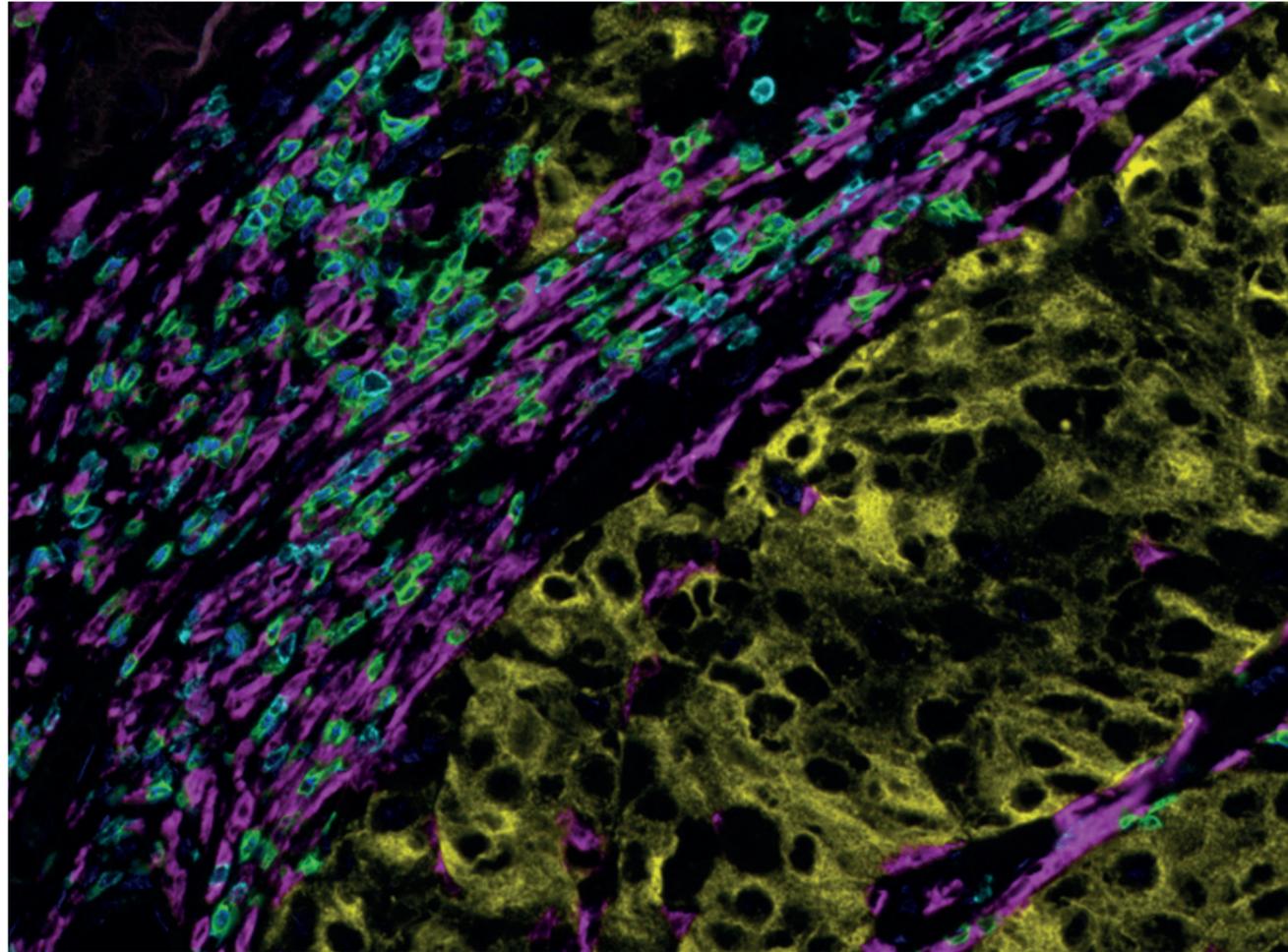
**Figure 2** Zn-deficiency induces miR-31 overexpression and promotes esophageal preneoplasia. Zn-deficiency induces miR-31 overexpression leading to downregulation of its direct target Stk40. Stk40 downregulation activates NF-κB and RAGE through S100a8/a9 dimer protein complex, and thereby promoting inflammation and cellular proliferation to produce the preneoplastic esophageal phenotype.

sensitivity to TRAIL-induced apoptosis (Abraham et al., 2005). In TRAIL-resistant cells, miR-148a induced apoptosis after TRAIL treatment by regulating MMP15 expression. miR-148a inhibited cell migration and invasion through the silencing of both MMP15 and ROCK1. Furthermore, we validated the inverse relationship between miR-148a and the two target genes in lung tumour tissue samples (Joshi et al., 2015).

## MicroRNAs in ESCC

Esophageal squamous cell carcinoma (ESCC) is a major cause of cancer death worldwide (Ferlay et al., 2008). Due to lack of early symptoms, ESCC is typically diagnosed at an advanced stage, and only 10% of patients survive 5-years. Thus, clarification of the mechanisms underlying the pathogenesis of ESCC and development of new prevention and therapeutic strategies are urgently needed. Risk factors for ESCC include alcohol and tobacco use and known nutritional factors such as zinc-deficiency (ZD). Together with collaborators at The Ohio State University, we tested the effect of ZD on the incidence of ESCC in rats. ZD induced esophagi hyperplasia and upregulation of genes involved in inflammation. Combination of a prolonged zinc deficient diet and low doses of an environmental carcinogen N-nitrosomethylbenzylamine (NMBA) gave rise to ESCC in 70% of the rats. Interestingly, esophagi of rats fed with a zinc deficient diet showed an increase of oncogenic microRNAs compared to esophagi of rats fed with a zinc sufficient diet, with miR-31 as the most upregulated one. We discovered that miR-31 by targeting STK40, an NF-κB inhibitor, induced NF-κB activation and upregulation of inflammatory genes that led to cell proliferation and ESCC (Figure 2) (Taccioli et al., 2015). In vivo miR-31 antagonism by systemically administered LNA-anti-miR decreased miR-31 overexpression in esophagus/tongue and circulating blood and prevented the development of a preneoplastic phenotype in esophagus and tongue in ZD rats.

**Publications listed on page 60**



Melanoma tissue fluorescently co-stained for melanoma-specific marker HMB45 (yellow) and immune cell markers CD163 (purple), CD8 (green) and CD4 (cyan).

*Image supplied by Gabriela Gremel (Molecular Oncology)*

# CANCER RESEARCH UK MANCHESTER INSTITUTE

RESEARCH SERVICES



Chief Laboratory Officer  
Stuart Pepper

It has been another busy year for the core facilities which has seen not only continued development of existing services, but the creation of a new facility for the production of novel transgenic murine models. The new transgenic facility will complement the expanded breeding and experimental facilities detailed in the pages below, giving researchers the potential to conduct a broad range of experimental protocols on novel transgenic models.

### Chief Laboratory Officer Stuart Pepper

Existing facilities have concentrated on exploiting the capabilities of instruments bought over the last two years to offer a broad range of services including multiplex histological analysis, new protein analysis services and protocols for sequence analysis of single cells.

Another exciting development has been the introduction of services to support small molecule screening. This work is supported by the Molecular Biology Core Facility who house several libraries and appropriate automation for library plating, and by Advanced Imaging using a high throughput, high content plate reader. Experimental design advice is available from the Drug Discovery Unit, ensuring optimal value is obtained from this resource.

### Advanced Imaging and Flow Cytometry

**Steve Bagley**, Jeff Barry (Deputy Manager, Flow Cytometry), Toni Banyard, Helen Bradley<sup>2</sup>, Helen Carlin<sup>1</sup>, Abi Johnson, Isabel Peset Martin<sup>1</sup>, Kang Zeng

<sup>1</sup>joined in 2015 <sup>2</sup>left in 2015

The Imaging and Cytometry Facility's remit is to provide state of the art tools for both the fundamental and translational study of cancer (from molecular interactions in primary cells through to tissue-wide responses), to introduce and develop new modalities, and assist in the application of these techniques to research endeavors. Microscopy data collection ranges

from the nanometre to centimetres of tissue; cytometry allows the sorting and analysis of up to 70,000 events a second; and high content screening permits the phenotypic analysis of cell populations across multiple conditions.

Over the year the facility has experienced rapid growth in data output, expansion of the methods available and importantly development of the team. This year the facility has provided over 3000 hours of microscopy, 5000 hours cytometry, 2500 hours of high content screening, 8000 histology slides have been imaged, and over 7000 hours of image analysis has been performed, across a total of 31 research groups.

Since 2009, the facility has been imaging histology using the automated whole slide method, where slides are received from the histology facility that have been handled and processed in a standardised manner; subsequently the whole tissue is digitised to produce very large, high detail images. Within the facility there are three systems to allow greater throughput of data. The original system was replaced this year with the Leica Versa, which has already extended our capabilities for histology based Fluorescence in situ Hybridisation (FISH) and histology that requires high magnification for the analysis of sub-cellular structures. Contrast based segmentation and image analysis is performed on all of the data to allow high content analysis, which enables complex questions to be asked regarding location, population, and phenotype.

The previous year, a Perkin Elmer Vectra was purchased which utilises a spectrophotometer rather than a camera to digitise histology data. By utilisation of a spectrophotometer, the researcher can mathematically model the light emitted from the labels attached to the molecules of interest and ask complex questions of the tissue. Over this year and in collaboration with the Histology facility and the Systems Oncology group, the modelling, imaging and analysis of seven fluorescent labels is now routine, again allowing complexity to be studied.

In histology and microscopy, the facility has introduced more automation for high throughput and high content analysis as there is a requirement to carry out standardised data capture and analysis, but also due to the high number of samples that need to be processed as quickly as possible. Over the last year Flow Cytometry has introduced automation for flow cytometry analysis, improved single cell sorting and worked alongside other facilities such as Molecular Biology Core Facility for sorting into specialised plates.

With many of the techniques available across the facility, automation is becoming invaluable for sample throughput and standardisation of output. The installation of automated histology screening and multi-well plate high content screening (with the Opera Phenix) has been expanded. The on-going development of flow cytometry automation with the BD Fortessa X20 and the BD Canto allows complex high throughput screening of non-adherent cells.

Over the year, as the supported techniques become more complex and in response to the users and the user group committee, several consultancy functions have been added to the role of the team. As imaging and flow cytometry becomes integrated into other techniques, facilities and fields of research, the requirement to work alongside the research groups and other facilities is essential. Support from the facility includes:

- panel design for flow cytometry to allow high parameter sorting and analysis
- screening design and method development (in collaboration with the Molecular Biology Core Facility and Drug Discovery)
- single cell sorting for molecular biology and biomarker functions
- expanded histology visualisation and image analysis support (in collaboration with the Histology facility)
- advice with in vivo imaging and analysis (in collaboration with the Biological Resources Unit)
- greater equipment standardisation and support for GLP based work

The coming year will see the installation of a micro-CT system in collaboration with the Biological Resources Unit; investment in a system for 3D cell and tissue cultures thus permitting the visualisation and analysis of organoids; expansion of the Institute's equipment management and booking system, investigative data collaboration tools; further improvements to single cell sorting; and to expand our cytometry sorting capacity, a third system is to be located under Class II containment.

### Biological Mass Spectrometry Facility

**Duncan Smith**, Yvonne Connolly, John Griffiths

The facility's role is to support research groups' protein analysis needs by easing access to cutting edge technologies, such as liquid chromatography (LC) and mass spectrometry (MS). These technologies are particularly valuable to researchers as they allow us to probe the proteome of any given biological model and answer questions such as which protein's expression levels are regulated and how the post-translational modification status of many proteins is modulated.

The facility has seen an exciting and challenging year with the complete replacement of our suite of mass spectrometers in February 2015. The successful bid to the UK Research Partnership



Investment Fund has allowed us to invest approximately £1M in two new state of the art LCMS systems. The Orbitrap Fusion has a unique tribrid architecture combining three independent mass analysers (a Quadrupole, an Orbitrap and a Linear Ion Trap). The system offers an unrivalled combination of mass accuracy, sensitivity and speed of data acquisition that makes this instrument an ideal choice for many proteomic workflows. The flexibility of the instrument's architecture also makes it a very powerful discovery tool when probing protein modifications. The 6600 Triple ToF represents the peak of abilities from Quadrupole-Time of Flight (QToF) based instruments. The platform offers excellent mass accuracy and resolution analyses at phenomenally high data acquisition rates making this instrument ideal for analysing highly complex 'whole proteome' samples. The fast acquisition rates of the 6600 mean we can now sample a far greater proportion of the proteome than was previously possible.

The installation of the two new platforms also saw the decommissioning of our existing platforms. The priority for 2015 was therefore to establish and optimise workflows of greatest demand onto the new platforms and build informatic data processing pipelines to accommodate the new data streams. The workflows of protein identification, phosphorylation mapping, acetylation mapping, methylation mapping, label free proteomic profiling and TMT/iTRAQ proteomic profiling, have been successfully optimised and can now be accessed as routine services. There will be significant further investment in data

processing and analysis pipelines in 2016 in collaboration with the groups of Claus Jorgensen and Crispin Miller to fully exploit the potential of these instruments.

**Biological Resources Unit  
Transgenic Breeding**  
Team Leader: Kim Acton

The transfer of the transgenic mouse breeding function to the University of Manchester Incubator Building was completed in 2015. Lines were initially maintained at the Paterson Building facility, until there were sufficient available to ensure a supply from the Incubator Building. With 20 breeding lines remaining at the Paterson Building in January 2015, this was reduced to just one by the end of the year. Approximately 50 new lines were set up at the Incubator Building during 2015, mostly by crossing existing lines, but included 12 newly imported lines.

With re-derivation of the facility complete, cryopreservation has been increased. Freezing of embryos and sperm has resulted in the archiving (closing of live colonies) for 21 lines during 2015. A further 26 live colonies were closed in 2015 (cryopreservation not required).

Currently, the Transgenic Facility has around 175 breeding lines of genetically altered mice, cared for by nine staff, for the Institute's scientists. Additional services include timed mating set up and the import and export of strains (three lines were exported in 2015 to USA, Australia and Austria).

Management of the genotyping service was transferred to the BRU Transgenic Team, where all samples are sent to an external company (Transnetyx, USA) and genotype results downloaded into the breeding records.

Transfers of stock mice to the BRU Experimental facility at the Paterson Building have now increased to twice weekly. Mice are transferred, on request and allowing a minimum of one week acclimatisation for the mice, from arrival at the Paterson Building, prior to starting an experiment.

**Biological Resources Unit  
Experimental Services**  
Team Leader: Lisa Doar

Over the last couple of years the BRU Experimental Unit has undergone a considerable number of changes to the operating procedures and facilities available. In 2015, it was gratifying to see these changes endorsed in visits by the Home Office Animals in Scientific Regulation Unit and following an in depth audit carried out by the University of Manchester. We have been working hard on improving the services and equipment available to the research groups. There is now more than double the space available for housing mice and a second procedure room has been opened up and refurbished. A strong focus has gone into developing our surgical facilities, as more and more surgical models are now being established.

The new in vivo imaging facility is in full swing. We have a Bruker in vivo Xtreme for optical and x-ray imaging alongside a VisualSonics LAZR for high resolution ultrasound and photo-acoustic imaging. We are also planning on adding a micro-CT scanner to the unit next year which will give us a comprehensive range of scientific capabilities.

The Experimental team have been out to several conferences/symposia this year in order to keep on top of new developments, and the aim is to make sure staff are involved in the expansion of any new techniques so they can better assist the research groups. A number of 3Rs (Replacement, Reduction and Refinement) initiatives have been put into place with improved cage enrichment, the introduction of ear-tags and the use of the ultrasound imaging system for monitoring internal tumours. Three posters were submitted by the BRU team to the Institute's annual 3Rs' competition with all of them receiving excellent feedback.

**Transgenic Production Facility**  
Natalia Moncaut<sup>1</sup>, Mark Willington

<sup>1</sup>joined in 2015

The Transgenic Production Facility is a new facility (established in September 2015) that will generate novel genetically-modified mice. The objective is to work closely with research groups to design the targeting approach based on their needs, and provide fully customisable support to enable the successful achievement of the experimental aims. The Facility is based at two different sites: the molecular biology and cell work is carried out at the Paterson Building, and the microinjection and mouse breeding at the University's Incubator Building. Currently the group includes a Manager and a Senior Scientific Officer. They will be joined by a support post during 2016. The facility is responsible for the targeting strategy design, generation of required reagents, breeding, genotyping and cryopreserving new mouse lines. Basically, it will use three main approaches to generate new transgenic mice: DNA pronuclear injection, ES cell targeting and CRISPR-Cas.

**Histology**

**Garry Ashton**, Caron Abbey, Michelle Greenhalgh<sup>1</sup> (MCRC, Tissue Biobank) David Millard<sup>1</sup>, Joanna Molenda<sup>1</sup>, Deepti Wilks (Haematological Malignancy Biobank)

<sup>1</sup>left in 2015

In 2015, the Histology unit has continued to develop and expand. The availability of new platforms/technologies, together with further equipment purchases and refurbishment, has allowed us to adopt and incorporate sophisticated labelling techniques. As the range and complexity of the services offered continues to grow, the continued professional development of staff has ensured the unit continues to offer a comprehensive and flexible service at all times. A new Scientific Officer was recruited in 2015 with two further posts being advertised.

The unit's remit is to offer a full range of both routine and advanced histological services for oncology research. Routine services include various fixation, tissue processing, microtomy, cryotomy and freezing regimes of tissue, embryo and cell pellets. The unit also houses the Leica BondMax & RX platforms together with two Ventana Discovery platforms. This allows us to offer a high throughput routine immunohistochemistry (IHC) service, plus troubleshooting and antibody validation services in line with current demand. In addition the flexibility of the both platforms has also

allowed us to develop, automate and offer both in situ hybridisation and multiplex immunohistochemistry as routine services. In situ hybridisation enables visualisation and quantification of multiplex mRNA expression at the single transcript/single-cell level, allowing the researcher to obtain gene expression information in the context of tissue/cell morphology, whilst multiplex immunohistochemistry allows researchers to study both the levels of expression of different proteins within the same tumour tissue/cells and the interactions between proteins, and for the first time on a large-scale to understand how these markers interact. Ultimately both these techniques allow the study of tumour heterogeneity from a different angle that complements efforts in understanding the genetic/molecular heterogeneity of tumours. Several groups are using both these services.

In addition, the facility now houses a Leica VT1200 vibratome which allows sections between 50–250 µm thick to be cut from fresh samples. This will aid standardisation and ensure better resolution of cellular interactions with the improved advanced imaging techniques now available. The technique is also being used to set up ex vivo cultures of pancreatic tumours. Multiple standardised sections of the same tumour are cultured and then treated with different inhibitors to study their effects side-by-side.

The microdissection/macrodisection of tissue and downstream extraction of both RNA and DNA, sufficient in quantity and quality for NGS from relatively small amounts of material, has become routine. DNA/RNA has now been extracted from hundreds of samples together with the pathological evaluation of the sample. In addition, improved methods for the microdissection of samples from both frozen and FFPE samples, based on immunophenotype, are now available.

Tissue microarray (TMA) construction using the ATA27 and MTA1 platforms continues to expand. High throughput, accurate TMA construction of tumour specific and custom arrays has resulted in the production of extremely high quality TMAs giving true representation. TMAs from disease groups including breast, melanoma, prostate (cores and chips), bladder, lymphoid, small cell and non-small cell lung cancer plus others have now all been constructed. In addition, mouse model and cell pellet control microarrays have been constructed.

The unit has been involved in using automated RNA in situ hybridisation to detect *gfi1*, *gfi1b* and *runx1* in E10.5/11.5 embryos for the Stem

Cell Biology group, who are studying the development of the blood system in the mouse and who have identified new regulators of this process.

In addition, determining the in vivo response to new compounds is a key part of the later stages of any drug discovery project. During the last year the unit has been closely interacting with the Drug Discovery group to establish an in vivo "Proof of Mechanism" IHC biomarker for their PARG project. PARG, like the PARPs, is an enzyme that is involved in ADP ribosylation. PARG breaks down the chains of poly ADP ribose (PAR) that have been made by PARPs at single strand breaks in DNA. PARG inhibition results in these PAR chains being maintained, which has been detected in cultured cells. IHC for PAR can be used to show that a PARG inhibitor is active in a tumour in vivo. The unit has been involved with this together with markers showing downstream consequences of PARG inhibition (inhibition of proliferation, apoptosis etc.).

The unit is heavily used by the CEP preclinical team and IHC biomarkers' teams. CDX models are phenotyped and then used in drug studies, such as the Olaparib Wee-1 study, where the automated IHC platforms and various detection technologies are used to ensure consistency, reproducibility and standardisation. CEP has a collaborative relationship with the Histology core facility which acts as an excellent platform for scientific discussion and knowledge exchange.

The unit continues to process FFPE and frozen samples for the MCRC Biobank. To date samples from over 10,000 patients have been collected. Blood, bone marrow and plasma (at various disease status time points) from haematological malignancy patients has also been collected and processed. The samples are of the highest quality ensuring maximum value to any research program.

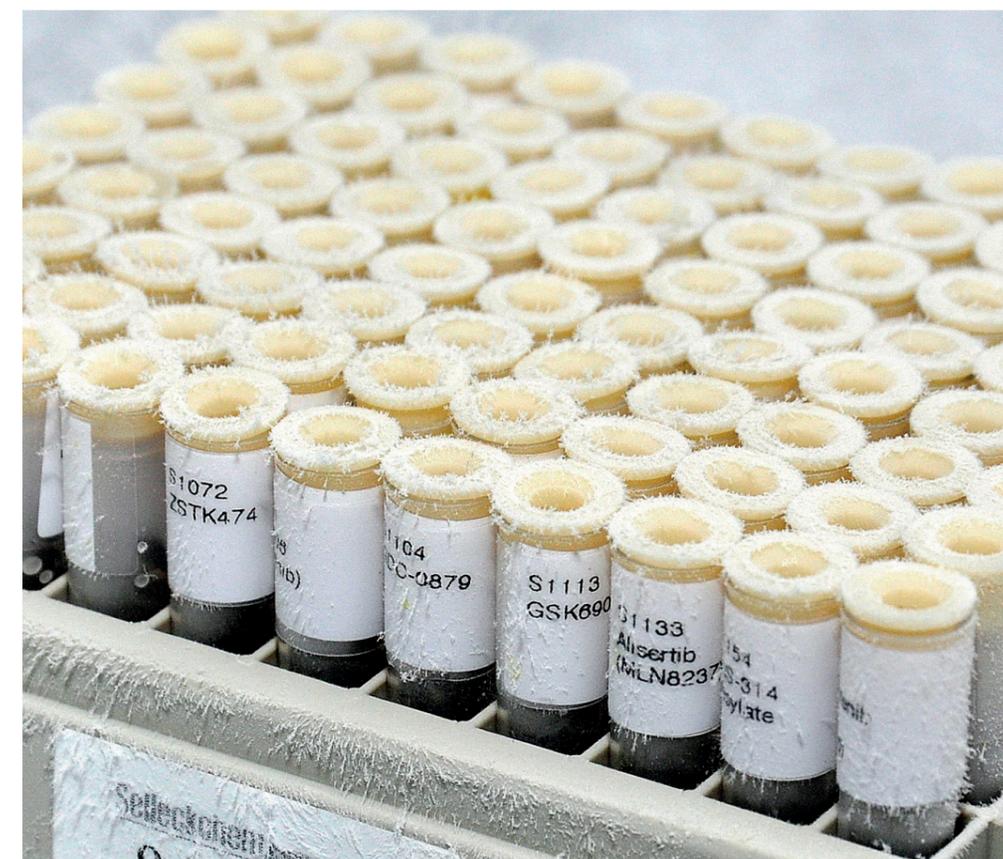
#### Laboratory Services

**Mark Craven**, Tony Dawson, Corinne Hand, John Higgins, Jolanta Organisciak<sup>1</sup>, Adriana Tudelo<sup>1</sup>, Christine Whitehurst

<sup>1</sup>joined in 2015

During 2015, the department has continued to support Research Groups and Service Groups within CRUK Manchester Institute and at the Wolfson Medical Imaging Centre, and as of autumn 2015, the new Manchester Cancer Research Centre Building. We have a new stand-alone Lab Services department in the MCRC Building, which now offers an equivalent service with the additional benefit of having an

The MIDaS (Manchester Institute Diversity Set) compound collection



autoclave capable of processing the new building's Category 111 lab waste prior to its disposal.

Across all three sites, our main role continues to be provision of sterile glassware and plastics. We also supply any required amounts of sterile water, simple buffers and bespoke bacteriological media and agar plates. By using standardised recipes we can maintain a reliable service and are able to adapt and incorporate new requests across the sites. We have a dedicated Clean Room based in CRUK MI to process requests across these newly expanded research facilities.

The department makes daily visits to laboratory areas to remove dirty glassware and to check and top up supplies of clean glassware and plastics. We also maintain the film developers across the CRUK MI and MCRC sites and are responsible for the provision of clean lab coats across both sites. In addition, we oversee the monthly Pipette Clinics to assist all the laboratories.

The Laboratory Services department also undertakes other tasks to support the Paterson Building. Each month we ensure all taps are opened as part of the Legionella testing programme. The department also checks and tops up the assorted first aid and eye wash supplies across the building.

#### Molecular Biology Core Facility

**Wolfgang Breitwieser**, Chris Clark, Toni Grady, Yvonne Hey<sup>1</sup>, Gillian Newton, Leanne Wardleworth, John Weightman

<sup>1</sup>left in 2015

The past year has seen a significant shift in the services for gene expression analysis provided by the Molecular Biology Core Facility (MBCF) when, after many years of success, the Affymetrix microarray service has finally been discontinued. In its place, Next Generation Sequencing has taken centre stage for genome and transcriptome analysis. Supported by three Illumina clonal sequencing platforms (HiSeq2500, Nextseq, and Miseq), the MBCF provides a wide range of clonal sequencing applications including whole genome and whole exome sequencing, as well as RNA sequencing applications for transcriptome analysis of mRNAs, and other RNA populations such as microRNAs and long non-coding RNAs. In addition we provide services for ChIP sequencing as well as sequencing of custom amplicons. The MBCF offers an all-inclusive service that integrates workflows starting from sample QC and library preparation to sample running on the distinct NGS instruments. The installation of an automated platform for sequence library preparation (Agilent Bravo) has had a significant impact on the rapid turnover and high throughput of samples. Furthermore, responding to unmet needs we have also

introduced protocols for low input samples such that we are currently able to offer services for reliable, high quality sequence library preparation from as low as sub-nanogram amounts of RNA, for example.

Providing PCR platforms and expertise for quantitative genome and gene expression analysis continues to be a cornerstone of the MBCF portfolio. In addition to the standard realtime quantitative PCR instruments (AB 7900), as well as single-cell analysis technology (Fluidigm C1/Biomark) we have, in 2015, installed a droplet digital PCR platform (Biorad QX200). Supported by an automated droplet generator for sample compartmentalisation (up to 20,000 droplets per sample), this set up allows scientists to determine absolute quantities of nucleic acid species and to detect rare genome variations, e.g. low abundance mutations in tumour samples.

A new direction in applications offered by the MBCF has been introduced with the installation of the Compound Library Service, with the aim of supporting scientists with drug compounds for screening purposes. Currently we hold four different sized compound libraries, the largest (MIDa5) having been designed by scientists within the Manchester Institute's Drug Discovery Unit and consisting of around 10,000 potentially bioactive molecules. Three smaller libraries consist of around 1,200 FDA approved drugs, as well as 350 known kinase inhibitors and 120 epigenetic inhibitors. The compound handling is administered by a Labcyte Echo 550 acoustic liquid dispensing instrument which is incorporated into the Access platform for automated plate processing. This robotic setup enables the high precision and high throughput

handling of compounds in nanoliter volumes for drug dosing experiments as well as supporting drug screening projects.

#### Scientific Computing

**Wei Xing<sup>†</sup>**, Zhi Cheng Wang, Christopher Smowton

<sup>†</sup>left in 2015

The Scientific Computing team continued to expand the Institute's High Performance Computing system. Working closely with the Institute's Estates team, a dedicated water-cooled machine room was established on site, and our High Performance Computing system (HPC), Troodon, was further expanded. HPC is now provided through a ~2000 core system with 33TB RAM. The system features a 40Gbs<sup>-1</sup> Infiniband interconnect to provide high-speed access to a 4PB multi-tier file system comprising a mixture of FLASH, GPFS, Lustre and Tape with remote archiving.

Research within the team has contributed to our understanding of how resources in remote computing clouds can be efficiently allocated to process genomics data (Smowton et al, 2015). A challenge with HPC is that it comes with additional programming demands in order to exploit the computational power offered by large amounts of parallelism. Chris Smowton in the team has worked closely with members of the Computational Biology Support team and RNA Biology group to develop implementations that run efficiently on our HPC hardware and to optimise software to run effectively on Troodon. This has led to substantial increases in the throughput of our data analysis pipelines.

# CANCER RESEARCH UK MANCHESTER INSTITUTE

## PUBLICATIONS AND ADMINISTRATION



**Iain Hagan** (page 14)  
Cell Division

### Refereed Research Papers

Grallert A, Boke E, Hagting A, Hodgson B, Connolly Y, Griffiths JR, Smith DL, Pines J, Hagan IM. (2015) A PP1-PP2A phosphatase relay controls mitotic progression. *Nature*, 517(7532):94-8.

### Other publications

Fu J, Hagan IM, Glover DM. (2015) The centrosome and its duplication cycle. *Cold Spring Harb Perspect Biol.*, 7(2):a015800.

**Nic Jones** (page 16)  
Cell Regulation

### Refereed Research Papers

Huang Q, Du X, He X, Yu Q, Hu K, Breitwieser W, Shen Q, Ma S, Li M. (2015) JNK-mediated activation of ATF2 contributes to dopaminergic neurodegeneration in the MPTP mouse model of Parkinson's disease. *Experimental Neurology*, S0014-4886(15)30111-4.

**Angeliki Malliri** (page 18)  
Cell Signalling

### Refereed Research Papers

Whalley HJ, Porter AP, Diamantopoulou Z, White GR, Castañeda-Saucedo E, Malliri A. (2015) Cdk1 phosphorylates the Rac activator Tiam1 to activate centrosomal Pak and promote mitotic spindle formation. *Nat Commun.*, 6:7437.

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Whalley HJ, Malliri A. (2015) Centrosome separation; a careful balancing act. *Cell Cycle*, 14(19):3001-2.

**Caroline Dive** (page 20)  
Clinical and Experimental Pharmacology

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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. (2015)

Application of sequencing, liquid biopsies and patient-derived xenografts for personalized medicine in melanoma. *Cancer Discovery*, pii: CD-15-1336. Epub 29 December 2015.

Greystoke A, Ayub M, Rothwell DG, Morris D, Burt D, Hodgkinson CL, Morrow CJ, Smith N, Aung K, Valle J, Carter L, Blackhall F, Dive C, Brady G. (2015) Development of a circulating miRNA assay to monitor tumor burden: From mouse to man. *Molecular Oncology*, doi: 10.1016/j.molonc.2015.10.004.

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Rothwell DG, Smith N, Morris D, Leong HS, Li Y, Hollebecque A, Ayub M, Carter L, Antonello J, Franklin L, Miller C, Blackhall F, Dive C, Brady G. (2015) Genetic profiling of tumours using both circulating free DNA and circulating tumour cells isolated from the same preserved whole blood sample. *Molecular Oncology*, doi: 10.1016/j.molonc.2015.11.006.

Symonds RP, Gourley C, Davidson S, Carty K, McCartney E, Rai D, Banerjee S, Jackson D, Lord R, McCormack M, Hudson E, Reed N, Flubacher M, Jankowska P, Powell M, Dive C, West CM, Paul J. (2015) Cediranib combined with carboplatin and paclitaxel in patients with metastatic or recurrent cervical cancer (CIRCCa): a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Oncology*, 16(15):1515-24.

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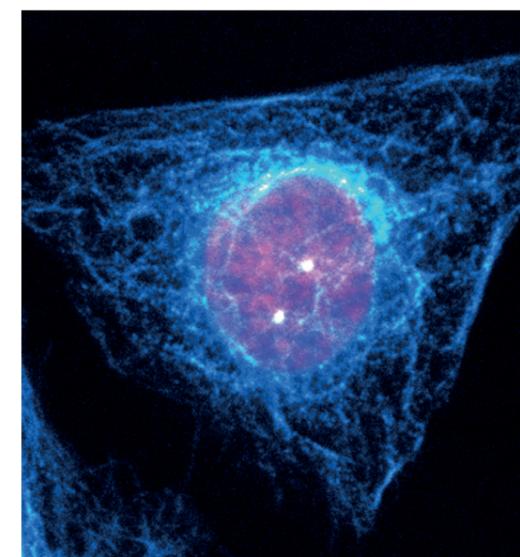
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Omlin A, Jones RJ, van der Noll R, Satoh T, Niwakawa M, Smith SA, Graham J, Ong M, Finkelman RD, Schellens JH, Zivi A, Crespo M, Riisnaes R, Nava-Rodrigues D, Malone MD, Dive C, Sloane R, Moore D, Alumkal JJ, Dymond A, Dickinson PA, Ranson M, Clack G, de Bono J, Elliott T. (2015) AZD3514, an oral selective androgen receptor down-regulator in patients with castration-resistant prostate cancer - results of two parallel first-in-human phase I studies. *Invest New Drugs*, 33(3):679-90.

MDCK II cells during early mitosis showing centrosome (white) separation.

Image supplied by Andrew Porter (Cell Signalling)



Krebs MG, Renehan AG, Backen A, Gollins S, Chau I, Hasan J, Valle JW, Morris K, Beech J, Ashcroft L, Saunders MP, Dive C. (2015) Circulating Tumor Cell Enumeration in a Phase II Trial of a Four-Drug Regimen in Advanced Colorectal Cancer. *Clinical Colorectal Cancer*, 14(2):115-22.

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**Donald Ogilvie** (Page 24)  
Drug Discovery

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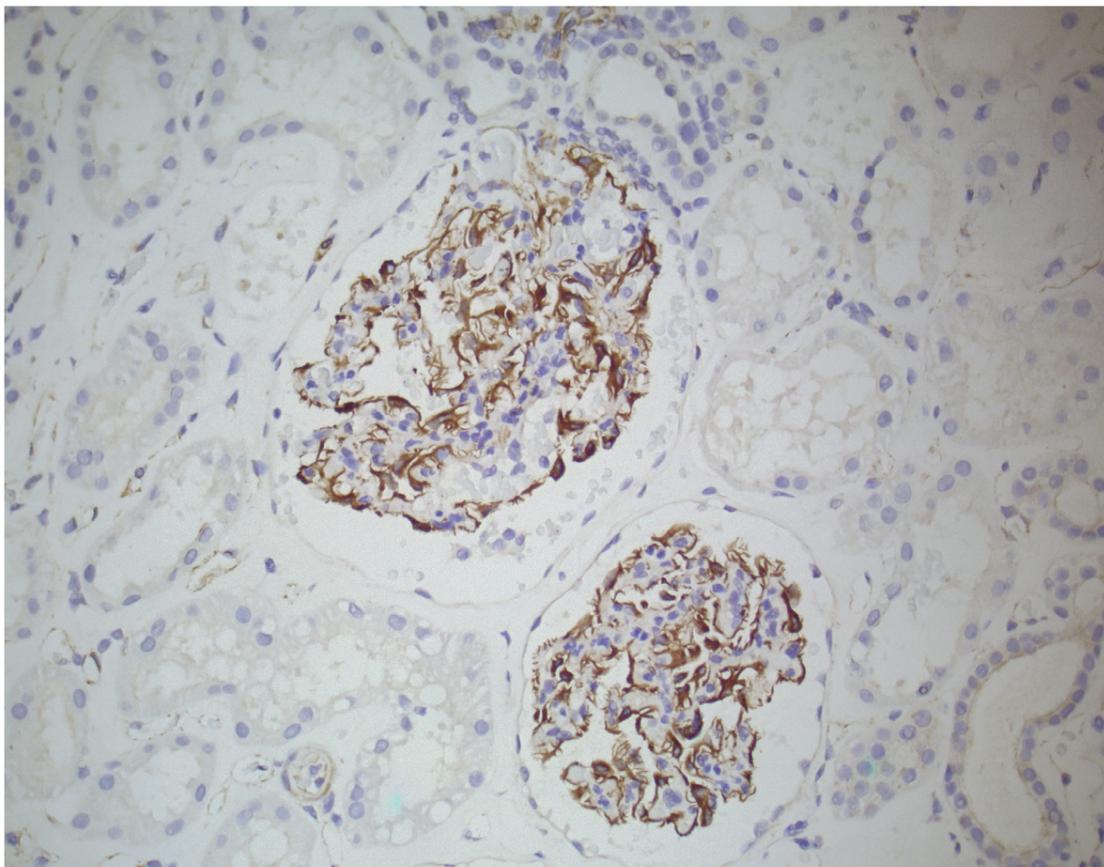
### Active Patents/Filings

Kristin Goldberg, Niall Hamilton, Stuart Jones, Allan Jordan, Amanda Lyons, Rebecca Newton, Donald Ogilvie, Bohdan Waszkowycz. "Quinazoline Compounds" PCT Int. Appl. WO2015079251, 4th June 2015.

Alison McGonagle, Allan Jordan, Bohdan Waszkowycz, Colin Hutton, Ian Waddell, James Hitchin, Kate Smith, Niall Hamilton. "Inhibitory Compounds" PCT/GB2015/053883, Filing date 11th December, 2015.

Staining for a cytoskeleton regulating protein kinases in kidney by Immunohistochemistry. Expression (brown) is specifically localised to the glomeruli.

Image supplied by Claus Jorgensen (Systems Oncology) and Garry Ashton (Histology)



Alison McGonagle, Allan Jordan, Bohdan Waszkowycz, Colin Hutton, Ian Waddell, James Hitchin, Kate Smith, Niall Hamilton. "Inhibitory Compounds" PCT/GB2015/054064, Filing date 17th December, 2015.

**Tim Somerville** (page 26)  
Leukaemia Biology

#### Refereed Research Papers

Somerville TD, Wiseman DH, Spencer GJ, Huang X, Lynch JT, Leong HS, Williams EL, Cheesman E, Somerville TC. (2015) Frequent Derepression of the Mesenchymal Transcription Factor Gene FOXC1 in Acute Myeloid Leukemia. *Cancer Cell*, 28(3):329-42.

Maes T, Mascaró C, Ortega A, Lunardi S, Ciceri F, Somerville TC, Buesa C. (2015) KDM1 histone lysine demethylases as targets for treatments of oncological and neurodegenerative disease. *Epigenomics*, 7(4):609-26.

Wiseman DH, Struys EA, Wilks DP, Clark CI, Dennis MW, Jansen EE, Salomons GS, Somerville TC. (2015) Direct comparison of quantitative digital PCR and 2-hydroxyglutarate enantiomeric ratio for IDH mutant allele frequency assessment in myeloid malignancy. *Leukemia*, 29(12):2421-3.

Korfi K, Mandal A, Furney SJ, Wiseman D, Somerville TC, Marais R. (2015) A personalised medicine approach for ponatinib-resistant chronic myeloid leukaemia. *Ann Oncol*, 26(6):1180-7.

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**Richard Marais** (page 28)  
Molecular Oncology

#### Refereed Research Papers

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. (2015) Application of sequencing, liquid biopsies and patient-derived xenografts for personalized medicine in melanoma. *Cancer Discovery*, pii: CD-15-1336. Epub 29 December 2015.

Baenke F, Chaneton B, Smith M, Van Den Broek N, Hogan K, Tang H, Viros A, Martin M, Galbraith L, Girotti MR, Dhomen N, Gottlieb E, Marais R. (2015) Resistance to BRAF inhibitors induces glutamine dependency in melanoma cells. *Molecular Oncology*, Epub 2015 Aug 20.

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Conde-Perez A, Gros G, Longvert C, Pedersen M, Petit V, Aktary Z, Viros A, Gesbert F, Delmas V, Rambow F, Bastian BC, Campbell AD, Colombo S, Puig I, Bellacosa A, Sansom O, Marais R, Van Kempen LC, Larue L. (2015) A caveolin-dependent and PI3K/AKT-independent role of PTEN in  $\beta$ -catenin transcriptional activity. *Nat Commun.*, 6:8093.

Miller BW, Morton JP, Pinese M, Saturno G, Jamieson NB, McGhee E, Timpson P, Leach J, McGarry L, Shanks E, Bailey P, Chang D, Oien K, Karim S, Au A, Steele C, Carter CR, McKay C, Anderson K, Evans TR, Marais R, Springer C, Biankin A, Erler JT, Sansom OJ. (2015) Targeting the LOX/hypoxia axis reverses many of the features that make pancreatic cancer deadly: inhibition of LOX abrogates metastasis and enhances drug efficacy. *EMBO Mol Med*, 7(8):1063-76.

Vizoso M, Ferreira HJ, Lopez-Serra P, Carmona FJ, Martínez-Cardús A, Girotti MR, Villanueva A, Guil S, Moutinho C, Liz J, Portela A, Heyn H, Moran S, Vidal A, Martínez-Iniesta M, Manzano JL, Fernández-Figueras MT, Elez E, Muñoz-Couselo E, Botella-Estrada R, Berrocal A, Pontén F, Oord Jv, Gallagher WM, Frederick DT, Flaherty KT, McDermott U, Lorigan P, Marais R, Esteller M. (2015) Epigenetic activation of a cryptic TBC1D16 transcript enhances melanoma progression by targeting EGFR. *Nat Med*, 21(7):741-50.

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Roulstone V, Pedersen M, Kyula J, Mansfield D, Khan AA, McEntee G, Wilkinson M, Karapanagiotou E, Coffey M, Marais R, Jebar A, Errington-Mais F, Melcher A, Vile R, Pandha H, McLaughlin M, Harrington KJ. (2015) BRAF- and MEK-Targeted Small Molecule Inhibitors Exert Enhanced Antimelanoma Effects in

Combination With Oncolytic Reovirus Through ER Stress. *Mol Ther*, 23(5):931-42.

Girotti MR, Lopes F, Preece N, Niculescu-Duvaz D, Zambon A, Davies L, Whittaker S, Saturno G, Viros A, Pedersen M, Suijkerbuijk BM, Menard D, McLeary R, Johnson L, Fish L, Ejima S, Sanchez-Laorden B, Hohloch J, Carragher N, Macleod K, Ashton G, Marusiak AA, Fusi A, Brognard J, Frame M, Lorigan P, Marais R, Springer C. (2015) Paradox-breaking RAF inhibitors that also target SRC are effective in drug-resistant BRAF mutant melanoma. *Cancer Cell*, 27(1):85-96.

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**Crispin Miller** (page 32)  
RNA Biology

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Thambyrajah R, Mazan M, Patel R, Moignard V, Stefanska M, Marinopoulou E, Li Y, Lancrin C, Clapes T, Möröy T, Robin C, Miller C, Cowley S, Göttgens B, Kouskoff V, Lacaud G. (2015) GFI1 proteins orchestrate the emergence of haematopoietic stem cells through recruitment of LSD1. *Nat Cell Biol*, Epub 2015 Nov 30.



Smowton C, Miller C, Xing W, Balla A, Antoniadou D, Pallis G, Dikaiakos MD. (2015) Analysing cancer genomics in the elastic cloud. Proceedings - 2015 IEEE/ACM 15th *International Symposium on Cluster, Cloud, and Grid Computing*, CCGrid 2015, art. no. 7152567, pp. 835-844.

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Antal CE, Hudson AM, Kang E, Zanca C, Wirth C, Stephenson NL, Trotter EW, Gallegos LL, Miller CJ, Furnari FB, Hunter T, Brognard J, Newton AC. (2015) Cancer-Associated Protein Kinase C Mutations Reveal Kinase's Role as Tumor Suppressor. *Cell*, 160(3):489-502.

**John Brognard** (page 34)  
Signalling Networks in Cancer

#### Refereed Research Papers

Hudson AM, Wirth C, Stephenson NL, Fawdar S, Brognard J, Miller CJ. (2015) Using large-scale genomics data to identify driver mutations in lung cancer: methods and challenges. *Pharmacogenomics*, 16(10):1149-60.

Marusiak AA, Stephenson NL, Baik H, Trotter EW, Li Y, Blyth K, Mason S, Chapman P, Puto LA, Read JA, Brassington C, Pollard HK, Phillips C, Green I, Overman R, Collier M, Testoni E, Miller C, Hunter T, Sansom OJ, Brognard J. (2015) Recurrent MLK4 Loss-of-Function Mutations

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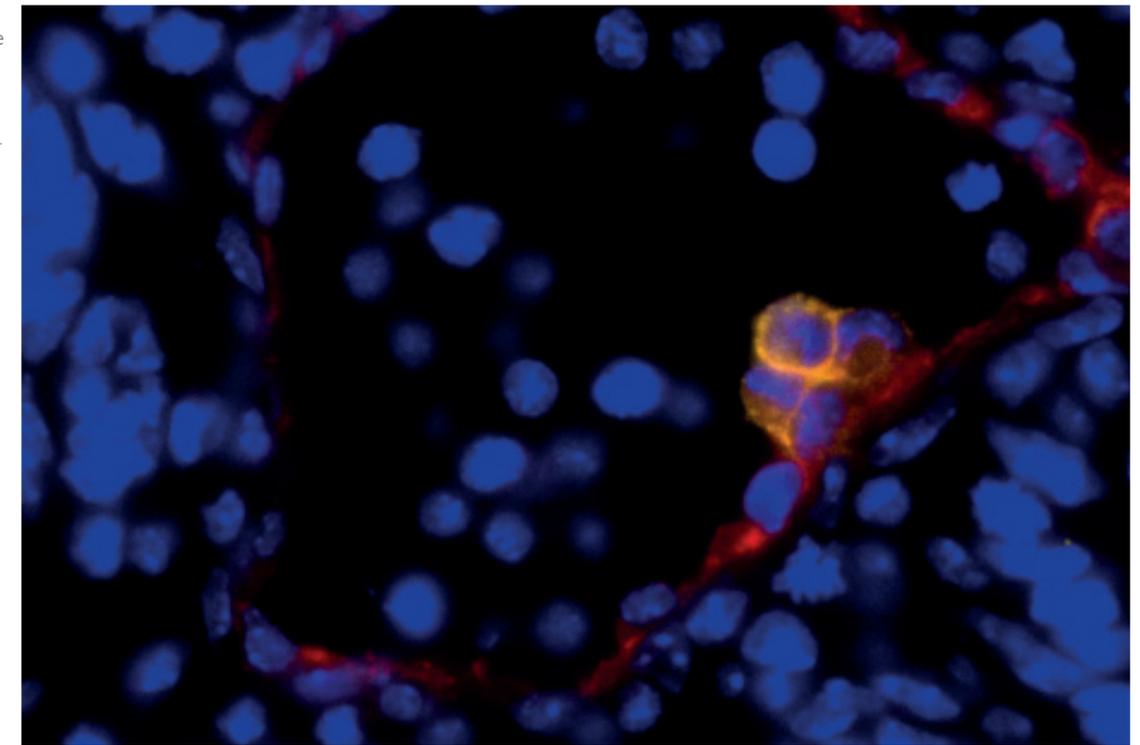
#### Active Patents/Filings

Kozikowski AP, Dennis PA, Brognard J, and Sun H Akt Inhibitors, Pharmaceutical Compositions, and uses Thereof. US Patent: 7,378,403, 2008. (WO/2004/022569)

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E10.5 AGM sections (10um) stained for the endothelial marker CD31(red) and the haematopoietic marker c-KIT (yellow). Counterstained with DAPI (blue).

Image supplied by Rahima Patel (Stem Cell Biology)



**Georges Lacaud** (page 36)  
Stem Cell Biology

#### Refereed Research Papers

Thambyrajah R, Mazan M, Patel R, Moignard V, Stefanska M, Marinopoulou E, Li Y, Lancrin C, Clapes T, Möröy T, Robin C, Miller C, Cowley S, Göttgens B, Kouskoff V, Lacaud G. (2015) GF11 proteins orchestrate the emergence of haematopoietic stem cells through recruitment of LSD1. *Nat Cell Biol.*, Epub 2015 Nov 30.

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Nasrallah R, Knezevic K, Thai T, Thomas SR, Göttgens B, Lacaud G, Kouskoff V, Pimanda JE. (2015) Endoglin potentiates nitric oxide synthesis to enhance definitive hematopoiesis. *Biol Open*, 4(7):819-29.

Piazza M, Williamson A, Lee CF, Pearson S, Lacaud G, Kouskoff V, McCubrey JA, Cocco L, Whetton AD. (2015) Quantitative phosphoproteome analysis of embryonic stem cell differentiation toward blood. *Oncotarget*, 6(13):10924-39.

Pearson S, Cuvertino S, Fleury M, Lacaud G, Kouskoff V. (2015) In vivo repopulating activity emerges at the onset of hematopoietic specification during embryonic stem cell differentiation. *Stem Cell Reports*, 4(3):431-44.

**Valerie Kouskoff** (page 38)  
Stem Cell Haematopoiesis

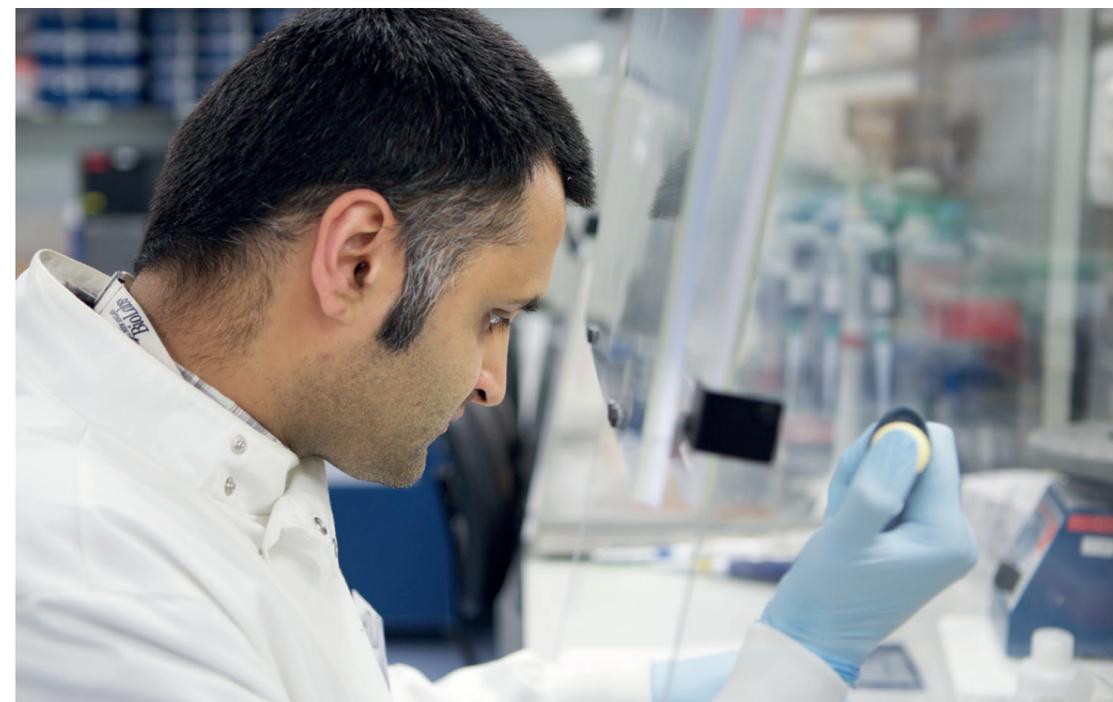
#### Refereed Research Papers

Thambyrajah R, Mazan M, Patel R, Moignard V, Stefanska M, Marinopoulou E, Li Y, Lancrin C, Clapes T, Möröy T, Robin C, Miller C, Cowley S, Göttgens B, Kouskoff V, Lacaud G. (2015) GF11 proteins orchestrate the emergence of haematopoietic stem cells through recruitment of LSD1. *Nat Cell Biol.*, Epub 2015 Nov 30.

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Nasrallah R, Knezevic K, Thai T, Thomas SR, Göttgens B, Lacaud G, Kouskoff V, Pimanda JE. (2015) Endoglin potentiates nitric oxide synthesis to enhance definitive hematopoiesis. *Biol Open*, 4(7):819-29.

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**Claus Jorgensen** (page 40)  
Systems Oncology

### Refereed Research Papers

So J, Pasculescu A, Dai AY, Williton K, James A, Nguyen V, Creixell P, Schoof EM, Sinclair J, Barrios-Rodiles M, Gu J, Krizus A, Williams R, Olhovsky M, Dennis JW, Wrana JL, Lindling R, Jorgensen C, Pawson T, Colwill K. (2015) Integrative analysis of kinase networks in TRAIL-induced apoptosis provides a source of potential targets for combination therapy. *Science Signaling*, 8(371):rs3.

**Michela Garofalo** (page 42)  
Transcriptional Networks in Lung Cancer

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Joshi P, Jeon YJ, Laganà A, Middleton J, Secchiero P, Garofalo M, Croce CM. (2015) MicroRNA-148a reduces tumorigenesis and increases TRAIL-induced apoptosis in NSCLC. *Proc Natl Acad Sci USA*, 112(28):8650-5.

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Taccioli C, Garofalo M, Chen H, Jiang Y, Tagliazucchi GM, Di Leva G, Alder H, Fadda P, Middleton J, Smalley KJ, Selmi T, Naidu S, Farber JL, Croce CM, Fong LY. (2015) Repression of esophageal neoplasia and inflammatory signaling by anti-miR-31 delivery in vivo. *J Natl Cancer Inst.*, 107(11). pii: djv220.

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Naidu S, Garofalo M. (2015) microRNAs: An Emerging Paradigm in Lung Cancer Chemoresistance. *Front Med (Lausanne)*, 2:77.

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**García-Rodríguez LJ, De Piccoli G, Marchesi V, Jones RC, Edmondson RD, Labib K.** (2015)

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Lamb R, Bonuccelli G, Ozsvári B, Peiris-Pagès M, Fiorillo M, **Smith DL**, Bevilacqua G, Mazzanti CM, McDonnell LA, Naccarato AG, Chiu M, Wynne L, Martinez-Outschoorn UE, Sotgia F, Lisanti MP. (2015) Mitochondrial mass, a new metabolic biomarker for stem-like cancer cells: Understanding WNT/FGF-driven anabolic signaling. *Oncotarget*, 6(31):30453-71.

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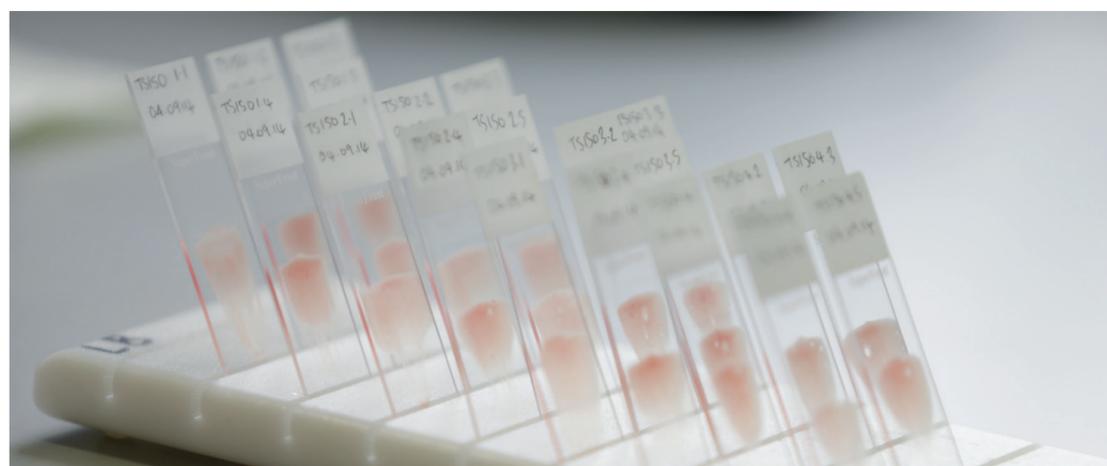
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# THESES



Laura Cove-Smith  
Clinical and Experimental  
Pharmacology

Cardiotoxicity from cancer therapy:  
A translational approach to biomarker  
development

Laura Cove-Smith



Danielle Potter  
Clinical and Experimental  
Pharmacology

Preclinical evaluation of rational drug  
combinations with BH3 mimetics in  
colorectal and small cell lung cancer

Danielle Potter



Zoe Edwards  
Signalling Networks in Cancer

The role of the mixed lineage kinase  
family in cancer and resistance to  
targeted therapies

Zoe Edwards



Maria José Villalobos Quesada  
RNA Biology/Cell Division

Characterisation of Pac1 function in the  
context of sexual differentiation and  
stress response

Maria José  
Villalobos Quesada



Magdalena Florkowska  
Stem Cell Biology

Direct reprogramming of murine  
fibroblasts to blood

Magdalena Florkowska



Tim Somerville  
Leukaemia Biology

Functional characterisation of the  
transcriptional regulators FOXC1 and  
IRX3 in AML

Tim Somerville



Danish Memon  
RNA Biology

The role of the non-coding genome in  
cancer

Danish Memon



Michael Tallis  
DNA Damage Response

Investigation of terminal ADP-Ribose  
protein Glycohydrolase-1 (TARG1) in the  
DNA damage response and  
neurodegenerative disease

Michael Tallis



Erinn-Lee Ogg  
Cell Signalling

The role of Tiam-1/Rac signalling in the  
centriole cycle

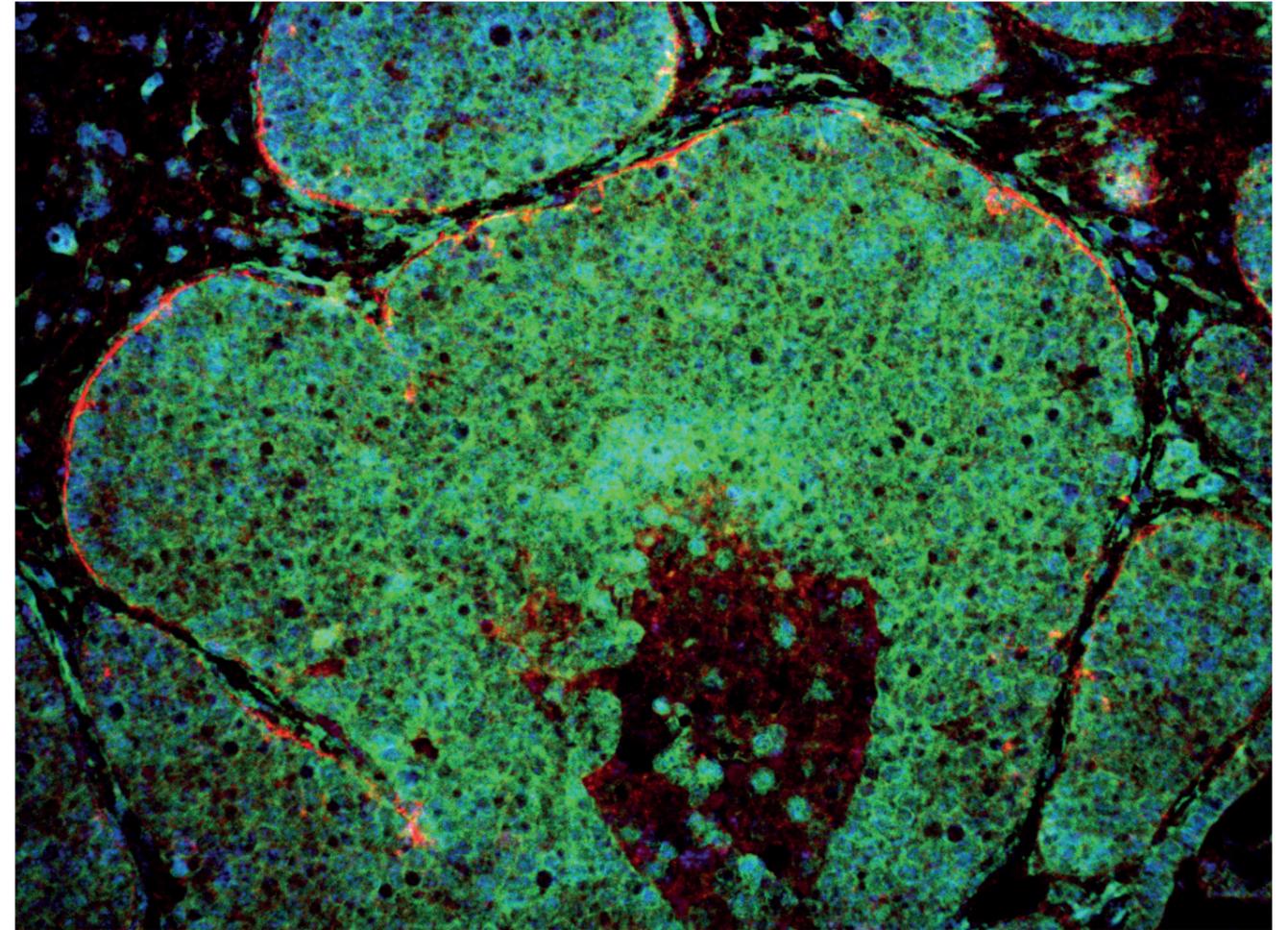
Erinn-Lee Ogg



Louise Carter  
Clinical and Experimental  
Pharmacology

Genomic profiling and characteristics  
of circulating tumour cells in patients  
with small cell lung cancer

Louise Carter



EGF receptor (green), extracellular matrix (red) and cell nucleus (blue) in a mammary  
tumour from a mouse model of breast cancer.

Image supplied by Haoran Tang (Molecular Oncology)

## EXTERNAL SEMINAR SPEAKERS 2015

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 interaction with an excellent set of internationally renowned speakers visiting the Institute. The Breast Cancer Now Research Unit seminar series 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.

**Rudolf Aebersold**  
Institute of Molecular Systems Biology

**Fatima Al-Shahrouh**  
Centro Nacional de Investigaciones Oncológicas (CNIO)

**Lorena Arranz**  
Arctic University of Norway

**Mariano Barbacid**  
Centro Nacional de Investigaciones Oncológicas

**Andrew Biankin**  
University of Glasgow

**Christin Burd**  
The Ohio State University

**Neil Carragher**  
University of Edinburgh

**Roger Daly**  
Monash University

**Greg Findlay**  
University of Dundee

**Ramiro Garzon**  
Ohio State University

**Andrew Gilmore**  
University of Manchester

**Adrian Hayday**  
King's College London

**Christopher Heeschen**  
Barts Cancer Institute

**Brian Huntly**  
University of Cambridge

**Philippe P. Juin**  
Université de Nantes

**Tony Kouzarides**  
The Gurdon Institute

**Lionel Larue**  
Institut Curie

**Douglas Lauffenburger**  
Massachusetts Institute of Technology

**Arian Laurence**  
National Institute of Arthritis and Musculoskeletal and Skin Disease

**Kathryn Lilley**  
Cambridge Centre for Proteomics

**Ian Macara**  
Vanderbilt University Medical Center

**Marc Mansour**  
University College London

**Michael Milsom**  
German Cancer Research Center

**Elizabeth Patton**  
Edinburgh Cancer Research Centre

**Laurence Pelletier**  
University of Toronto

**Jorge Reis-Filho**  
Memorial Sloan-Kettering Cancer Center

**Erik Sahai**  
Cancer Research UK London Research Institute

**Yardena Samuels**  
Weizmann Institute of Science

**Carmela De Santo**  
University of Birmingham

**Victoria Sanz-Moreno**  
King's College London

**Joan Seoane**  
Vall d'Hebron Institute of Oncology

**Manuel Serrano**  
Centro Nacional de Investigaciones Oncológicas (CNIO)

**Hugues de The**  
Paris Diderot University

**Roman Thomas**  
University of Cologne

**Paul Tumeah**  
UCLA's Jonsson Comprehensive Cancer Center

**Stefano Volinia**  
University of Ferrara

**Britta Weigelt**  
Memorial Sloan Kettering Cancer Center

### Breast Cancer Now Seminars

**Renée van Amerongen**  
University of Amsterdam

**Generoso Bevilacqua**  
University of Pisa and Pisa University Hospital

**Mark Clemons**  
Ottawa Hospital Cancer Centre

**Stephen Hursting**  
University of North Carolina

**Chris Lord**  
Institute of Cancer Research

**Bin-Zhi Qian**  
MRC University of Edinburgh Centre for Reproductive Health

**Shyam Sharan**  
NIH: National Cancer Institute Center for Cancer Research

**Eva Gonzalez Suarez**  
IDIBELL

**Sara Zanivan**  
Cancer Research UK Beatson Institute

# POSTGRADUATE EDUCATION

www.cruk.manchester.ac.uk/education



**Julie Edwards**  
Postgraduate  
Education Manager

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 clinical 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. Ninety-nine percent (99%) of our PhD students over the past five years have found employment after graduation, over half continuing in academia (54%) or within scientific industry (20%), whilst 25% continue to progress in their clinical careers in the NHS. Students leave the CRUK MI with excellent prospects in academic research, financial and technology careers, scientific writing and industrial management, securing positions in destinations across the UK, Europe and the USA.

In 2015, we welcomed ten graduate students and two clinical research fellows to our PhD programme, working in a variety of fields from drug discovery, to stem cells and leukaemia 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 Cancer Cell, Leukemia, Oncogene and Current Biology. During the course of this year, a total of seven PhD students and one Clinical Fellow were awarded their PhDs, a 100% success rate. From the seven graduates, four students continued their careers in academia successfully attaining post-doc positions at institutions such as the University of Cambridge, Cold Spring Harbor Laboratory, New York and the Dana-Farber Cancer Institute, Harvard Medical School, Boston. Two students

continued their scientific careers by gaining posts in industry.

## 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. Further support is also available individually from the Education Committee Chair, 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.

The CRUK Manchester Institute Colloquium, held annually in September, 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. Poster prizes are awarded, including the Lizzy Hitchman Prize for the best poster presented by a PhD student or clinical fellow. In 2015, clinical research fellow Andrew Hudson from the Signalling Networks in Cancer group was the recipient of this Prize for his work identifying novel driver mutations from The Cancer Genome Atlas (TCGA) dataset. Andrew developed a novel bioinformatic screen that identified kinases mutated in critical regions responsible for catalytic function. This has enabled the discovery of driver mutations in novel kinases in lung and upper GI cancers that are now being investigated in the lab.

## 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 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, the 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 three-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. This year, both CRUK/AZ clinical fellows Louise Carter and Rob Metcalf on completion of the fellowship projects joined

## POSTGRADUATE EDUCATION (CONTINUED)

the Christie Early Clinical Trials Unit and remain closely associated with biomarker research in the CEP group.

### Education Committee 2015

The Education Committee acts for the postgraduate students and consists of group leaders, the Chief Operating Officer, the Postgraduate Tutor and the Postgraduate Education Manager of 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

#### Valerie Kouskoff

Postgraduate Director & Chair, Education Committee [until January]

#### Tim Somerville

Postgraduate Director & Chair, Education Committee [from January]

#### Ian Waddell

Postgraduate Tutor [until October]

#### Angeliki Malliri

Postgraduate Tutor [from October]

#### Richard Marais

Ex-Officio Member

#### Wolfgang Breitwieser

[from February]

#### John Brognard

[from January]

#### Julie Edwards

#### Claus Jorgensen

[from January]

#### Donald Ogilvie

#### Caroline Wilkinson

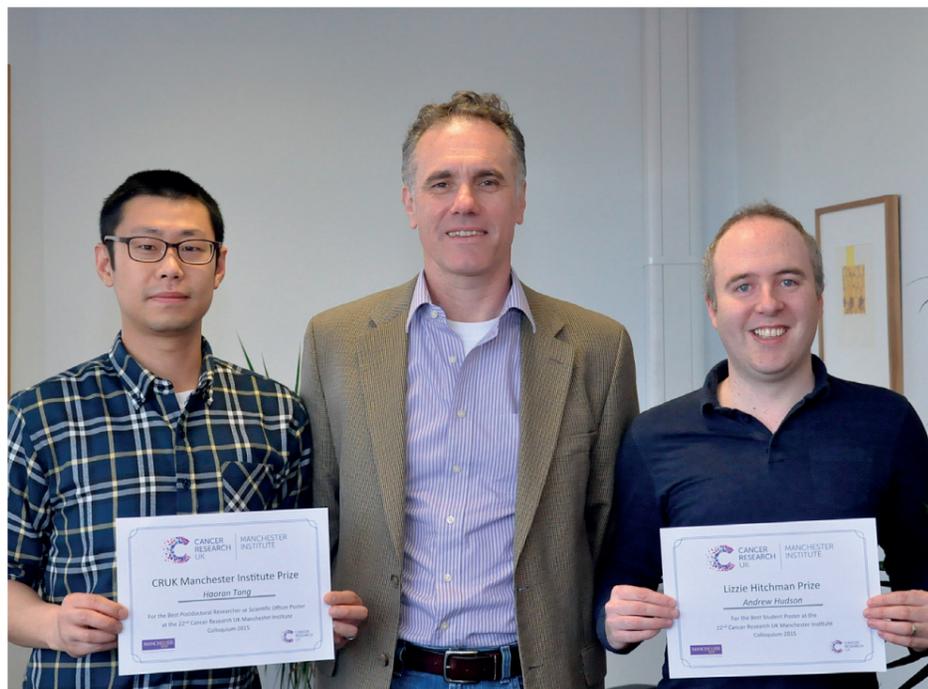
### Student Representatives

**Alekh Thapa** [until October]

**Emma Williams**

**Amy McCarthy** [from October]

Colloquium Poster Prize winners Haoran Tang (best poster by a postdoctoral researcher) and Andrew Hudson (Lizzy Hitchman Prize for best poster by a PhD student) receiving their awards from Institute Director Richard Marais.



Our students, here with keynote speaker Bruce Alberts, organised the 9th International PhD Student Cancer Conference (IPSCC)

## OPERATIONS



**Caroline Wilkinson**  
Chief Operating Officer



**Stuart Pepper**  
Chief Laboratory Officer



**Margaret Lowe**  
Head of Finance



**Rachel Powell**  
Head of Human Resources

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 right across the Operations team have assisted with arrangements to help set up the new Manchester Cancer Research Centre Building and to aid some of our research teams that are moving in. Other major projects included the HASMAP health and safety audit, launch of the new intranet and a significant upgrade of our IT infrastructure.

### Director's Office and Administration Services

**Ruth Perkins**, Ekram Aidaros<sup>1</sup>

<sup>1</sup>left in September 2015 to take up role as Personal Assistant to the Deputy Director, Professor Caroline Dive.

The office provides administrative support to the Director and to the Institute Group Leaders. In addition, the department has assisted with the organisation of several events over the course of the year, including the Institute Colloquium, a quinquennial review, the first meeting of the Belfast-Manchester Movember Centre of Excellence Scientific Advisory Board, and the first MCRC Autumn School.

Administrative support is provided for the external seminar series, which has been a great success in 2015, increasing from one to 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](http://www.cruk.manchester.ac.uk/seminars).

### Estates

**Steve Alcock**, Graham Hooley<sup>1</sup>, Lewis Parkinson<sup>1</sup>, Steven Powell<sup>2</sup>, Tony Woollam

<sup>1</sup>left in 2015

<sup>2</sup>joined in 2015

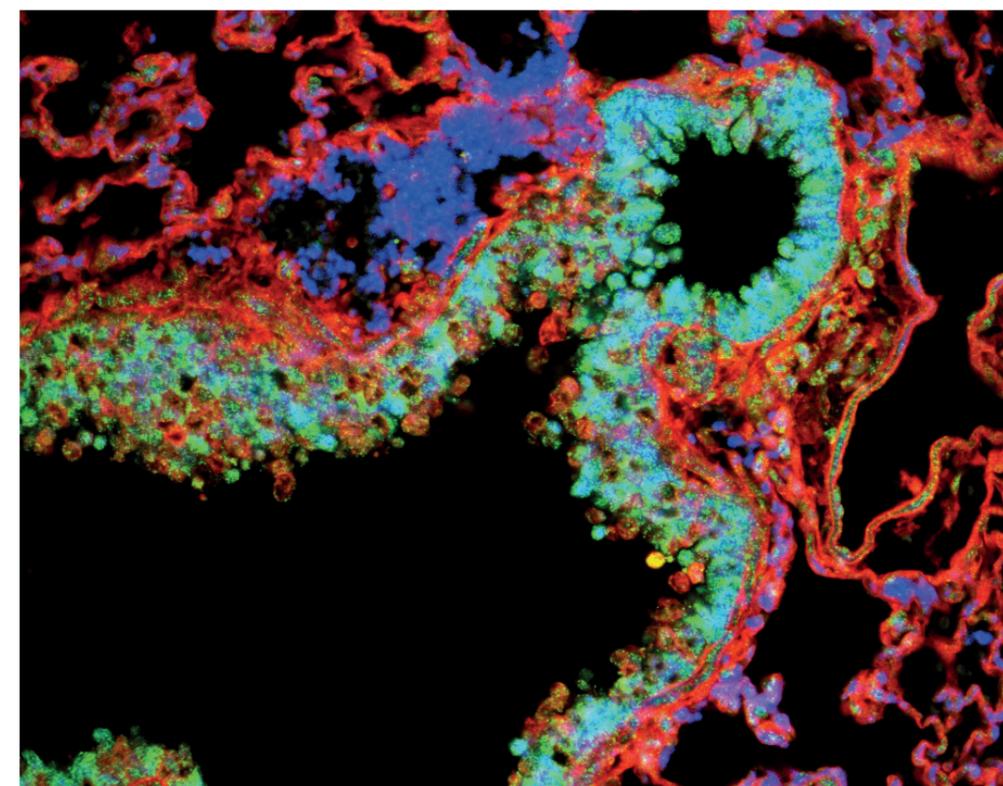
The Estates team has had a successful year in 2015 balancing the provision of routine service support for the Paterson Building alongside completing refurbishment projects.

The Estates team worked closely with external contractors and the Scientific Computing team to complete the development of a new server room for High Performance Computing, with accompanying office space. One of the key challenges for any server room is the provision of sufficient cooling to allow the operation of a large amount of IT equipment in a relatively small area. For this project we used water cooling rather than the more traditional air cooling, which reduces significantly the amount of energy used for cooling. By using water, the cooling is essentially free on all but the warmest of days. Completing a design that gave the required specification for the server room, whilst furthering the sustainability aims of the Institute, was a real success.

Another significant project has been planning the replacement of an autoclave and cage washer. This work will take place in 2016 following a successful design and tendering process.

Lysyl oxidase (green), elastin (red) and cell nucleus (blue) in normal mouse lungs.

*Image supplied by Haoran Tang (Molecular Oncology)*



Aside from these major projects the Estates team play a vital role in keeping the electrical and mechanical systems that support the building in good working order. An important aspect of this function is to ensure legislative compliance and to this end the team members have attended relevant courses to improve their skills and keep their knowledge up to date with current working practices and changing legislation. In the last year this has included training in both Legionella monitoring and gas handling.

### Finance & Purchasing

**Margaret Lowe**, Neepa Begum<sup>1</sup>, David Jenkins, Denise Owen, Muhammad Raja, Vikki Rosheski<sup>2</sup>, Debbie Trunkfield

<sup>1</sup>left in 2015

<sup>2</sup>joined in 2015

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 2015 or will be activated early 2016. 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 carry out the breadth of research that we need to 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 & Safety

**Colin Gleeson**

Monitoring of our health and safety performance figured large in 2015. There was a laboratory self-inspection programme undertaken by all groups in the Institute resulting in co-ordinated remedial action plans to minimise disruption to research. This programme was run in addition to the formal planned rolling programme of inspections by the Health and Safety Manager. In addition, the University has also undertaken a health and safety audit of the Institute (HASMAP). It focused on how we manage health and safety, and included a detailed assessment of our processes and procedures. It also involved interviewing

people at all levels of the organisation to ascertain how well engrained health and safety knowledge and practice is within the organisation. We await the audit report and will implement any necessary actions accordingly.

There have been compliance exercises including checks on stocks held of chemical weapons, de-sensitised explosives, dangerous pathogens and toxins, Euratom materials and radioactive waste disposal records. The building-wide data was fed back to the University Compliance Officer.

The Environment Agency (EA) visited the building in early 2015 to undertake an audit in relation to our possession and disposal of open and closed sources of radioactivity. This was a successful visit resulting in no formal actions required by the EA. The Home Office also visited in early 2015 to undertake a compliance visit for our possession of chemicals listed as controlled drugs and the renewal of a licence for them. This visit was also successful. No remedial actions were necessary and the new licence has now been issued.

Much preparation work was undertaken for the newly opened Manchester Cancer Research Centre Building. This included the assessment and selection of major equipment and creating a framework by which the building will operate both on a day to day basis and in terms of health and safety. During the coming months the framework will be monitored and tweaked, if necessary, to facilitate efficient and safe operation of the building.



## Human Resources

**Rachel Powell**, Laura Jones, Julie Jarratt, Emma Lloyd<sup>1</sup>, David Stanier<sup>2</sup>

<sup>1</sup>joined in 2015

<sup>2</sup>joint with Scientific Operations

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 2015, 67 individuals were successfully appointed to enhance the work of the Institute. This included the start-up of a new research group and a core facility which highlights the continued growth and development of CRUK MI. The HR Department itself also increased its headcount in September when Emma Lloyd joined the team as the Recruitment Administrator. The department administered the successful promotion of five individuals and supported the retirement of four members of staff from the Institute.

The department has delivered several internal training sessions for managers on key management areas; these were well received and we plan to provide this training to other managers moving forward. We have continued to promote the training and development opportunities provided by The University of Manchester on areas such as Equality and Diversity.

We have sustained our commitment to joint partnership working with the union which has resulted in the revision of ten HR policies and procedures and the implementation of a new policy on Shared Parental Leave. The Institute values the thoughts and opinions of its staff and in March 2015 the University of Manchester staff survey was undertaken to provide an opportunity for employees to feed back on their experiences in working for the Institute, and to highlight issues they feel should be addressed in the future. Accordingly, an action plan was generated and initiatives are being implemented.

Next year, we will be developing and launching a new in-house online performance review system and working towards the Athena SWAN accreditation. A self-assessment team has been established and will be chaired by Professor Caroline Dive. We look forward to the positive work of this group and the benefits that it will bring to the Institute and its staff.

## Information Technology

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

The CRUK Manchester Institute IT team has continued to provide a full catalogue of IT services to the high standard expected by our service users, who now number in excess of 500 researchers and service providers.

During 2015, we have focused our activities on building a new enterprise class IT infrastructure capable of providing high performance, scalable and fault tolerant IT services into both the Paterson and MCRC Buildings. We have built a new 10G core network spanning both buildings. This supports hundreds of researchers, providing high-speed access to CRUK MI, The University of Manchester and internet-based IT services. We have also replaced our ageing server farm with a new datacentre class facility. This provides our researchers with the high performance servers required to manage and analyse their rapidly growing research datasets. It also gives us capacity to grow to meet the needs of both our existing and new researchers in the MCRC Building. Replacing IT infrastructure whilst maintaining a service is always a challenge; all this work was done without any disruption to IT services during normal business hours. Work has also started to replace legacy access layer switches, which connect all our desktop computers. This work will provide researchers with fast, fault-tolerant connectivity to all our IT services.

The past year also saw some of our research groups relocate to the MCRC Building. The planning and execution of IT service provision into this site was a significant undertaking by the whole team to build a new IT infrastructure in a new building. This work is on-going as the number of researchers on this site continues to increase in 2016.

Looking forward, planning commenced in 2015 for significant IT infrastructure upgrades in 2016. This is primarily to replace our legacy file storage facility plus several other critical services.

## Logistics

**Andrew Lloyd**, Michael Alcock, Edward Fitzroy, Sedia Fofana, Stephen Keane, Jonathan Lloyd

The past year has seen changes within the team's structure. At the beginning of the year, Andy Lloyd was appointed Logistics Manager and in the summer, Jonathan Lloyd was promoted to the role of Supervisor.

The team has continued to deliver an efficient and effective service providing support for the research carried out in the Institute. 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 the Institute 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 from 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, Fisher kits and Qiagen). We now stock over 260 products, and new products are being added all the time to meet the demands 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 therefore helping facilitate internal rearrangements and the arrival of new groups. This includes reconfiguration of meeting rooms for numerous events being held in the Holt Major/Conference Room. The team also looks after the Institute's "Shred It" service and water coolers.

This year also saw the opening of the MCRC Building. The team has been heavily involved in taking deliveries of all the large pieces of equipment and distributing them around the building. Since June the team has worked closely with the MCRC Operations Committee and most recently the University of Manchester's Technical Operations Manager, preparing the building for its scientific occupants. The team is currently in process of moving some groups from the Paterson Building to the MCRC Building, which will continue into early 2016.

### Scientific Operations

**Caroline Wilkinson**, Tom Bolton<sup>1</sup>, Gillian Campbell, Julie Edwards, Steve Morgan, David Stanier<sup>2</sup>

<sup>1</sup>joint with MCRC

<sup>2</sup>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 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. Talks and tours are also provided for a packed programme of fundraisers' events and other visitors throughout the year.

In addition to receiving core funding from CRUK, our researchers also apply for external awards to extend the portfolio of research that we can undertake. All grant submissions submitted by our researchers are screened for the appropriate ethical approvals, as well as the ability of the Institute to accommodate the proposed programme of work. We also have a rigorous internal peer review process for grant applications which is organised by Gill Campbell, the Institute's Grants Advisor. Gill also provides support to the Institute's researchers through the grant preparation and submission process. This past year, saw the number of external funding submissions double including successful applications to the ERC, the Pancreatic Cancer Research Fund, The Lung Cancer Research Foundation and Worldwide Cancer Research.

Our new intranet which was produced in house was launched during the year and has proved popular providing us with a great deal of flexibility around internal communications. The team played a major role in organising the MCRC Autumn School which was held in October around the theme of personalised medicine.

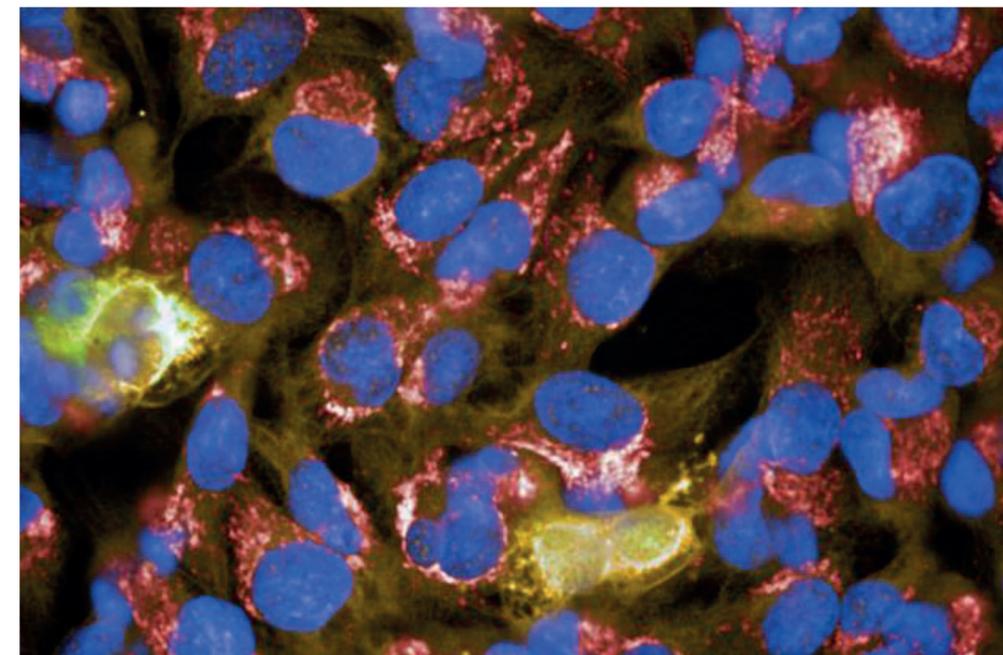
### Cancer Research Technology Martyn Bottomley

Cancer Research Technology (CRT) is a specialist oncology-focused development and commercialisation company wholly owned by Cancer Research UK. 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

Lung cancer cells (stained orange; nuclei stained blue), are full of mitochondria (red). Image acquired on the Opera Phenix High Content microscope allowing many thousands of cells to be imaged in a short space of time. Mitochondria can be identified and tracked in each individual cell using associated software.

*Image supplied by Andrew Porter and cells prepared and stained by Aishwarya Payapilly (Cell Signalling)*



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 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 the ongoing collaborations with

Pharmaceutical partners such as GSK, HitGen and AZ 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. 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. 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.

# CANCER RESEARCH UK'S RESEARCH ENGAGEMENT



Cancer Research UK's Research Engagement Manager

Sive Finlay<sup>1</sup>



Hannah Leaton<sup>2</sup>

<sup>1</sup>joined in 2015  
<sup>2</sup>left in 2015

Public engagement remained an important part of the Institute's work during 2015. We welcomed a record number of visitors and our researchers brought their science to life for more people than ever before.

We opened our doors to over 1,250 visitors this year including donors, CRUK ambassadors, CRUK Friends and Legacies supporters, fundraising committees, corporate partners and members of the public. Visitors thoroughly enjoyed exploring the labs, getting an insight into our work and seeing how we make the best use of their generous support.

In July we marked the official opening of the Manchester Cancer Research Centre Building with a week-long programme of events and activities. Donors and fundraising committees who had supported the construction of the building received special tours and tried their hand at science-themed activities. Local school children also got a chance to be a scientist for the day and to see their cell-themed cushions take pride of place throughout the building.

Our annual open days for CRUK supporters were as popular as ever. Over 50 researchers were involved with hosting lab tours, delivering activities and guiding visitors around the Institute. The introductory presentation at

November's open day was a great hit, with one supporter commenting that "it was witty, funny and illuminating". They suggested that Allan Jordan who gave the presentation should be on TV!

Away from the Institute, scientists have showcased our research to an even greater number of people, directly engaging with over 12,000 people at more than 50 external events in 2015, over four times more than in the previous year.

The Institute hosted a very popular stand at the Royal Society Summer of Science exhibition, engaging more than 10,100 people during a week-long programme of activities based on the Clinical and Experimental Pharmacology group's research into circulating tumour cells. The video we created to accompany the exhibition, featuring an interview with group leader Professor Caroline Dive, received over 1,500 views on YouTube.



Visitors at the Royal Society Summer Exhibition learning about the Clinical and Experimental Pharmacology lab's research into circulating tumour cells.



Top, left to right: Researcher volunteers during the Manchester Science Festival for our behind the scenes tours and science spectacular activity stand; Allan Jordan, dressed as Gwen the Gorilla, ready to take on the Manchester 10k run. Bottom, left to right: Stuart Pepper enjoying our new virtual reality lab tour; Some of the Relay for Life team at their plant sale; Some of our fantastic supporters visiting us for a lab tour.

We expanded our now annual participation in October's Manchester Science Festival to include four different events. Our public lab tours were very popular and showcased our work to people who hadn't previously interacted with the charity. Chatting with researchers was a highlight for many people, with one visitor commenting that "we got to see some amazing, innovative approaches and were shown around by some very enthusiastic and knowledgeable staff".

Our CRUK Grand Challenge evening at Manchester Art Gallery included fun activities to explain the key research questions to be addressed and also invited the audience to contribute their ideas about how research money should be spent. Taking part in the Cravings Late evening at the Museum of Science and Industry was a great success and sparked a lasting partnership with the museum. A team of CRUK staff and researcher volunteers showcased the Institute's research to over 900 visitors during the evening with lots of fun activities and demonstrations. We rounded off the Festival with our #Cellfie stand at the University of Manchester's Science Spectacular where visitors could use their smartphone as a microscope, learn about personalised medicine and create their own cell balloon.

Staff at the Institute continue to be generous supporters for the charity through a variety of fundraising activities and events. The staff team for the Stockport Relay for Life braved the weather throughout the night to raise a fantastic £3,000 for CRUK, while a flurry of cake sales,

yoga classes, moustache-growing and raffles in November raised over £1,500 to support the Movember Centre of Excellence. Our now annual #sciencecakes competition also attracted some impressive culinary creations and raised much-needed funds in support of Stand Up to Cancer.

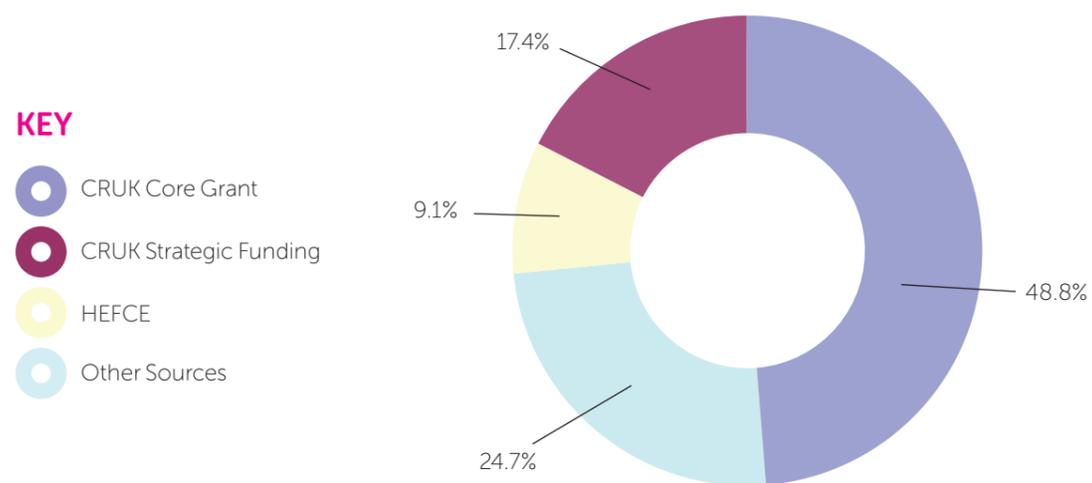
In an exciting first for CRUK, the Institute created a virtual reality lab tour which will allow even more people to get an inside view of our research. Hosted by Marina Parry and filmed in the Drug Discovery and Leukaemia Biology labs, the tour brings viewers into the heart of the Institute and provides a unique insight into our work. Initial feedback about the tour from fundraisers, philanthropy partners and the general public has been extremely positive and we aim to make it available nationally across the charity in 2016.

With the new MCRC building up and running, continued partnership with the Museum of Science and Industry and new opportunities to reach wider audiences as part of Manchester's European City of Science celebrations, we are looking forward to bringing our research to life for many more people in 2016.

## ACKNOWLEDGEMENT FOR FUNDING OF THE CANCER RESEARCH UK MANCHESTER INSTITUTE

The total funding of the Cancer Research UK Manchester Institute for 2015 was £25m. The major source of this funding was awarded by Cancer Research UK (CRUK) via a core grant of £11.3m plus additional strategic funding of £5.4m. This funding enables the various scientific groups and service units within the Institute to carry out their research.

### CRUK MANCHESTER INSTITUTE FUNDING 2015



The infrastructure of the CRUK Manchester Institute is funded by HEFCE generated income at a cost of £2.3m.

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:

- Worldwide Cancer Research
- AstraZeneca
- Biotechnology and Biological Sciences Research Council
- Bloodwise
- British Lung Foundation

- Christie Hospital NHS Foundation Trust
- Clearbridge Biomedicals
- European Commission
- John Swallow Fellowship
- Kay Kendall Leukaemia Fund
- Lung Cancer Research Foundation
- Medical Research Council
- Menarini Biomarkers Singapore
- Moulton Charitable Trust
- Pancreatic Cancer Research Fund
- Parsortix
- Prostate Cancer UK
- Roy Castle Lung Cancer Foundation
- Wellcome Trust

We are immensely grateful to all our sponsors.

## 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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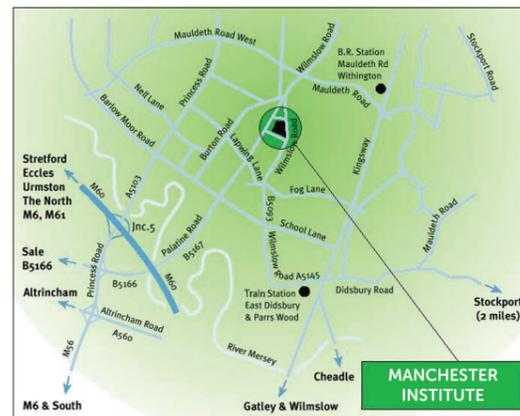
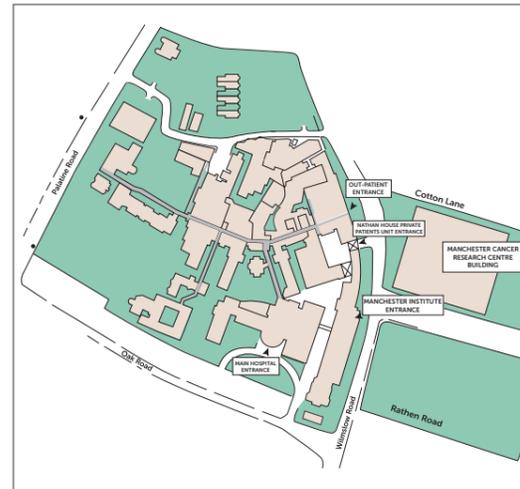
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Electronic version of this report can be found at:  
[www.cruk.manchester.ac.uk/About/](http://www.cruk.manchester.ac.uk/About/)

### Cancer Research UK

Cancer Research UK is a registered charity in England and Wales (1089464), Scotland (SC041666) and the Isle of Man (1103).  
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Tel 44(0) 20 1234 5678  
[www.cruk.org](http://www.cruk.org)



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