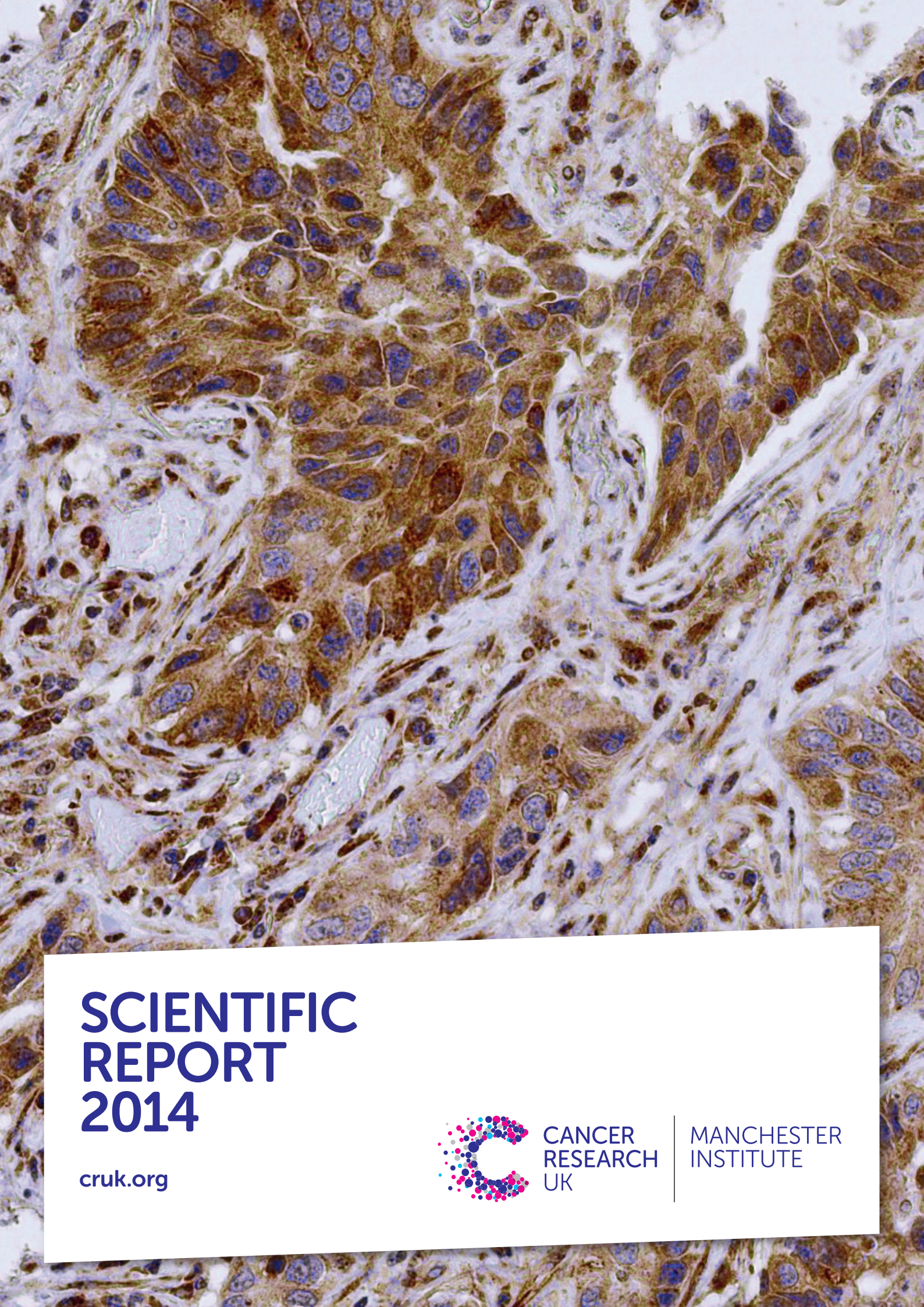

SCIENTIFIC REPORT 2014

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DIRECTOR'S INTRODUCTION

Professor Richard Marais
Director of the Cancer Research UK Manchester Institute

Highlights in 2014 included the arrival of three new Junior Group Leaders, the establishment of our prostate cancer and lung cancer Centres of Excellence and significant investment from the CRT Pioneer Fund into the Drug Discovery Unit.

Claus Jorgensen joined the Institute as a Junior Group Leader at the start of the year and is working on the interactions between tumour and stromal cells in pancreatic cancer. Esther Baena and Michela Garofalo joined in the summer. Esther is heading a team investigating the mechanisms underpinning prostate cancer and the development of novel therapeutics and personalised treatments. Her activities will complement the *Belfast-Manchester Prostate Cancer UK Movember Centre of Excellence* which we inaugurated during the year. The aim of the Centre is to improve prostate cancer survival rates via personalised delivery of therapies based on DNA damage. Michela Garofalo is establishing a team to study the role of non-coding RNAs in lung cancer development and resistance to chemotherapy. This appointment will complement our other activities in the area of lung cancer. A further exciting development was the establishment of a CRUK Lung Cancer Centre of Excellence between the University of Manchester and University College London. The Manchester efforts will be led by Professor Caroline Dive, Deputy Director of CRUK MI, and will contribute towards the key role of the Centre which is to help strengthen and coordinate lung cancer research across the UK. Lung cancer remains an area of priority for CRUK as highlighted in their new research strategy, which was published early in the year and introduced to the Manchester research community in an event hosted at the Institute.

Major research highlights from 2014 included a bioinformatics study that found discrepancies in cancer genomic sequencing that could lead to the discovery of novel driver mutations; the upregulation of mixed-lineage kinases as a potential mechanism by which melanoma cells develop resistance to BRAF inhibitors; identification of a key cell-cell adhesion regulatory pathway whose disruption promotes lung cancer cell invasion and the identification of a phosphoinositol kinase, PIP4K2A as a potential therapeutic target in acute myeloid leukaemia.

Exploration of the role of ultraviolet radiation in the development of melanoma revealed that mutations in the tumour suppressor TP53 (p53) cooperate with BRAF to drive development of the disease. This work, published in Nature, also explored the use of sunscreen and showed that it can delay, but not prevent UV-driven melanoma, highlighting the importance of combining sunscreen application with other sun avoidance strategies. We also developed a novel preclinical model for the study of small cell lung cancer (SCLC)–a disease with a clear unmet clinical need and associated with a lack of relevant material available for research. We demonstrated that circulating tumour cells isolated from patients with SCLC gave rise to genotypically and phenotypically indistinguishable tumours when injected into immunocompromised mice. The study published in *Nature Medicine* also showed that the CDX tumours (CTC-derived explant) responded in the same way to chemotherapeutic treatments as the donor patients.

We also congratulate Georges Lacaud, and the Stem Cell Biology Group, on a successful quinquennial review of his research programme, the aim of which is to study the role of the transcriptional activators RUNX1 and MOZ in haematopoietic development and maintenance in order to better understand better how alterations of these functions lead to leukaemogenesis.

A key aim over the past two years has been the development of the Institute's core facilities to fully support our translational research agenda. This development has been made possible by an award, in 2012 that included £8.7m from HEFCE via the UK Research Partnership Investment Fund. The majority of the funds have now been drawn down and the new equipment is being fully utilised by researchers on site. To ensure that the facilities are running optimally, an external review was held at the end of 2013 and over the past year, several of the resulting recommendations have been implemented. These include expansion of the Histology facility, recruitment of a full time manager for the Molecular Biology Core Facility and the continuing development of a Scientific Computing core facility. The Biological Resources Unit has been reorganised into two sections, a transgenic and an experimental unit each with their own manager along with the recruitment of a Regulatory Liaison and Training Officer.

It is always a pleasure to record various prizes and awards made to members of the Institute. Travel awards were granted from the British Association for Cancer Research to Ewelina Testoni, a graduate student in the Signalling Networks in Cancer Group and to Romina Girotti, a Postdoctoral Research Fellow in Molecular Oncology. It was a very successful year for Romina who also received a travel bursary from the Society for Melanoma Research (SMR) to present her work on the development of preclinical models of melanoma at the SMR congress in Zurich. Romina's success throughout the year was also recognised by CRUK MI as she received the Institute's Dexter Award for Young Scientists. Andrew Porter, a Postdoctoral Research Fellow in the Cell Signalling group, was awarded a prize for his poster at the ESF-EMBO Cell Polarity and Membrane Trafficking meeting.

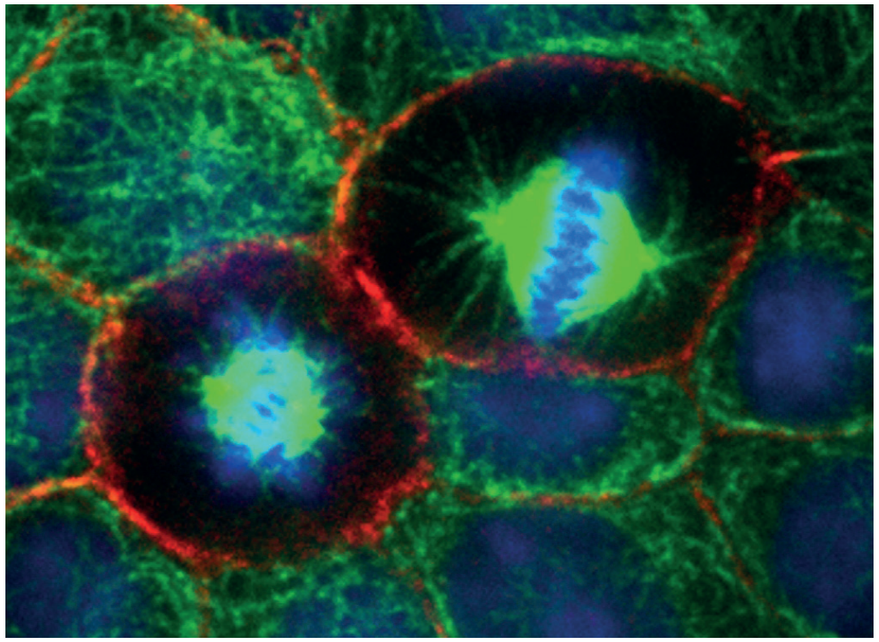
Tim Somerville, a PhD student in the Leukaemia Biology group received an Abstract Achievement Award from the American Society of Hematology (ASH) which allowed him to attend, and present his data, on transcriptional regulation in acute myeloid leukaemia at the annual ASH meeting in California. Tim Somervaille, a clinician scientist who heads the Leukaemia Biology group was elected to the Royal College of Physicians.

I was elected as President of the European Association of Cancer Research (EACR), sitting from 2014 to 2016. During my presidency, I will take the lead role in organising the 24th EACR Biennial Congress, which will be held in Manchester in July 2016. This prestigious event will help raise the profile of cancer research in Manchester on a global level and coincides with Manchester becoming the European City of Science for 2016. Another personal highlight during 2014 was my role as Chair of the annual National Cancer Research Institute (NCRI) conference held in Liverpool. The NCRI brings together stakeholders from across the research spectrum which allows for a broad discussion of many of the issues relevant to cancer research. This year marked the 10th anniversary of the meeting, a milestone that presented a good opportunity to reflect on the progress made over the previous decade and also to look forward to what the future may hold. It was particularly pleasing to see a high level of participation at the meeting from members of the Institute, both in oral and poster presentations.

We have continued our drive to supplement our core funding from CRUK with other grants in order to increase the breadth of research that we undertake. Successful applications were made to various funding bodies including the British Lung Foundation, the Lung Cancer Research Foundation, the Moulton Charitable Foundation and the MRC. The Drug Discovery Unit was awarded £1.6m from Sixth Element Capital, who manage the Cancer Research Technology Pioneer Fund which is aimed at progressing research derived from CRUK-funded projects. The award will be used for the development of RET inhibitors for non-small cell lung cancer patients with relevant mutations.

Our public engagement programme continues to go from strength to strength and was led during 2014 by Hannah Leaton. Over 1300 visitors came to the Institute throughout the year – a significant increase on numbers for 2013. Over 50 researchers were involved in the Institute's Open Day, which has become a bi-annual event. Our scientists also engaged with more than 3000 people at a range of external events while our social media presence continued to grow (@CRUK_MI). Three labs took part in 'virtual' engagement activities, ensuring that our progress can be communicated to a wider audience. Esther Baena was involved in an interesting project to take scientific research to a new audience when she teamed up with a Pennsylvanian fashion designer to create a dress showing how prostate cancer spreads in the body. Their work was featured on live models at various events in America.

Allan Jordan, Head of Chemistry in the Drug Discovery Unit, has been a very committed participant in our public engagement programme so it was a pleasure to see his efforts and enthusiasm rewarded by CRUK through their Flame of Hope Special Commendation Award for Research Engagement.



Mitotic MDCK II cells. *Image supplied by Andrew Porter (Cell Signalling).*

In the coming year we shall welcome Santiago Zelenay as a new Junior Group Leader working on melanoma immunology. Further group leader recruitment will continue especially in the area of molecular pathology which is of critical importance to the personalised medicine agenda that lies at the core of the Institute's strategy.

Our PhD students will be organising and hosting the 9th International PhD Student Cancer Conference (IPSCC), which aims to provide high calibre PhD students from cancer research institutes across Europe with the opportunity to network, present their research and interact with leading scientists. The students are organising all aspects of the event which promises to be an interesting and rewarding experience.

The new MCRC research building is set to open in the summer of 2015 and we look forward to the opportunities that this will bring, both in terms of room for expansion for the Institute and the increase in cancer research activity on this site.The coming year looks to be every bit as promising as 2014 proved to be.

RESEARCH HIGHLIGHTS

In this section we highlight some research publications from 2014 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.

Hodgkinson CL, Morrow CJ, Li Y, Metcalf RL, Rothwell DG, Trapani F, Polanski R, Burt DJ, Simpson KL, Morris K, Pepper SD, Nonaka D, Greystoke A, Kelly P, Bola B, Krebs MG, Antonello J, Ayub M, Faulkner S, Priest L, Carter L, Tate C, Miller CJ, Blackhall F, Brady G, Dive C.
Tumorigenicity and genetic profiling of circulating tumor cells in small-cell lung cancer.
Nature Medicine, 2014; 20(8):897-903.

Small cell lung cancer (SCLC) is a disease with dismal prognosis whilst treatment options for patients with SCLC have not improved since the 1980s. This is due, in part, to a lack of SCLC biological material available for research which has led to few preclinical models that truly reflect the clinical disease. One option for obtaining clinical SCLC samples, in the absence of resected or biopsied material, is via the isolation of circulating tumour cells (CTCs) from SCLC patients' blood; previous research from the Clinical and Experimental Pharmacology group has demonstrated that CTCs are more prevalent in SCLC patients than in patients with any other tumour type. In this study, the team demonstrated that when SCLC patient CTCs were injected into immunocompromised mice they gave rise to CTC derived explant (CDX) tumours that were genotypically and phenotypically indistinguishable from SCLC tumours. Importantly, when CDX tumours were challenged with cisplatin and etoposide, the standard of care for SCLC patients, they responded in the same way as the donor patient. This is the first description of SCLC CTCs being tumourigenic and provides a novel approach for the generation of clinically relevant preclinical models to study a disease with a clear unmet clinical need.

Hudson AM, Yates T, Li Y, Trotter EW, Fawdar S, Chapman P, Lorigan P, Biankin A, Miller CJ, Brognard J.
Discrepancies in cancer genomic sequencing highlight opportunities for driver mutation discovery.
Cancer Research, 2014; 74(22):6390-6.

Despite hundreds of lung cancers having been sequenced we still lack knowledge about common drivers for a majority of these cancers. Whilst mining online cancer genomics data for novel genes, the Signalling Networks in Cancer and RNA Biology groups noticed a discrepancy between the outputs of the two most prominent resources (COSMIC and CCLE) and wondered if this was causing targets to be missed. They therefore carried out a comparison of 568 mutually sequenced cell lines and found a poor concordance rate (57.38 %) of reported mutations. Looking more closely, they found the most common reason for the discrepancy was poor sequencing of GC-rich regions. This is important in lung cancers where GC nucleotides are more likely to be mutated due to the mutagenic effects of cigarette smoke. They identified over 400 large regions (sequencing cold-spots) in known cancer-causing genes and kinases, where next generation sequencing methods fail to read the DNA. Extrapolating the data, they estimate an average of three mutations per cell line is being missed in this small subset of genes alone. The groups identified a gain-of-function mutation in one of the cold-spots (PAK4) that had not been reported by COSMIC or CCLE. The study suggests that specifically optimising the sequencing of GC-rich regions may lead to enhanced discovery of commonly mutated genes in lung cancer.

Vaughan L, Tan CT, Chapman A, Nonaka D, Mack NA, Smith D, Booton R, Hurlstone AF, Malliri A.
HUWE1 ubiquitylates and degrades the RAC activator TIAM1 promoting cell-cell adhesion disassembly, migration, and invasion.
Cell Reports, 2014; Epub 2014 Dec 24.

Malignant conversion of epithelial tumour cells is incompletely understood. Prior to this study the Cell Signalling group, amongst others, had demonstrated that the RAC activator TIAM1 promotes cell-cell adhesion and thereby suppresses invasiveness of epithelial cells. They then wanted to understand how TIAM1/RAC signalling can be down-regulated in malignant cells. In this paper they uncovered a role for HUWE1, an E3 ubiquitin ligase previously implicated in tumour formation, in cell migration and invasion through degrading TIAM1, implying an additional function for HUWE1 in malignant progression. They showed that in MDCKII cells in response to HGF, HUWE1 catalyses TIAM1 ubiquitylation and degradation predominantly at cell–cell adhesions, facilitating junction disassembly, migration and invasion. Depleting HUWE1 or mutating the TIAM1 ubiquitylation site prevented TIAM1 degradation, antagonising scattering and invasion. Moreover, simultaneous depletion of TIAM1 restored migration and invasion in HUWE1-depleted cells. Significantly, the group showed that HUWE1 stimulates human lung cancer cell invasion through regulating TIAM1 stability. Finally, they demonstrated that HUWE1 and TIAM1 protein levels are inversely correlated in human lung carcinomas. Thus, they elucidated a critical role for HUWE1 in regulating epithelial cell–cell adhesion and provided further evidence that ubiquitylation contributes to spatio-temporal control of RAC.

Marusiak AA, Edwards ZC, Hugo W, Trotter EW, Girotti MR, Stephenson NL, Kong X, Gartside MG, Fawdar S, Hudson A, Breitwieser W, Hayward NK, Marais R, Lo RS, Brognard J.
Mixed lineage kinases activate MEK independently of RAF to mediate resistance to RAF inhibitors.
Nature Communications, 2014; 5:3901.

Recent progress in cancer treatment is based on the development of targeted therapies, including vemurafenib that targets mutated BRAFV600E, a well-known oncogene present in melanomas. RAF inhibitor therapy results in significant clinical response in the majority of V600E-positive melanoma patients, however resistance occurs within 2-18 months. Therefore, identifying and understanding the mechanisms of resistance is essential to achieve long-term progression-free survival. This work demonstrated that the mixed lineage kinases (MLK1-4) can mediate resistance to vemurafenib in BRAFV600E-positive melanomas. The study described MLKs as direct MEK kinases that reactivate the MEK/ERK pathway in the presence of RAF inhibitors and promote survival of V600E-positive melanoma cell lines. Furthermore, MLKs promote resistance to RAF inhibitors in cell line and mouse models and upregulation of MLKs was detected in drug-resistant tumours from melanoma patients. Finally, several MLK1 mutations observed in melanoma patients were characterised and found to be intermediate-activating mutations that could predispose patients to de novo resistance. These findings will have broad implications as the MEK/ERK pathway is a critical pathway required to promote cell proliferation and survival in many different types of cancer. In the future, the Signalling Networks in Cancer group will characterise novel inhibitors that target this family of kinases in collaboration with pharmaceutical companies.

Viros A, Sanchez-Laorden B, Pedersen M, Furney SJ, Rae J, Hogan K, Ejiama S, Girotti MR, Cook M, Dhomen N, Marais R.
Ultraviolet radiation accelerates BRAF-driven melanomagenesis by targeting TP53.
Nature, 2014; 511(7510):478-82.

Cutaneous melanoma is epidemiologically linked to ultraviolet radiation (UVR), but the molecular mechanisms by which UVR drives melanomagenesis have not been clear to date. The most common somatic mutation in melanoma causes a V600E substitution in the signalling protein BRAF, which is an early event. To investigate how UVR accelerates oncogenic BRAF-driven melanomagenesis, the Molecular Oncology group used a BRAF(V600E) mouse model. In mice expressing BRAF(V600E) in their melanocytes, a single dose of UVR that mimicked mild sunburn in humans induced clonal expansion of the melanocytes, and repeated doses of UVR increased melanoma burden. The team showed that sunscreen (UVA superior, UVB sun protection factor (SPF) 50) delayed the onset of UVR-driven melanoma, but only provided partial protection. The UVR-exposed tumours showed increased numbers of single nucleotide variants and they observed mutations (H39Y, S124F, R245C, R270C, C272G) in the Trp53 tumour suppressor in approximately 40% of cases. TP53 is an accepted UVR target in human non-melanoma skin cancer, but is not thought to have a major role in melanoma. However, this study found that in mice, mutant Trp53 accelerated BRAF(V600E)-driven melanomagenesis, and that TP53 mutations are linked to evidence of UVR-induced DNA damage in human melanoma. Thus, the group were able to provide mechanistic insight into epidemiological data linking UVR to acquired naevi in humans. Furthermore, they identified TP53/Trp53 as a UVR-target gene that cooperates with BRAF(V600E) to induce melanoma, providing molecular insight into how UVR accelerates melanomagenesis. This study validates public health campaigns that promote sunscreen protection for individuals at risk of melanoma but also underscores the importance of combining this with other sun avoidance strategies.

Wiseman DH, Small HF, Wilks DP, Waddell ID, Dennis MW, Ogilvie DJ, Somervaille TC.
Elevated plasma 2-hydroxyglutarate in acute myeloid leukaemia: association with the IDH1 SNP rs11554137 and severe renal impairment.
British Journal of Haematology, 2014; 166(1):145-8.

Somatic mutations in isocitrate dehydrogenase (IDH) enzymes IDH1 and IDH2 occur in 10–20% of cases of acute myeloid leukaemia (AML) and result in neomorphic production of the oncometabolite D-2-hydroxyglutarate. This inhibits α-ketoglutarate-dependent dioxygenases promoting leukaemic transformation. In collaboration with Helen Small in the Drug Discovery Unit, the Leukaemia Biology group screened diagnostic plasma and cell samples from 102 patients presenting with AML or myelodysplasia to The Christie NHS Foundation Trust for their plasma 2-HG levels and IDH mutation status. Samples were from the Manchester Cancer Research Centre's Biobank. The team identified threshold levels of 2-HG, which predicted the presence of an IDH mutation with high sensitivity and specificity and identified two additional, novel associations of elevated plasma 2-HG in AML patients: acute renal impairment and the presence of a single nucleotide polymorphism in *IDH1* that has previously been associated with poor prognosis. These data will inform on the identification and appropriate management of patients suitable for treatment with specific molecular inhibitors of mutant IDH1 and IDH2, which are advancing through early phase clinical trials.

Jude JG, Spencer GJ, Huang X, Somerville TD, Jones DR, Divecha N, Somervaille TC.
A targeted knockdown screen of genes coding for phosphoinositide modulators identifies PIP4K2A as required for acute myeloid leukemia cell proliferation and survival.
Oncogene, 2014 Mar 31. doi: 10.1038/onc.2014.77. [Epub ahead of print]

Identifying genes and cellular pathways which are essential for the proliferation and survival of acute myeloid leukaemia (AML) cells but which are not required for normal bone marrow cells is an important approach to the discovery of novel candidate therapeutic targets. In collaboration with Nullin Divecha's Inositide Laboratory, the Leukaemia Biology group performed a targeted knockdown screen of genes that regulate phosphoinositide signalling lipids in human AML cells. They took this approach because phosphoinositide signalling is known to be deregulated in leukaemic haematopoiesis. One of the screening hits was the lipid kinase phosphatidylinositol-5-phosphate 4-kinase, type II, alpha (PIP4K2A) which regulates cellular levels of phosphatidylinositol-5-phosphate and phosphatidylinositol 4,5-bisphosphate. They found *PIP4K2A* to be essential for the clonogenic and leukaemia-initiating potential of human AML cells, including primary cells. Its knockdown results in accumulation of the cyclin-dependent kinase inhibitors CDKN1A and CDKN1B, G_1 cell cycle arrest and apoptosis. Both CDKN1A accumulation and apoptosis were partially dependent on activation of the mTOR pathway. Critically, however, *PIP4K2A* knockdown in normal haematopoietic stem and progenitor cells did not adversely impact either clonogenic or multilineage differentiation potential, indicating a selective dependency. Thus, PIP4K2A is a novel candidate therapeutic target in myeloid malignancy.

Sanchez-Laorden B, Viros A, Girotti MR, Pedersen M, Saturno G, Zambon A, Niculescu-Duvaz D, Turajlic S, Hayes A, Gore M, Larkin J, Lorigan P, Cook M, Springer C, Marais R.
BRAF inhibitors induce metastasis in RAS mutant or inhibitor-resistant melanoma cells by reactivating MEK and ERK signaling.
Science Signaling, 2014; 7(318):ra30.

Melanoma is a highly metastatic and lethal form of skin cancer. In approximately half of all melanoma cases, the tumour contains a mutation in the BRAF gene. BRAF inhibitors improve progression-free and overall survival in these patients. However, after a relatively short period of disease control, most patients develop resistance because of reactivation of the

RAF-ERK (extracellular signal-regulated kinase) pathway, mediated in many cases by mutations in RAS. BRAF inhibitors such as vemurafenib are known to have different effects in BRAF mutant cells depending on the additional presence of RAS mutations. In this study, the Molecular Oncology group further explored the effect of both genetic and pharmacological inhibition of BRAF on RAS mutant melanoma cells. The team observed increases in ERK-interleukin 8 mediated signalling and secretion of extracellular proteases, leading to invasion in vitro and metastasis in vivo. In addition, the dominant morphology of the tumour cells switched from rounded to spindle-shaped cells. This behaviour also occurred in cells that were resistant to BRAF inhibitors. Finally, the group were able to block this invasion and metastasis using MEK inhibition. Their results support the use of BRAF and MEK inhibitors in combination in the clinic.

Lie-A-Ling M, Marinopoulou E, Li Y, Patel R, Stefanska M, Bonifer C, Miller C, Kouskoff V, Lacaud G.
RUNX1 positively regulates a cell adhesion and migration program in murine hemogenic endothelium prior to blood emergence.
Blood, 2014; 124(11):e11-20.

The gene encoding the AML1/RUNX1 transcription factor is frequently rearranged or mutated in human leukaemias. Consistent with its implication in leukaemia, RUNX1 has also been shown to be critical for haematopoietic development. RUNX1 has an essential function in enabling precursors of blood cells to emerge from specialised cells called haemogenic endothelium (HE). However, HE cells are rare and short-lived, making them hard to study. The Stem Cell Biology and Stem Cell Haematopoiesis teams used a technique known as DamID (DNA adenine methyltransferase identification) to deposit 'tags' at points on the DNA that interact with the RUNX1 molecule, and then detected the location of these tags. By looking at the location of the tags, and combining them with observed alterations of gene expression following *Runx1* deletion, they identified genes which are directly switched on by RUNX1. The work found an unexpected role for RUNX1 in regulating cell adhesion- and cell migration-associated genes within the HE, as it had previously been thought to be more of a haematopoietic regulator. They identified the RUNX1b form of the protein as responsible for this activity. Outside of the haematopoietic context, this cell adhesion- and cell migration RUNX1 signature in HE might also reflect the emerging new role of RUNX1 in epithelial-based tumour formation, progression and in particular metastasis.

Potter DS, Kelly P, Denneny O, Juvin V, Stephens LR, Dive C, Morrow CJ.
BMX acts downstream of PI3K to promote colorectal cancer cell survival and pathway inhibition sensitizes to the BH3 mimetic ABT-737.
Neoplasia, 2014; 16(2):147-57.

The phosphatidylinositol-3-kinase (PI3K) signalling network is frequently upregulated in many cancers and is widely regarded as preventing apoptotic cell death leading to cancer cell survival. However, in colorectal cancer (CRC) cell lines inhibition of PI3K signalling does not lead to cell death. To determine whether PI3K inhibition might prime cells for apoptosis the Clinical and Experimental Pharmacology group investigated the combination of PI3K inhibitors with the BH3 mimetic ABT-737, which directly induces apoptosis via inhibition of some anti-apoptotic BCL2 family proteins. This research demonstrated that the combination of PI3K inhibitor and ABT-737 caused increased apoptosis; however, inhibitors of AKT and mTOR, canonical downstream effectors of PI3K signalling, did not increase ABT-737 induced apoptosis, suggesting an alternative PI3K-dependent pro-survival pathway. A siRNA screen of potential downstream targets of PI3K demonstrated that knockdown of the non-receptor tyrosine kinase BMX sensitised to ABT-737, although BMX knockdown did not further sensitise cells to ABT-737 when PI3K activity was also inhibited. This study revealed the presence of a novel pro-survival PI3K signalling pathway and suggests a new rational drug combination that could be taken forward in clinical trials for the treatment of colorectal cancer patients.

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[1]left in 2014
[2]joined in 2014
[3]joint with RNA Biology

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.

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.

Commitment to mitosis is instigated by the activity of the 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 invoke the formation of the mitotic spindle. As the spindle forms, the chromosomes condense and their specialised kinetochore regions attach to the microtubules that emanate from either pole of the bipolar spindle. Kinetochore attachment continues until each chromosome has been captured by microtubules from opposing centrosomes, whereupon Cyclin B is targeted for destruction by the Anaphase Promoting Complex (APC). Cyclin B destruction abolishes Cdk1-Cyclin B activity to mark the end of the chromosome capture and alignment phase (prophase and metaphase) and promote the separation of the two sister chromatids to either spindle pole (anaphase).

The timing of APC activation is regulated by kinetochore attachment to the spindle because each unattached kinetochore generates an APC inhibitory signal in a phenomenon called the Spindle Assembly Checkpoint (SAC) (Figure 1). Extended SAC signalling is symptomatic of major problems in chromosome transmission that would probably lead to genome instability if they were ever to be resolved. To ensure that such dangerous cells are eliminated from the body, prolonged SAC signalling triggers programmed cell death (apoptosis).

Although we know very little about the mechanism by which prolonged mitotic arrest triggers apoptosis, this form of cell death has been widely exploited in chemotherapy for decades through the imposition of mitotic arrest by anti-microtubule drugs (e.g. vinca-alkaloids and taxols). The genome instability and rearrangements of tumour cells means that they invariably have greater problems in genome segregation than normal cells and so have heightened sensitivity to the additional burden of spindle disruption invoked by anti-microtubules. In other words, doses of anti-microtubules that simply delay mitotic progression in untransformed cells, combine with the inherent segregation issues of transformed cells to induce death.

Unfortunately, however, anti-microtubule drugs invoke undesirable side effects. Because microtubules play major structural roles in neurons, anti-microtubule agents are dose limited by painful *chemotherapy induced peripheral neuropathy.* This pathological side effect prompted the development of *next generation* anti-mitotic drugs that specifically target mitotic spindle function without impacting upon the interphase functions of microtubules upon which the neuronal system relies. However, these novel anti-mitotics are proving less effective than anticipated as neutropenia has emerged as a dose limiting toxicity. These disappointments have prompted a re-evaluation of the means by which the mitotic arrest imposed by anti-microtubules could be emulated or enhanced by targeting other molecules. Two avenues have come to the fore of this quest for a further generation of mitotic drugs: APC inhibitors and delaying mitotic exit. Either approach should harness the natural apoptotic networks to eliminate cancer while preserving the viability of the neighbouring normal tissues.

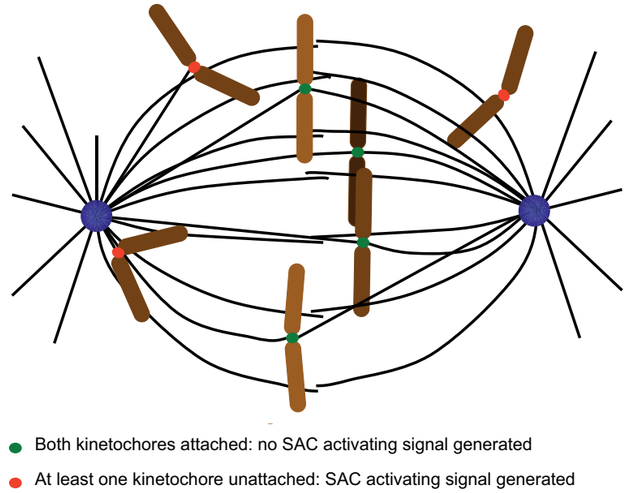

Figure 1
The mitotic spindle and SAC signalling
A schematic of the mitotic spindle with representation of the centrosomes (purple), chromosomes (brown) and microtubules (black). Kinetochores are shown as either red or green ovals depending upon their SAC checkpoint status. Unattached kinetochores that generate APC inhibitory SAC signals are shown in red while the attached kinetochores that emit no signal are green.

Mitotic exit is enforced by phosphatases that remove the phosphate that mitotic kinases put on targets in order to drive the rearrangements of cellular architecture that generated the mitotic apparatus. Just as the sequence of phosphorylation events that drives spindle formation must be highly co-ordinated, the removal of these phosphates must be equally well controlled to ensure the staged progression from one phase of division to the next.

Identification of a phosphatase relay controlling mitotic progression

Over 95% of phosphatase activity in human cells has been attributed to Protein Phosphatase 1 (PP1) and Protein Phosphatase 2A (PP2A). The direct inhibition of PP1 by Cdk1-Cyclin B has long highlighted the potential of this phosphatase for mitotic control, while functional studies identified a key role for PP2A phosphatases in driving mitotic exit. We recently uncovered an unanticipated link between these two major classes of phosphatases. We found that the staged recruitment of PP1 to docking sites in the regulatory subunits of the PP2A-B55 and PP2A-B56 phosphatases drove the sequential activation of the PP2A enzymes. PP1 bound PP2A-B55 from mitotic commitment to genome segregation in anaphase.

Because Cyclin B destruction enables PP1 to auto-catalytically remove the Cdk1-Cyclin B imposed inhibitory phosphorylation, it also supports the reactivation of PP2A-B55 by PP1. PP1 also bound and reactivated PP2A-B56 phosphatase, however the presence of a phosphate within the PP1 docking site of the B56 subunit blocked PP1 recruitment until the end of mitosis. It was only when this phosphate was finally removed that PP1 could dock and reactivate this second PP2A phosphatase. Strikingly, the phosphatase that removed the inhibitory phosphate from PP2A-B56 to promote PP1 docking was PP2A-B55. Thus, Cyclin B destruction set in train a phosphatase relay that sequentially restored PP2A-B55 and PP2A-B56 activities to ensure the efficacy of mitotic exit. An engaging collaboration with the CRUK funded team of Professor Jonathan Pines at the Gurdon institute in Cambridge, established that human PP1 binds the docking site of human PP2A-B56 to suggest that the core principles of the phosphatase relay we have identified in fission yeast are conserved in human cells.

Lessons from yeast

The identification of this intimate relationship between PP1 and PP2A enzymes now raises an obvious set of questions including the identity of the target for PP1 in PP2A holoenzyme complexes and the control of PP1 recruitment to PP2A-B55. The high degree of conservation between the yeast and human phosphatases suggests that yeast work will once more prove instrumental in guiding our progress towards the manipulation of the human phosphatases that could trap cells in mitosis with high levels of SAC signalling that would, in turn, trigger apoptosis of genomically unstable transformed cells.

Publications listed on page 52

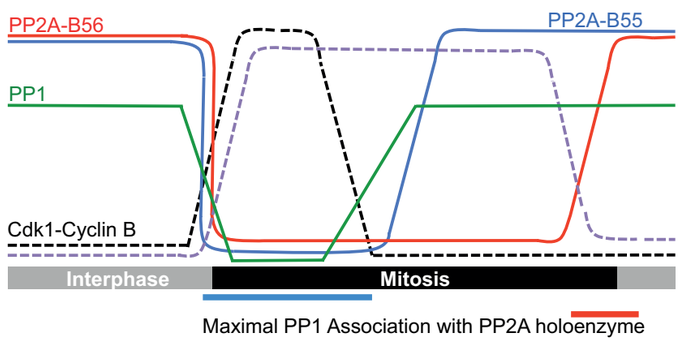

Figure 2
The mitotic PP1-PP2A phosphatase relay
PP1 and PP2A activities are all repressed upon entry into mitosis. The mode of PP2A repression is unclear, however, it is well established that Cdk1-Cyclin B phosphorylation represses PP1 activity. Cyclin B destruction then allows PP1 to auto-catalytically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

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

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Over a number of years the Cell Regulation group has focused on delineating the functions of stress activated signalling pathways primarily involving JNK and p38 kinases. Using molecular and genetic approaches we have focused on members of the AP-1 family transcription factor, principally ATF2 and ATF7, which are crucial substrates for these kinases and are involved in development and, critically, in oncogenic transformation. We have developed cellular and genetic mouse models of human cancer and confirmed that stress kinases, through the activation of transcriptional programmes, are critical in positive or negative regulation of oncogenic transformation.

The stress activated MAP kinase pathway comprises a multi-tiered signalling cascade involved in a wide range of biological activities. At its heart lie two distinct kinase families, JNK and p38, that are activated not only by cellular stress and inflammation, but also by growth factors and cytokines, and function in the regulation of proliferation, differentiation, cellular senescence and apoptosis. They exert this through the activation of a multitude of substrates ranging from transcription factors, including c-Jun, ATF2, and p53, to mitochondrial apoptotic regulators, including Bcl-2 and Bim. Consequently, in the context of cancer, JNK and p38 have been shown to be critical in oncogenic transformation, tumour metastasis, as well as tumour-stroma interactions.

Not surprisingly, given the multitude of substrates, the roles of kinase families are complex and often seemingly contradictory. While many of these functions for JNK dependent signalling have been delineated using cellular and genetic model systems, recent advances in cancer genome sequencing have highlighted the high frequency at which components of stress signalling pathways are deregulated, or mutated in various tumour types including breast, prostate, endometrial and ovarian cancer. The nature of these mutations suggests that the pathway is aberrantly activated in some types of cancer but, to a far greater extent, inactivated in many other types. Thus, stress signalling pathways are thought to be pro-oncogenic or tumour suppressive depending on the cancer context.

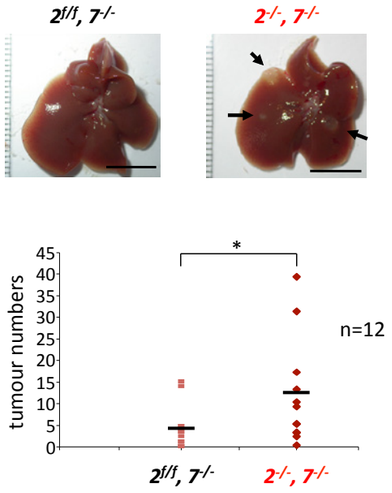

Figure 1
Loss of ATF2 and 7 sensitises to oncogenic transformation in mouse liver cells. ATF2-wt/7-ko or ATF2/7 double knockout hepatoblasts were transformed with oncogenic HRas and reintroduced into mice by orthotopic injection. Resulting liver tumours were significantly increased in number in the absence of functional ATF2 (arrows in right panel).

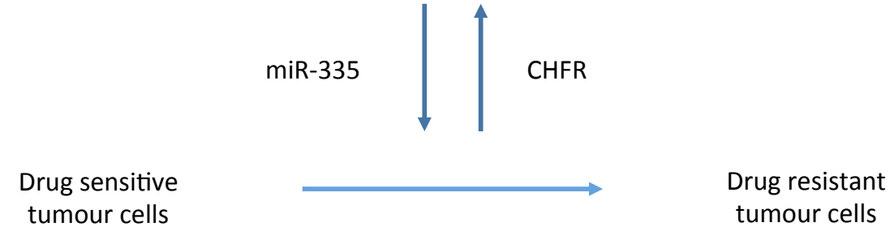

Figure 2
Inverse correlation of miR-335 expression and CHFR expression in the cisplatin-sensitive A2780 ovarian tumour cell line and the cisplatin-resistant CP70 cell line.

ATF2 and ATF7 in development and tumour initiation

One focus of research in the Cell Regulation group has been the AP-1 transcription factors ATF2 and ATF7, which are effector substrates of both JNK and p38 (reviewed in Gozdecka and Breitwieser, 2012). Our research showed that ATF2/7 have essential functions during development of the embryonic liver and that this is dependent on its activation by the SAPK signalling cascade (Breitwieser et al., 2007). This work, as well as a further project describing the deficiencies in mouse brain development (Ackermann et al., 2011) in the absence of functional ATF2/7, uncovered a role for these transcription factors in a negative feedback loop to regulate the potentially hazardous activities of its upstream acting stress kinases.

High levels of phospho-ATF2 have been detected in both human melanoma and prostate carcinoma samples, and a role for ATF2 in driving progression of these tumours has been suggested in the literature. Conversely, low levels of ATF2 expression in human breast tumours have also been reported. Experimental analysis using mouse tumour models carried out by ourselves, as well as by other research groups, has uncovered diverse roles for ATF2 in tumourigenesis. Accordingly, ATF2 was shown to contribute to melanoma development through its role in melanocyte-specific gene activation (Shah et al, 2010). In contrast, ATF2-deficient mice are sensitised to carcinogen-induced skin tumourigenesis, underlining the tumour context dependent activities of the stress signalling pathway. In a mouse model of Myc oncogene-induced B-cell lymphoma development, we showed that ATF2 deficiency results in accelerated disease onset. Further in vitro analysis revealed that ATF2 responds to oncogene-induced cellular stress by inducing programmed cell death (Walczynski et al., 2013).

ATF2 drives transcriptional programmes in tumour suppression

A recent research project has focused on the role of components of the SAPK pathway in hepatocellular carcinoma (Gozdecka et al, 2014). Here, we demonstrated that JNK dependent activation of ATF2 is critical in blocking the oncogenic transformation of hepatocyte precursors (hepatoblasts) as well as in suppressing their tumourigenicity after orthotopic transplantation into recipient mouse livers. In addition, we defined a JNK and ATF2 dependent transcriptional programme that acts in a tumour suppressive manner. Further analysis revealed that this programme is frequently found inactivated or genetically altered in a variety of human tumours types, including breast, brain, colorectal and lung carcinoma. This analysis therefore confirmed that the experimental tumour models reflect human cancer scenarios. Further analysis of SAPK dependent effectors as well as other components of stress signalling pathways will therefore be the focus of future research.

MiR-335 in therapeutic drug response
MicroRNAs (miRs) are important regulators of gene expression and their deregulation is a common feature in tumour cells. We identified miR-335 among microRNAs that are transcriptionally down-regulated upon expression of oncogenic Ras. Uniquely, miR-335 is up-regulated transcriptionally in cells after treatment with the DNA damaging agent cisplatin. Frequently, in ovarian tumour cells miR-335 expression is epigenetically silenced by methylation that can be reversed by treatment with demethylating drugs. In a cisplatin resistant derivative (CP70) of the ovarian tumour cell line A2780, expression of miR-335 significantly re-sensitises to cisplatin-induced apoptosis. This correlates with the finding of epigenetic silencing of the endogenous miR-335 gene locus in CP70 cells. In contrast, a putative miR-335 target gene, CHFR, is epigenetically silenced in A2780 cells but shows significant expression in CP70 cells. Thus, CHFR expression in A2780 cells may be suppressed by both epigenetic silencing through methylation and post-transcriptionally by miR-335. Suppression of CHFR in CP70 cells by siRNA leads to significant sensitisation to cisplatin. CHFR is a known modulator of chromatin modifying enzymes and we find that both expression of miR-335 and suppression of CHFR leads to altered expression of histone deacetylase, HDAC1. Our data therefore suggest that CHFR is a critical target for miR-335 mediated sensitisation to cisplatin.

Publications listed on page 52

CELL SIGNALLING

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Tumour initiation and progression result from the inappropriate activity of intracellular signalling cascades. RHO-like GTPases are molecular switches in signalling pathways that regulate the organisation of the actin and microtubule cytoskeletons, junctional complexes, and extracellular matrix attachments, as well as regulating gene transcription. In this way, RHO proteins influence cell morphology, adhesion, motility, as well as cell cycle progression and survival. Data has emerged to directly implicate RHO proteins in tumourigenesis. We investigate the mechanisms by which certain regulators of the RHO-like GTPase RAC control cell cycle progression and cell–cell adhesion, how their activities, as well as activity of RAC itself, are controlled and how deregulated RAC signalling contributes to tumourigenesis.

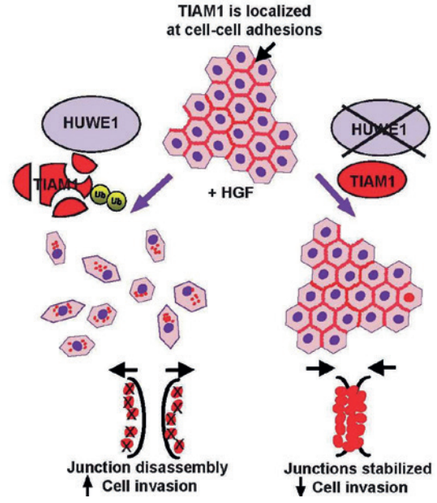
RAC cycles between a GDP- and a GTP-bound state. When GTP-bound, it interacts with various effector molecules that elicit downstream responses including, notably, actin cytoskeletal reorganisation. Multiple mechanisms control RAC activity, including control of nucleotide binding and hydrolysis by Guanine nucleotide Exchange Factors (GEFs) and GTPase Activating Proteins (GAPs) respectively, regulation of subcellular localisation, modulation of RAC protein levels, and post-translational modification including isoprenylation and, as we and others have recently demonstrated, ubiquitylation and SUMOylation (Castillo-Lluva et al., 2013; Castillo-Lluva et al., 2010).

RHO GEFs are typically large proteins harbouring multiple protein–protein interaction domains. Besides stimulating guanine nucleotide exchange, GEFs act as molecular scaffolds targeting RHO molecules to particular subcellular locations and increasing the local concentration of selective effector molecules, thereby influencing downstream processes. Through influencing selectivity in RHO signalling, GEFs perform non-redundant signalling roles which can confer a significant involvement in tumourigenesis. TIAM1 is a guanine nucleotide exchange factor that selectively activates RAC. Mice deficient for 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., 2002). Thus TIAM1, we infer, plays a unique role in mediating RAS transformation, which the cell signalling group is currently elucidating.

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., 2010). Subsequent to this study, we have found that TIAM1 is phosphorylated by Cyclin B/CDK1 at the onset of mitosis. We have now mapped the dominant phosphorylation site on TIAM1 and by creating a non-phosphorylatable mutant, demonstrated that while not required for TIAM1 localisation to centrosomes, phosphorylation is essential for its role in regulating centrosome separation. Currently we are investigating the mechanism by which phosphorylation of TIAM1 influences its role at centrosomes and have delineated a novel function of phosphoTIAM1 in stimulating the activity of an important RAC effector that we also implicate in centrosome separation.

Figure 1
In benign epithelial cells, TIAM1 localises to cell–cell junctions where it stimulates cell adhesion. Cytokines like HGF promote the migration and invasion of epithelial cells by triggering degradation of TIAM1 by HUWE1. Without TIAM1 at cell–cell junctions, the junctions disassemble giving cells an opportunity to disaggregate. Disassociation of epithelial cells can enhance their motility and invasiveness.



TIAM1 antagonises malignant progression

Despite their slower growth, tumours arising in Tiam1-deficient mice progress more frequently to malignancy (Malliri et al., 2002). One mechanism by which TIAM1 and RAC suppress malignant progression is through promoting cell–cell adhesion. We recently identified β2-syntrophin, a component of the dystroglycan adhesion complex, as a TIAM1 binding partner. Our study (Mack et al., 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., 2009).

In a recently published study, 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 through 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 (Vaughan et al., 2014). 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.

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CLINICAL AND EXPERIMENTAL PHARMACOLOGY

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The CEP group places emphasis on the discovery, development and validation of circulating biomarkers to facilitate drug development and to aid cancer patient treatment decision making. This year we also placed considerable emphasis on the development of lung cancer patient Circulating tumour cell Derived eXplant models (CDX) which faithfully recapitulate patient responses to standard of care chemotherapy. CDX are being used to explore the biology of both Small Cell and Non Small Cell Lung Cancer, to identify new drug targets and to test novel treatments and biomarkers in collaboration with Pharma partners and the CRUK Centre for Drug development.

Promising novel drugs and drug combinations will be translated to clinical trials within our newly established Manchester and UCL CRUK Lung Cancer Centre of Excellence. This year the nucleic acids biomarkers team has developed our capability for single circulating tumour cell molecular analysis facilitating exploration of tumour heterogeneity. We have also developed sensitive circulating tumour DNA (ctDNA) assays for deployment in the upcoming Christie NHS Foundation Trust TARGET protocol, working with our clinical colleagues to optimise selection of the most appropriate phase I trial for their patients.

Preclinical Pharmacology Team – Research Highlights

(i) CDX models Circulating tumour cells (CTCs) are highly prevalent in SCLC patients. We asked whether CTCs, enriched from Small Cell Lung Cancer (SCLC) patients and implanted into immune-compromised mice, could give rise to tumours and whether these tumours reflected the response of the donor patient to therapy. To date we have implanted CTCs from 35 extensive stage SCLC patients prior to treatment, with follow-up relapse samples from 10 patients. These gave rise to 13 CTC derived explant (CDX) models. CellSearch enumeration of CTCs in a parallel blood sample revealed a range of EpCAM+/CK+ CTCs in patients who gave rise to CDX was 160-7687 CTCs/7.5 ml blood; 8/9 samples with >400 CTCs/7.5 ml generated CDX. When CDX were treated with cisplatin and etoposide, the standard of care for SCLC patients, they responded in the same way as their donor patient (see Hodgkinson et al., 2014, Figure 1) and represent improved models for therapy testing in a disease where tumour biopsy is very challenging. We have initiated academic collaborations with the Dana Farber Cancer Institute, Memorial Sloan Kettering Cancer Center and the Karolinska Institute to test novel agents in our CDX panel and to evaluate tumour epigenetics. We are also testing novel therapeutic strategies in collaboration with a number of pharmaceutical companies, including those targeting DNA damage repair pathways. We initiated a CDX programme for Non Small Cell Lung Cancer (NSCLC) where the number of CellSearch EpCAM+/CK+ CTCs is considerably lower. The first NSCLC CDX we developed was derived from a patient with no CellSearch detectable EpCAM+/CK+ CTCs. However, when their blood sample was filtered and stained with epithelial and mesenchymal markers, >150 CTCs/ml blood were detected with ~80% expressing the mesenchymal marker indicative of an epithelial to mesenchymal transition (EMT).

(ii) Lowering the threshold for apoptotic cell death in SCLC

We have a long standing interest in the regulation of apoptosis in SCLC and the action

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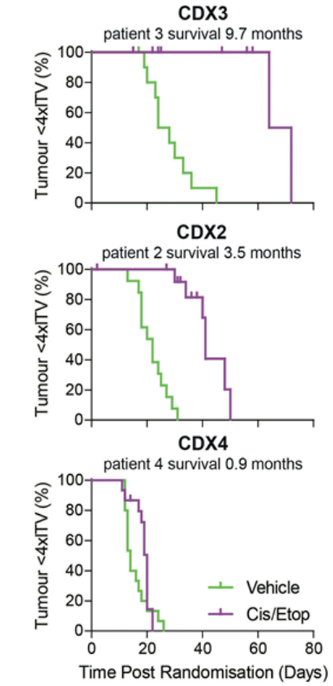


Figure 1
SCLC CDX models recapitulate patient response to treatment with cisplatin and etoposide. Mice bearing CDX3, 2 or 4 were treated with cisplatin and etoposide or vehicle control and the length of time taken to reach 4 times initial tumour volume (4xITV) monitored. Patient 3 survived 9.7 months and only 2 CDX3 tumours reached 4xITV after treatment with cisplatin and etoposide. Patient 2 survived for 3.5 months and CDX2 exhibited an intermediate response to cisplatin and etoposide while patient 4 progressed through therapy and survived 0.9 months and CDX4 did not respond to therapy.

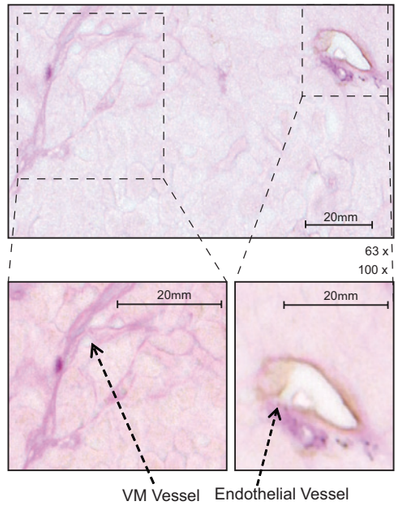


Figure 2
VM networks are present in SCLC. Representative images of a human SCLC TMA stained with modified PAS/CD31 (central panel) and higher magnification panels showing PAS+/CD31 VM vessels (left) and PAS+/CD31+ endothelial vessels (right).

of the BH-3 mimetic class of drugs. PIM kinases promote cell survival by disrupting the interactions between the pro- and anti-apoptotic Bcl-2 family members as well as stabilising c-Myc and causing increased c-Myc driven transformation. Given this co-operation between PIM kinase signalling and Myc driven oncogenesis, together with the survival promoting phosphorylation by PIM kinases of the pro-apoptotic Bcl-2 family member Bad, we investigated the effect of the PIM kinase inhibitor AZD1208 in combination with the BH3 mimetic AZD4320 (which targets the BH3 domain of anti-apoptotic Bcl-2 family members) in collaboration with colleagues at AstraZeneca. Furthermore, both MYC and Bcl-2 are frequently amplified in SCLC. We found that the PIM kinase inhibitor AZD1208 caused cytostasis across a panel of 9/11 SCLC cell lines, down-regulated global protein translation and although it reduced the pro-survival phosphorylation of the pro-apoptotic protein BAD, it did not (as a single agent) induce cell death. In five SCLC cell lines, the combination of AZD1208 and AZD4320 caused significant sensitisation to cell death and significantly impaired the growth of H2171 SCLC xenograft tumours in vivo. These data suggest that PIM kinase inhibition combined with BH-3 mimetic mediated lowering of the apoptotic threshold could hold promise for the treatment of SCLC.

(iii) Vasculogenic mimicry in SCLC

Vasculogenic mimicry (VM) describes the ability of aggressive tumour cells to 'mimic' properties of endothelial cells and enable de novo generation of tumour-derived vascular networks that provide micro-circulation independently of non-cancer cells/stroma. VM is evaluated in clinical specimens by immunohistochemical analysis of Periodic Acid Shiff (PAS) positive, CD31 negative vessels. In common with angiogenesis, VM can be driven by a hypoxic tumour microenvironment providing an alternative to angiogenesis for the delivery to tumour of oxygen and nutrients, and a potential escape mechanism for tumours treated with anti-angiogenic therapies.VM was first associated with poor prognosis in aggressive melanomas. Using a SCLC tissue microarray from 41 limited stage (LS) patients, we have shown for the first time that VM occurs in SCLC and that a high VM score is associated with worse patient overall survival (Figure 2). We also observed VM in 11 SCLC CDX models established from patients with extensive stage (ES) disease. Furthermore, CTCs from 37/38 SCLC patients contained a subpopulation of CTCs expressing Vascular Endothelial-Cadherin (VE-cadherin), a VM associated protein. Using shRNA VE-cadherin knockdown, we demonstrated its functional role in formation of VM-like networks by the SCLC cell line

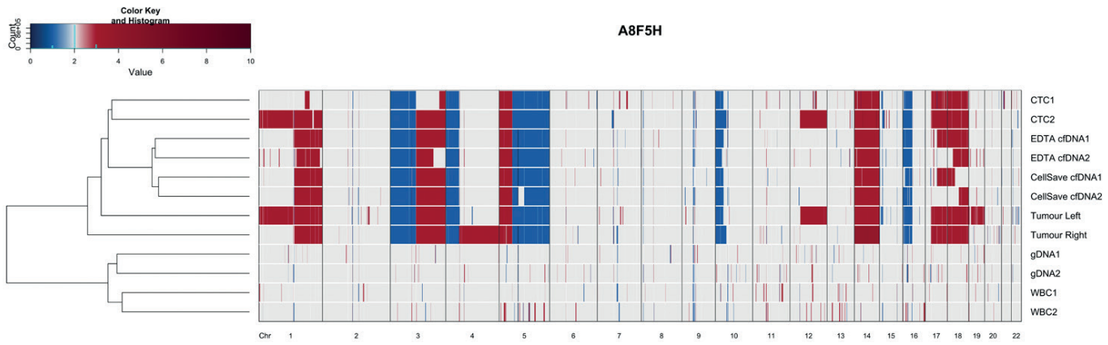
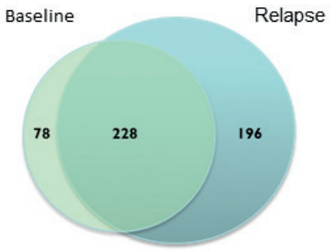


Figure 3
A. Heat map showing CNA of SCLC patients. CNA profiles were generated from isolated CTCs, EDTA cfDNA, CellSave cfDNA, two CDX tumours, germline gDNA and isolated WBC from a SCLC patient. Matching patterns of gain (red) and loss (blue) were seen across all tumour material. Chromosomal locations are shown below.
B. Venn diagram showing distribution of somatic mutations identified in baseline and relapse samples from a SCLC patient.



NCI-H446 in matrigel. VM warrants further investigation in SCLC where it may contribute to the prevalence of CTCs in this highly metastatic disease, and where components of the VM pathway may be targets for SCLC therapeutics.

Update from the Nucleic Acids Biomarkers Team (NAB)

Tumour heterogeneity and subsequent emergence of drug resistance is increasingly recognised as a major barrier for improving cancer patient treatment. The NAB team is developing and applying molecular profiling methods suitable for monitoring tumour heterogeneity and evolution in a patient's blood sample. Use of circulating biomarkers to determine the molecular status of the patient's tumour also reduces reliance on tumour biopsies. In some cancers as little as 1 ml of patients' blood samples may contain 0-1000s CTCs amongst 10^8 normal white blood cells (WBCs) along with ng quantities of circulating DNA (cfDNA) of which some is tumour-derived. The approaches being evaluated within the NAB team now include:

- Processing of a single blood sample to deliver CTC mRNA, CTC gDNA and cfDNA up to 4 days post blood sample draw
- Marker dependent and independent enrichment and isolation of single CTCs to enable molecular analysis
- Routine whole genome amplification of single patient derived CTCs and subsequent next generation sequencing (NGS) based copy number analysis (CNA)
- Application of targeted and whole exome sequencing (WES) NGS to identify potential clinically addressable mutations in patient CTCs
- Routine plasma cfDNA isolation and quantification
- Tailored NGS library generation to maximise sensitivity and reproducibility of molecular analysis of cfDNA
- Targeted pull-down and PCR based NGS panels for mutational analysis of +600 cancer associated genes from patient cfDNA
- Both WES and targeted NGS of cfDNA including detection of potential mechanisms of resistance to targeted therapies
- Single cell mRNA profiling by Fluidigm, Nanostring and RNA-Seq
- A bioinformatics pipeline for NGS analysis in collaboration with Dr Crispin Miller

NAB place emphasis on improving blood collection and ensuring minimal loss or change in cellular and extracellular nucleic acids following storage of whole blood for four days at room temperature, ensuring compatibility with multisite clinical trials. To this end we have developed protocols for improving the detection of the circulating tumour DNA (ctDNA) fraction of cfDNA by avoiding the inadvertent lysis of WBCs and consequent dilution of low abundance ctDNA. We confirmed that our protocol avoids WBC lysis and delivers ctDNA amenable to both targeted sequencing and genome-wide NGS analysis (Figure 3A). We increased the sensitivity of NGS capture and developed a targeted 600-gene panel of cancer associated genes. This panel is ready for application in 2015 to identify clinically important mutations in cfDNA isolated from cancer patients on The Christie NHS Foundation Trust Early Clinical Trial Unit TARGET protocol. In collaboration with Professor Richard Marais, we have performed targeted and WES analysis of cfDNA from melanoma patients and identified potential mechanisms of resistance to targeted therapies. In collaboration with Dr Fiona Blackhall, we have accumulated and analysed a unique collection of CTCs from SCLC patients collected prior to and post chemotherapy treatment. NGS analysis of these samples has identified potential molecular signatures of resistance that are currently undergoing further analysis (Figure 3B). Similar NGS analysis is underway comparing SCLC CTC samples from patients who responded well to initial treatment to those CTC samples from SCLC patients who failed to respond.

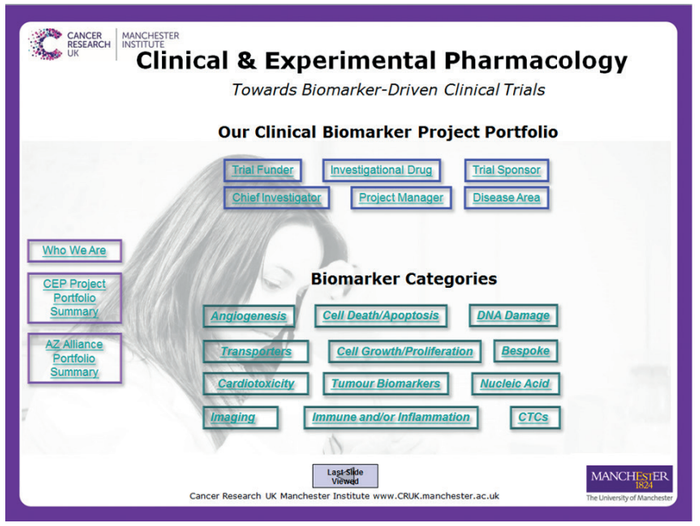


Figure 4
The CEP 'dashboard' front-page. This web-based interface provides the user with easy access to information contained with CEP portfolio of clinical trials with biomarkers based on a range of searches, such as disease area or biomarker type.

Using our protocol for the accurate and reproducible transcriptional profiling at the single cell level (Rothwell et al., in press), we are RNA profiling CTCs. These studies include analysis of RNA signatures which may help guide the choice of treatment for prostate cancer within our Movember Centre for Excellence with partners in Belfast. In addition, we are also examining whether CTC mRNA profiling can be used to identify transcriptional changes in CTCs that arise as a consequence of treatment. To this end we are developing both focused approaches, utilising the Fluidigm and NanoString platforms to analyse pre-defined transcriptional signatures and whole genome RNA-Seq approaches to identify global transcriptional changes. We are currently refining approaches to enable this analysis on both enriched populations and at the single cell level.

Underpinning the NGS studies in NAB is strong bioinformatics collaboration including support from both CEP-based bioinformaticians and Dr Crispin Miller's group at CRUK MI. Regular interactions between the groups have led to the development of robust pipelines for the analysis of NGS-based CNA that enables the accurate identification of tumour cells and detailed mutational profiling of samples. We became the oncology hub of the Manchester MRC Single Cell Centre of Excellence following this award in late 2014.

A Snapshot of our Clinical Trials Biomarker Portfolio

The CEP Clinical Trials Portfolio encompasses 60 active clinical trials and experimental medicine projects, with another 13 at the planning stage. To assist effective project management and to provide user-friendly rapid access to key project and trial information, a customised 'Dashboard' has been developed. This allows the user to navigate quickly to one-page trial summaries using a range of queries, for example disease area, investigational drug, biomarker type, etc. The Dashboard 'front page' is shown in Figure 4. As our portfolio continually expands we are revising our processes and document management systems, to more easily achieve and monitor our compliance to Good Clinical Practice for Laboratories (GCPL). As part of this process, we are implementing Q-Pulse, a proven compliance solution providing a suite of integrated modules to manage business functions effectively and efficiently, including:

- Document Control
- Audits and Findings
- Issues and Corrective / Preventative Action (CAPA)
- Staff Competency, Training and Development
- Asset Management

During the last year our specific user requirements have been comprehensively 'mapped' and the training and experience gained during Q-Pulse configuration will ensure we have an effective solution in place for ongoing maintenance and future developments, including our ambitious plans to build and develop, over the next two to four years, a CRUK Manchester Centre for Biomarker Sciences.

There has been significant activity this year assessing the value of enumerating and characterising CTCs as prognostic, predictive and pharmcodynamic biomarkers. We have also worked with a number of CTC technology providers in the past year, evaluating new approaches to CTC enrichment and isolation. Notably, in collaboration with the CRUK Manchester Institute Molecular Oncology Group, melanoma patient samples have been processed, enumerated and stored for detailed molecular analysis. Here we have worked to facilitate the development of the Clearbridge ClearCell FX device, a novel marker-independent spiral chip which has been used successfully to detect and enrich melanoma CTCs.

Publications listed on page 52

DRUG DISCOVERY

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The past year has been an incredibly busy time for the Drug Discovery Unit (DDU). It was capped by the signing of an agreement between the group and Sixth Element Capital resulting in a £2M investment from the CRT Pioneer Fund to accelerate development of a novel lung cancer therapy targeting the RET oncogene.

The DDU portfolio continues to develop and in our other advanced project, PARG, we have made significant progress, developing a biomarker strategy for patient selection and delivering compounds showing an acute pharmacodynamic effect. At the other end of the spectrum, new target identification and validation are essential to the future portfolio and we have taken steps to focus these efforts around DNA Damage Response (DDR)/ Synthetic Lethality and Epigenetics. In the years ahead we will expand our partnerships and collaborations both within and beyond the Manchester Institute.

Progressing our portfolio; the way forward for the Drug Discovery Unit

At the time of this report last year, our most advanced project PARG had just moved into the lead optimisation phase. Over the past year, our lead series have been refined and optimised to deliver over a 1000x improvement in cellular potency and now also display more "drug-like" features (Figure 1). These compounds are well tolerated and show promising pharmacokinetic and pharmacodynamic profiles which will allow us to test the therapeutic potential of PARG inhibition in more relevant disease models. In addition we have developed a better understanding of the underlying genetic defects that confer cellular sensitivity to PARGi. This information will help us determine which patients are likely to respond to these agents.

Whilst PARG offers an opportunity to understand and exploit novel biology, our efforts on the RET oncogenic kinase recognises a therapeutic need for improved agents against a drug target which is already clinically validated. The RET (Re-arranged during transfection) receptor tyrosine kinase is a known oncogene which is implicated in about 1% of non-small cell lung cancers (NSCLC). In addition to developing our existing series, during 2014 we began a new project targeting potential resistance mechanisms. Through computational and bioinformatics analysis, we have identified the likely clinical resistance mechanism and have begun the hunt for inhibitors of the resistant enzyme. Funding from Sixth Element Capital will accelerate our progress. As an indication of our success, chemical patent applications have been filed for our first lead series in both RET and PARG.

2014 has also seen the first full year of our collaboration with the GlaxoSmithKline (GSK) Epigenetics drug discovery group based in Pennsylvania, USA. In January we held our first full project team meeting looking at the output of their 1.7 million compound screen against a target of joint interest. In spite of the geographical and time differences we have developed a strong working relationship with our partners in GSK and have recently made significant progress against a very challenging epigenetics target, such that 2015 should see us make an early project phase transition.

With two projects in lead optimisation it has been difficult to grow our early portfolio and often tough priority choices have had to be made. Despite these challenges we have made good progress opposite SMARCA and mtIDH1. In terms of SMARCA, our first target derived from bioinformatics, significant progress has been made to confirm the biology around the target. For mtIDH1 a 1.4 million compound screen conducted by the DDU at AstraZeneca's Alderley Park facility, has delivered an exciting drug-like compound series with potential for rapid progression. G6PD remains of interest

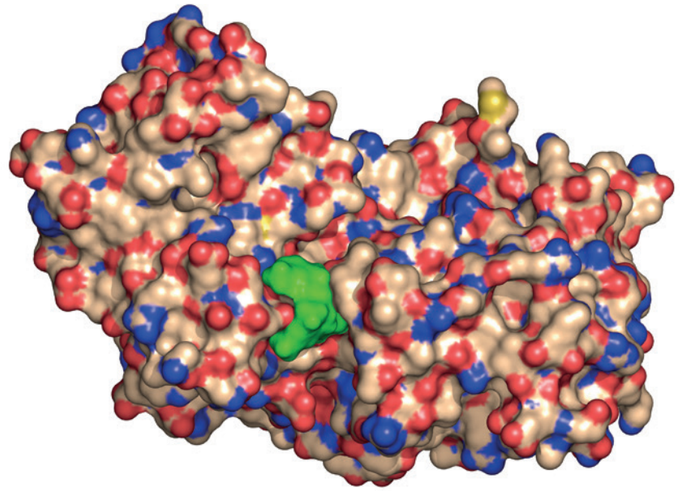


Figure 1
Crystal structure of PARG with a DDU inhibitor (in green) bound.

Figure 2
Custom analysis of IncuCyte cell growth data, using a bespoke analysis package created in R by Phil Chapman. Cell confluency data is taken directly from the IncuCyte (1) and data from undosed control cell growth is analysed to determine the precise time for a specified number of cell doublings (2). The percent confluency for cells treated with different doses of experimental agents are then determined at this specific time point (3). The data are then uploaded into our informatics system and curve fitting carried out to determine an EC50 value, comparable against different cell lines.

biologically but is the most chemically challenging target we have worked on to date. Through our Chinese biotech partner HitGen, we have screened over 0.7 billion compounds but have yet to deliver a chemically viable series.

As mentioned in the introduction, in order to maintain a steady flow of new projects, novel target identification and validation remains of vital importance to the group. However with the resourcing pressures on the rest of the portfolio, we have decided to focus our efforts around DNA Damage Response /Synthetic Lethality, Epigenetics and Lung Cancer. To facilitate this we have empowered subgroups to reach out to potential collaborators both within and outside the Institute. We are already beginning to see the benefits of this new focus and we anticipate that our efforts will result in a strengthened early portfolio in 2015.

Technical Progress

Whist it is important to focus on the project portfolio, a great deal of technical work goes on in the background that gives meaning to the data we produce. A good example of this involves our use of the IncuCyte cell imaging system. The IncuCyte produces a kinetic read-out of cell confluency. It is ideal for the long term monitoring of cell proliferation after treatment with compounds. However when targeting the DDR we would prefer to know the exact number of cell doublings that have occurred so that we can compare effects across multiple cell lines. Using the scripting language R, we have built an algorithm that takes the exported confluency data and allows us to generate dose-response IC50 curves for a given number of cell divisions. With multiple cell lines, this then allows us to control for the effect that the cell cycle may have on the response to a drug or DNA damaging agent (Figure 2). Furthermore, the arrival at the Institute of the first fully automated Opera Phenix will be a great boost to the operational capabilities of the DDU cell biology group.

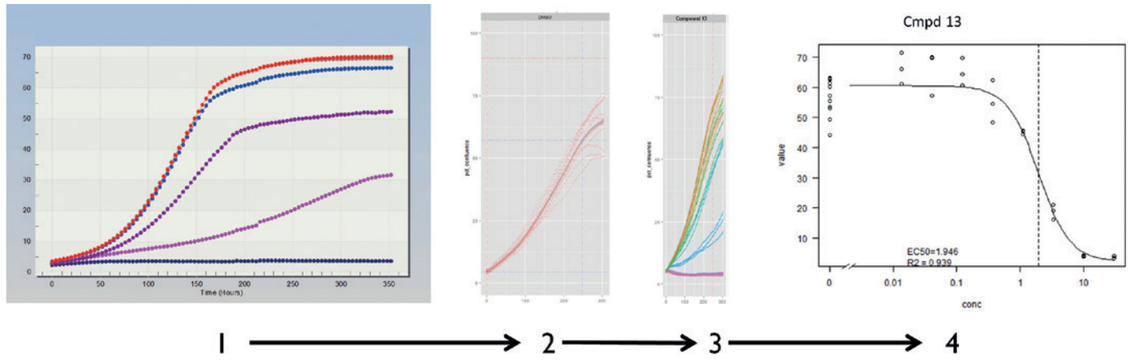
People

The continuing development of our staff remains of vital importance to the DDU leadership. In the summer, with the help of The Research Network, we held our first Drug Discovery Workshop which received extremely positive feedback. In 2014 more than 50% of our staff attended and presented our work at scientific meetings and as a group we have authored or contributed to eight peer reviewed publications and two filed patent applications.

The Future

The stated aim of the DDU over the next five years is to deliver our first candidate drug into pre-clinical development and with the progress made on PARG and RET we are on course to achieve this. However we will only be truly successful if we can deliver our first candidate drug whilst also maintaining a sustainable ongoing portfolio. The latter is only possible through support and collaboration with our colleagues in the local and national CRUK networks.

Publications listed on page 54



LEUKAEMIA BIOLOGY

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A key focus of the group's work is the identification of genes selectively required for the function of leukaemia cells but not normal bone marrow cells: this is an important strategy to pinpoint new therapeutic targets for drug development. Over the past 12 months there has been significant progress in two projects which will lead to publications in 2015. In the first, we have identified an unexpected mechanism by which inhibitors of LSD1 promote myeloid differentiation. In the second, we have identified FOXC1 as a transcriptional regulator which is aberrantly derepressed in human acute myeloid leukaemia to functional effect.

LSD1

Lysine Specific Demethylase 1 (LSD1) is one of a number of epigenetic regulators which have recently emerged as candidate therapeutic targets in cancer. It was initially identified as a core component of a RCOR1 (CoREST) histone deacetylase (HDAC) corepressor complex and later found to have lysine-specific demethylase activity. LSD1 is a flavin adenine dinucleotide (FAD) dependent homologue of the amine oxidase family which is known to demethylate monomethyl or dimethyl lysine 4 (K4) of histone H3, releasing hydrogen peroxide and formaldehyde.

The interest in LSD1 as a therapeutic target in cancer arose from the observation of its high level expression in poor prognosis sub-groups of prostate, lung, brain and breast cancer, as well as in certain haematological malignancies. The first drug found to inhibit LSD1 was tranylcypromine (TCP), a monoamine oxidase inhibitor used in the treatment of depression. TCP is a mechanism-based suicide inactivator of LSD1 which covalently attaches to the N(5) and C(4a) residues of the isoalloxazine ring of FAD, which is itself located deep within the active site of LSD1. To improve the potency and selectivity of TCP towards LSD1, derivatives active in the nanomolar range have been developed and these show promise as differentiation-inducing agents in pre-clinical studies in acute myeloid leukaemia (AML), as we previously reported in Cancer Cell in 2012. With LSD1 inhibitors already in early phase clinical trials, and others in development, an appreciation of their mechanism of action is essential. The assumption has been that LSD1 contributes to gene repression by removing monomethyl and dimethyl histone marks from lysine 4 of histone H3 and that this is the key activity targeted for potential therapeutic effect. However, in work led by James Lynch and Gary Spencer, we observed that drug-induced changes in transcription preceded changes in histone modifications targeted by LSD1 and that AML cell proliferation did not require the catalytic activity of LSD1. Instead, concomitant with up regulation of a myeloid differentiation program, a tranylcypromine-derivative inhibitor induced physical separation of LSD1 from the transcription factors GFI1 and MYB, and more generally from chromatin (Figure 1). Physical separation of GFI1 from LSD1 was required for drug-induced differentiation, and LSD1 sustained the association of MYB with chromatin. Complete resistance to the effects of LSD1 inhibition could be achieved by co-induction of MYB and a GFI1 DNA binding domain-LSD1 fusion protein. Thus, pharmacological inhibition of LSD1 promotes differentiation by disabling the activity of key myeloid transcription factors, through abrogation of protein:protein interactions rather than blockade of histone demethylase activity. A report detailing these discoveries is currently out for review.

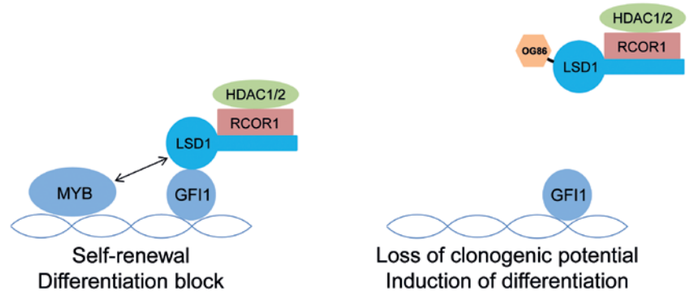


Figure 1
Model of the mechanism of action of LSD1 inhibitors in myeloid leukaemia. OG86, an exemplar tranylcypromine-derivative inhibitor of LSD1, irreversibly binds LSD1 leading to its physical separation from transcription factors and chromatin, and resulting loss of MYB from chromatin.

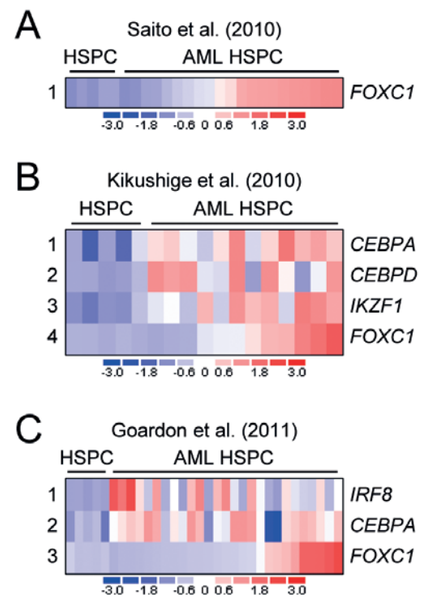
In the clinical arena, there is ongoing progress (in collaboration with Oryzon Genomics) with our first-into-man Phase 1 trial of a first-in-class LSD1 inhibitor, ORY1001. The trial began its recruitment in June 2014 and is proceeding satisfactorily at The Christie NHS Foundation Trust, as well as at sites throughout Spain.

FOXC1

To identify transcriptional regulators expressed in human acute myeloid leukaemia haematopoietic stem and progenitor cells (AML HSPC) but not normal HSPC, we analysed the expression levels of known or candidate transcription factor genes in recently published datasets. Of those exhibiting significantly higher expression in AML HSPC versus HSPC, *FOXC1* was among the most highly up regulated genes in each study when ranked by fold-change increase in expression (Figure 2). *FOXC1* is a member of the forkhead box family of transcription factors which regulate processes such as development and differentiation. In keeping with a requirement for FOXC1 in mesenchymal differentiation, *Foxc1* null mice die perinatally with skeletal, cardiac and renal abnormalities, hydrocephalus, iris hypoplasia and open eyelids. Humans with germ line mutations in FOXC1 develop the Axenfeld-Rieger syndrome which includes developmental anterior segment abnormalities of the eye.

Critically, *FOXC1* is not expressed in the haematopoietic system and so a question was raised as to whether its derepression in ~15-20% of cases of human AML contributes to transformation. In work led by Tim Somerville, we have shown that shRNA-mediated knockdown of *FOXC1* in human AML cells impairs clonogenic potential and induces myeloid differentiation, whereas normal HSPC are spared. In forced expression experiments, *FOXC1* induced a transient enhancement of both the clonogenic potential and myeloid differentiation block of normal murine HSPCs cultured in serial replating assays, and myeloid skewing in in vivo transplantation assays. In silico and qPCR analyses showed that high level *FOXC1* expression strongly associates with high level *HOXA9* expression in human AML. We demonstrated this co-expression to be of functional significance because retroviral co-overexpression of *FOXC1* and *HOXA9* in murine HSPC strongly enhanced their clonogenic potential and myeloid differentiation block in serial replating assays, and accelerated leukaemia initiation in in vivo transplantation assays. These data demonstrate that FOXC1 functions to accelerate and enhance the development of AML in collaboration with HOXA9. Thus FOXC1 is a hitherto unappreciated transcriptional regulator in human AML which is inappropriately derepressed to functional effect. A report detailing these discoveries is also currently out for review.

Figure 2
Expression of *FOXC1* in human AML. Transcription factor genes differentially expressed in AML HSPCs versus normal HSPCs were identified using an unpaired t-test (with $p<0.005$) and were ranked according to the mean fold change increase in expression. Heat maps show the most highly up regulated genes in the indicated datasets, in order of rank (indicated by number to the left of each heat map row).



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MOLECULAR ONCOLOGY

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The primary focus of our studies is melanoma, a cancer that affects over 12,000 people and causes over 2,000 premature deaths each year in the UK. Common cutaneous (skin) melanoma is the most common form of melanoma. It occurs on hair-bearing skin and is associated with exposure to the ultraviolet light that is present in sunlight and produced by sunbeds.

The two most commonly mutated oncogenes in melanoma are NRAS (about 20% of cases) and *BRAF* (about 45% of cases). The proteins that these mutant genes produce activate a conserved signalling pathway that controls cell growth and survival and drives melanoma development. It was not known how activation of this pathway interacts with exposure to ultraviolet light to drive melanoma development, but drugs that inhibit the pathway can stop the growth of melanomas when *NRAS* or *BRAF* are mutated. However, although most patients respond to these treatments, they all eventually fail treatment and relapse with drug-resistant disease. Over the past year, we have studied how NRAS and BRAF interact with each other and with ultraviolet light to drive melanoma development. We have also continued to study drug resistance in order to improve our knowledge of melanoma biology so that new treatment strategies can be developed.

In 2014 we reported our investigations into the relationship between ultraviolet light and the NRAS/BRAF signalling pathway. We used a mouse model of melanoma in which expression of mutant BRAF in the melanocytes (the pigment cells in the skin that are the precursors of melanoma) induced melanoma in about half of the mice in about 12 months. When we exposed these mice to weekly low doses of ultraviolet light, they all developed melanoma within seven months (Viros et al). Since ultraviolet light alone did not induce melanoma, these data show that even low doses of ultraviolet light that mimic weak sunburn in humans are sufficient to accelerate the development of melanoma in melanocytes that express mutant BRAF.

To investigate how ultraviolet light accelerates melanoma development, we sequenced the tumours and found that those from mice exposed to ultraviolet light presented a significantly higher number of mutations than those that had not been exposed. In particular, we observed a signature of DNA damage that is characteristic of DNA following exposure to ultraviolet light, suggesting that ultraviolet light directly damages melanocytes' DNA. In agreement with this, we found that about 40% of the tumours that had been exposed to ultraviolet light carried mutations in the tumour suppressor TP53, whereas no TP53 mutations were seen in the tumours that had not been exposed. We validated this result by showing that mutant BRAF and mutant TP53 cooperate to drive melanoma development even without ultraviolet light, establishing that TP53 is a bona fide target of ultraviolet light in melanoma and that it cooperates with BRAF to drive this disease. Finally, we demonstrated that while sunscreen delays melanoma onset, it only provided partial protection. This is because although sunscreen is very efficient at absorbing ultraviolet light, a small amount escapes and damages the DNA. Sunscreen clearly plays an important role in sun protection, but it is vital to note that it does not offer complete protection and so it should be used in combination with other sun avoidance strategies to provide full protection from the damaging effects of the sun's rays.

Although epidemiological studies have implicated ultraviolet light in common cutaneous melanoma, the role of this carcinogen in other forms of melanoma is less clear. In 2014 we reported the genomic landscape of acral melanoma, a relatively rare disease that affects the non-hair bearing skin of palms, soles and nail beds. In line with its distinct epidemiological and clinical profiles, and compared to common cutaneous melanoma, we found acral melanoma has a relatively low mutation burden. Moreover, we did not observe the UV DNA damage signature that is a feature of the genomes of common cutaneous melanoma and we found a distinct spectrum of mutations. Consistent with epidemiological and chromosomal-level genomic studies, our findings show that acral and common cutaneous melanoma are distinct diseases that will likely need to be treated differently. Notably, the genomic landscape of acral melanoma is more similar to mucosal melanoma than to common cutaneous melanoma, and in agreement with these findings, mucosal melanoma is also not associated with exposure to ultraviolet light.

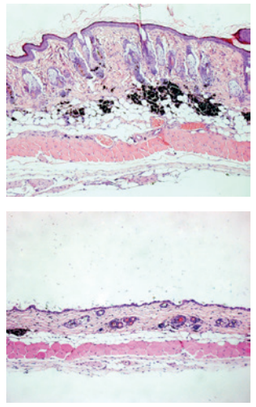

Figure 1
Skin from animals expressing $BRAF^{V600E}$ in their melanocytes 7 days after exposure to UVR. Exposed skin (top) and protected skin (bottom).

As described above, genomic approaches can provide insight into the biology and aetiology of melanoma. Another use of this technology is to investigate mechanisms of drug resistance. In 2014, we reported a case of a patient who did not respond to a BRAF drug despite the presence of a BRAF mutation. We performed whole genome sequencing of the patient's tumours and this revealed that in addition to the *BRAF* mutation, the tumours also carried mutations in two genes called *PTEN* and *GNAQ*. Our functional studies demonstrated that the mutant proteins these genes encoded reduced the tumour's response to the BRAF drug. Thus, in this patient the tumours were resistant before the treatment even started (intrinsic resistance). We posit that knowledge such as this will be critical to optimising treatment for patients by providing a platform of knowledge that can be used to individualise or personalise treatment to ensure the best outcomes for each patient.

Finally, although NRAS and BRAF mutations each occur in a high proportion of melanomas, they are rarely coincident, suggesting that they satisfy similar requirements to melanoma cells. In rare cases however, NRAS and BRAF mutations are coincident, but this always involves rare mutations that inactivate rather than activate BRAF. To study these rare forms of melanoma, we again used mouse models. We found that inactive mutants of BRAF cooperated with mutant NRAS and mutant KRAS to induce melanoma, (Pedersen; Sanchez-Laorden). We have previously reported that this cooperation occurs because if BRAF is inhibited in the presence of mutant RAS, it drives paradoxical activation of the pathway. This occurs because when drugs inactivate BRAF, it binds to and stimulates a closely related protein called CRAF, which then drives hyper-activation of the pathway. Importantly, we found that in addition to accelerating the development of RAS-driven tumours, paradoxical activation of the pathway stimulated melanoma dissemination (metastasis) in the mice. More importantly, we found that when melanoma develops resistance to BRAF inhibitors, the inhibitors mediate a similar re-activation of the pathway and this then stimulates tumour dissemination. Our data suggests therefore that not only do the tumours stop responding to the drugs when they develop resistance, but that they use the drugs to drive tumour dissemination through the body. Clearly, knowledge such as this is critical for optimising patient treatment and for knowing when to withdraw treatments that are no longer working.

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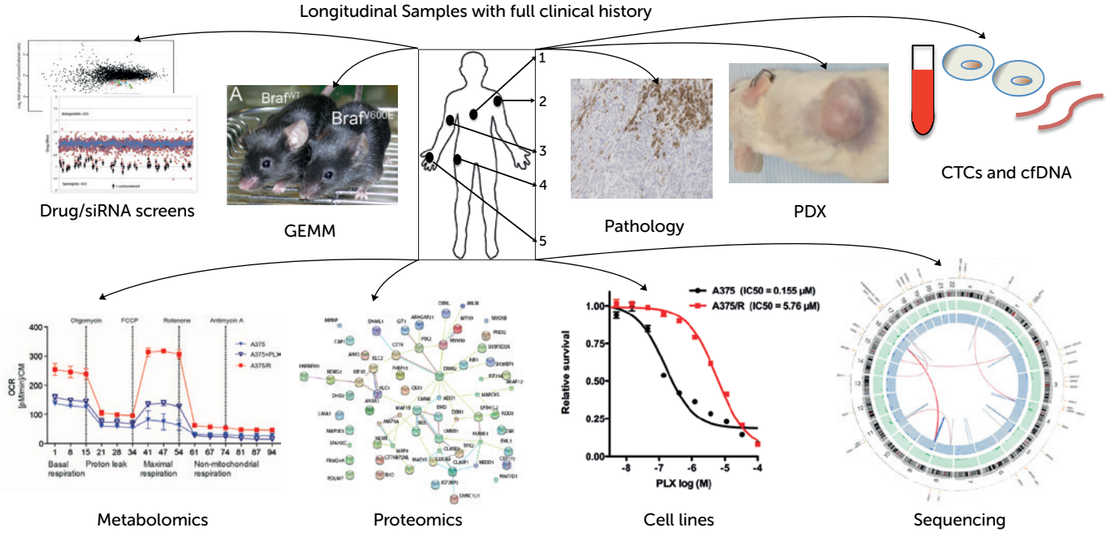
Figure 2
Personalised medicine in melanoma.
Early detection of relapse and elucidation of the mechanisms of resistance to therapies are needed to guide clinical care and personalise treatment decisions for individual patients. The Molecular Oncology group integrates new diagnostic tools using a combination of techniques to optimise personalised care and improve patient outcomes. These include proteomics, metabolomics, genetically engineered modified mice (GEMM), pathology, cell lines, whole exome sequencing (WES), patient derived xenografts (PDXs), and circulating tumour DNA (ctDNA).



PROSTATE ONCOBIOLOGY

www.cruk.manchester.ac.uk/Research/CRUK-MI-Groups/Prostate-Oncobiology/Home

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Prostate cancer (PCa) is the third leading cause of cancer-related mortality. Initial response to hormone therapy is almost universally good but progression to castration-resistance PCa (CRPC) is inevitable and lethal. Failure to develop effective therapies is a consequence of genetic heterogeneity and a lack of pathologically defined subtypes that predict patient outcome.

Inadequate knowledge of the genetic lesions that drive tumour progression has hampered efforts for effective treatment strategies for PCa, resulting in overtreatment or treatment failure. Thus, the main focus of the Prostate Oncobiology group is to advance the understanding of the molecular mechanisms and cellular heterogeneity in prostate tumourigenesis to predict PCa patients' outcomes and pursue the development of better and personalised therapeutics.

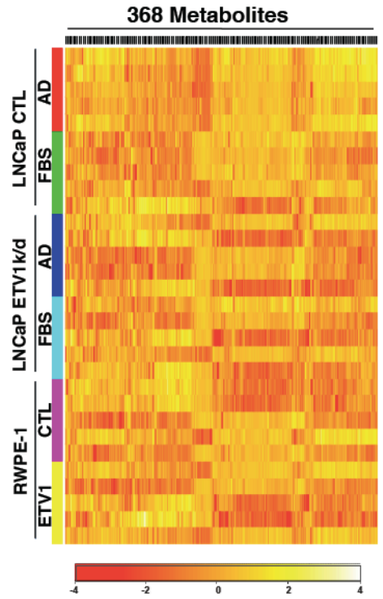
Molecular mechanisms driving prostate tumourigenesis

Understanding the molecular mechanisms underlying tumour initiation and progression is critical for development of more effective prostate cancer therapy. The recent technologies that combine pathological and morphological changes with cancer genome sequencing are providing the means to highlight multiple somatic alterations that occur in cancer, such as mutations in metabolic pathways. One of the key alterations identified in PCa pathways are the ETS transcription factor gene fusions in ~50% of human prostate cancer cases, making them the most frequent gene fusion associated with human malignancy. The recently developed genetically engineered mouse models (GEMM) based on these key ETS fusions, which more closely replicate specific disease phenotypes, suggest a different role for ERG and ETV1 in prostate tumourigenesis. Specifically, ETV1 directs androgen synthesis, providing new insights into how this oncogene acts to promote hormone unresponsive lethal disease. Indeed, findings from our genetically engineered mice and genomic analysis led us to predict a worse outcome in patients by ETV1 over-expression and the ETV1-associated network.

A major reprogramming of cellular energy metabolism is observed in cancer cells to support continuous cell growth and proliferation, replacing the metabolic program that operates in most normal tissues. This elevation in the rates of glucose uptake, but reduced rates of oxidative phosphorylation by tumours in the presence of oxygen known as aerobic glycolysis, was first noted by Otto Warburg. Since then, glycolytic fuelling has been shown to be associated with activated oncogenes (e.g., RAS, MYC) and mutant tumour suppressors (e.g., TP53). Moreover, gain-of-function mutations in metabolic enzymes such as the isocitrate dehydrogenase 1/2 (IDH) have been reported as driver mutations in glioma and other human tumours. Such findings suggest that targeting metabolic reactions could be a promising therapeutic strategy.

As an initial step in identifying novel or understudied roles of genomic aberrations such as the ETV1 fusion in cancer metabolism, we will focus on its targets involved in steroid biosynthesis, and energy metabolism. We have performed mass spectrometry-based metabolome analysis on human prostate cancer cells after ETV1 overexpression and silencing (Figure 1). The initial metabolome data analysis, in comparison with gene expression profiling obtained in the same cell lines, support our transcriptome findings, and indicates that ETV1 promotes glucose intake and lactate production, and increases lipid metabolism. Thus, we will focus on enzymes that are (i) ETV1 direct targets showing altered expression in ETV1-expressing non-tumorigenic and cancer prostate cells; (ii) highlighted in metabolome and gene expression studies as involved in the reprogrammed de novo lipid synthesis; and (iii) scored in lipid metabolism drug screening. Then, we will test the requirements for selected ETV1 metabolism-associated targets for tumour growth in vivo using our GEMM and preclinical xenograft mouse models. We anticipate that ETV1 metabolic targets and their combinatorial effect with current treatments will improve current therapeutics. Thus, studying and elucidating the relationship between metabolic disorders and cancer should provide new avenues for molecular intervention and may also help to promote a new field for ETV1-expression tumours (e.g. prostate cancer, melanoma, and gastro-intestinal stromal tumours) that will provide metabolism-based targets for cancer patients.

Figure 1
Metabolic profiling of human prostate cell lines. RWPE-1 cells, human immortalised normal prostate cells; control (CTL) and ETV1-expressing (ETV1) conditions. LNCaP, human androgen-dependent prostate cancer cells growing in normal (FBS) and androgen-deprived (AD) conditions; shRNA silencing ETV1 (ETV1 k/d) and non-targeting shRNA control (CTL).

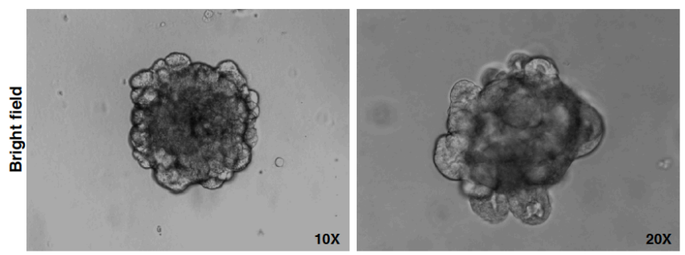


Identification of prostate tumour-initiating cells as novel biomarkers and targets for therapeutics

A central challenge in cancer is whether oncogenic transformation of different cells of origin within an adult tissue gives rise to distinct tumour subtypes that differ in their prognosis and/or heterogeneous treatment response. Thus, a key problem remains the identification of the cell type capable of initiating and sustaining growth of the tumour. Being able to target neoplastic cell populations based on unique surface-marker expression patterns has provided a new approach in cancer research to study directly the cells thought to be at the root of the disease. Our group aim to identify cells of origin of prostate cancer and the pathways responsible for the transformation of normal target cells into self-renewing cancer cells.

As a first step to identify tumour-initiating cells, further characterisation of the prostate compartment is needed. Thus, we will initially focus on the characterisation of the basal and luminal epithelial cell compartments in normal and regenerating androgen-deprived mouse prostate. Given the limitation of cell surface markers and histological features defining prostate epithelial cells, gene expression analyses of the cell populations based on current markers may not adequately reflect the difference between the basal and luminal compartments. Single cell analysis overcomes this limitation, and allows identification of novel surface markers to classify the prostatic epithelial compartments. The establishment of self-organising organoids in ex vivo culture has become an emerging paradigm for the study of tissue stem cells and tumorigenic potential. In addition, organoids can reconstitute either normal or transformed prostate in vivo. These findings indicate that prostate organoids represent an excellent system for investigating prostate biology. Accordingly, to test their differentiation potential and lineage specifications ex vivo, we are establishing organoids cultures from primary mouse prostate cells (Figure 2). Next, we will investigate whether the novel basal and/or luminal subpopulations defined in our single-cell screening might have differential tumorigenic potential by shRNA and CRISPR/Cas9 targeting for genetic engineering of PCa tumour phenotypes in vitro, as shown for intestinal organoids. Furthermore, we will perform mechanistic studies of therapeutic response and resistance comparing organoids from different cells of origin. Thus, the individuality of the single-cell, and the information it contains, is likely to be the key to therapeutically targeting every cell in a tumour.

Figure 2
Generation of mouse prostate epithelial organoids. Bright field images of a Day-8 prostate organoid.



The focus of the Prostate Oncobiology group's research is to facilitate the development of patient-specific therapies, and serve as a valuable resource in understanding the roles of cancer metabolism and cancer stem cell regulatory networks. In addition, the data generated from this research will be a valuable resource for novel biomarkers and therapeutic targets for epithelial tumours.

RNA BIOLOGY

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The RNA Biology group is interested in how cells regulate gene expression, and how these processes are disrupted in tumours. We have a particular focus on noncoding RNAs (ncRNAs). These are transcripts that are expressed, but never translated, into proteins. Recent work investigating individual loci has shown that ncRNAs can be functional in their own right, raising the prospect that they provide an additional layer of cellular machinery that acts alongside protein-based mechanisms, and suggesting that they may act as significant players in the processes that regulate tumours.

The RNA Biology group is highly interdisciplinary, and comprises a mix of computer scientists, computational- and bench- biologists. We make extensive use of deep sequencing and mass spectrometry to explore changes in gene expression in both clinical and in vitro derived datasets, using computational approaches to identify candidate regulatory interactions involving important cancer-associated genes. We then pursue individual loci identified from these analyses at the bench using conventional molecular biology. We make heavy use of high performance computing in order to analyse the high volumes of genomics data generated from our deep sequencing and mass spectrometry platforms and write novel software tools to support data analysis, much of which we contribute to R/Bioconductor.

A global non-coding RNA system modulates fission yeast protein levels in response to stress

Fission yeast is a model eukaryote in which many regulatory pathways are conserved with human cells. These include the RNAi machinery, MAPK signalling pathways, and chromatin modifiers. This conservation with human cells makes it an excellent system in which to explore the basic biology of regulatory noncoding RNAs. In previous studies we demonstrated that adjacent gene-pairs that overlap at their 3´ end can co-regulate through the generation of sense-antisense pairs, and that this is used to control the expression of the MAPK Spk1, a key kinase on the pheromone signalling cascade. This process was dependent on components of the RNAi machinery. These initial observations also raised the question as to whether these mechanisms might be employed more generally across the genome.

Since we had observed substantial expression of ncRNAs and antisense transcripts that were under the control of the ATF/CREB family transcription factor Atf21, we speculated that they might act to regulate protein expression in a stress-dependent manner. In a collaboration with the Cell Regulation group, we therefore combined tandem mass spectrometry with RNA sequencing to examine transcript and protein expression through a timecourse of osmotic stress. We found substantial changes in sense/antisense pairs and multiple loci where the 3´ transcriptional extent of the gene was altered in a stress dependent way. By integrating these RNA sequencing data with label free quantitative proteomics data, we were able to identify a global system in which changes to ncRNAs and 3´ overlaps between adjacent genes were associated with modulations in the corresponding protein levels, and to identify ncRNAs with expression patterns strongly correlated to those of functionally related sets of protein coding genes (Figure 1; Leong et al., 2014). These data are interesting because they indicate that noncoding RNAs are an important component of the stress response.

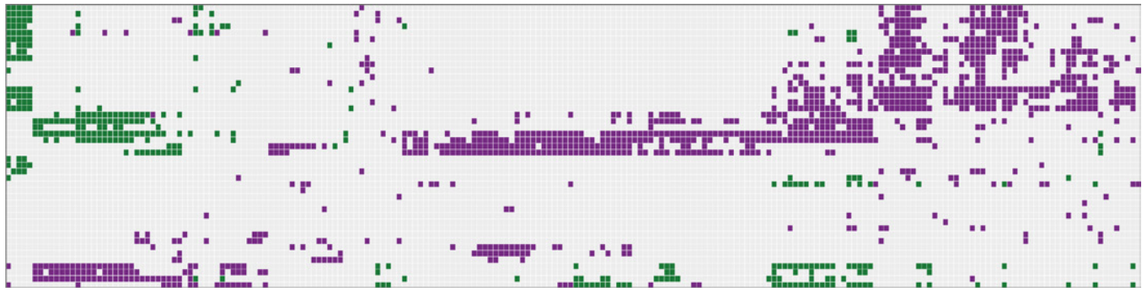

Figure 1
Columns represent individual ncRNAs changing over a time course of osmotic stress. Rows represent functionally related sets of protein coding genes, as defined by GOSlim category. Cells are coloured when the expression profile of a noncoding RNA was highly correlated (purple), or anti-correlated (green), with a significant number of members of the given protein family.

The stress response is complex, temporally staged, and modulated according to the level of stress, and can include changes within the cell that prepare them for future insults that often occur following the current condition. Overall this requires substantial regulatory control, and alterations to gene expression must be achieved within physiological timescales. The ubiquity of noncoding RNAs, combined with the speed at which cells can effect a change in transcript levels, suggests that ncRNAs have the potential to add substantial information processing capacity to the signalling systems within a cell. Since many of the pathways involved are conserved between fission yeast and humans, it is likely that similar processes will occur in higher eukaryotes. Work in the group is currently underway to explore these phenomena in human cells.

Computational analysis

While less than 2% of the genome encodes proteins, as much as 90% is now known to be transcriptionally active. Our work in fission yeast has demonstrated that the boundaries of the expressed portion of a gene can change according to context. In addition, numerous noncoding loci are present that have yet to find their way into the reference genome databases; while in previous work we were able to identify hundreds of novel protein coding genes by analysing tandem mass spectrometry data to identify peptides originating from unannotated regions of the genome. Together, these data indicate the potential to miss important features within the data if analysis is restricted only to those parts of the dataset that map directly to a static representation of the consensus genome annotation. We therefore perform de novo re-annotation of the genome for each individual sample in an RNA sequencing experiment, and have developed computational strategies in R/Bioconductor to facilitate this.

A further challenge with deep sequencing data is that the ability to routinely profile every single nucleotide of the diploid genome across hundreds of samples generates large datasets that demand substantial computational power to analyse.

We are also interested in the consequences of mutations on the proteome and have been collaborating with the Signalling Networks in Cancer group to identify regions of the exome that have been missed by existing sequencing studies. We identified hundreds of DNA 'blind spots' that have the potential to harbour cancer-causing mutations that have yet to be detected by current approaches.

Computational Biology Support

Bioinformatics analysts:
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A separate computational biology support team provides access to pre-processing and data analysis expertise across the Institute. This includes genome aligners, annotation databases and analysis software. In addition, the team provides analytical support to numerous groups within the CRUK MI, and acts as a hub around which informaticians from other groups can embed. The team works closely with the Molecular Biology Core Facility and the Scientific Computing Team, led by Wei Xing (p49), to ensure the timely and efficient analysis of deep sequencing data. The majority of the group's work is performed in R/Bioconductor.

Publications listed on page 56

SIGNALLING NETWORKS IN CANCER

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Approximately 70% of all lung cancer patients present with late stage disease and there is a pressing need to develop better therapies for these patients. This remains a major challenge as the underlying genetic causes of greater than half of all lung cancers remain unknown. Compounding this problem, it is becoming clear that initiating events are dispensable for maintenance of tumorigenic phenotypes, for example mutationally activated KRAS (one of the more prevalent driver mutations in NSCLC) is not required to maintain tumour cell survival in a subset of cancer cell lines.

Given the dismal success rates of current treatments, it is imperative that genetic mechanisms driving maintenance of tumorigenic phenotypes are discovered, new drug targets identified, and targeted therapeutics developed to improve treatment for lung cancer patients. Recent success with therapies targeting mutationally activated EGFR and constitutively active ALK serve as proof of principle that drugs targeting activated drivers of lung cancer result in better, more durable therapeutic responses in patients. Recent clinical trials, such as the TRACERx trial, are allowing unprecedented flexibility in tailoring therapeutic treatments to the underlying genetic aberrations that drive an individual's specific lung cancer. This is based on patient mutational profiles and other biomarkers from real time biopsies. This type of personalised clinical trial is revolutionising the way we treat patients with lung cancer. Unfortunately, as we do not understand the mechanisms that drive more than 50% of lung cancers, we lack personalised therapies for a large proportion of the patient population. A major aim of our research is to systematically fill in these knowledge gaps by identifying novel mutationally activated oncogenes, focusing mainly on kinases, as they are readily druggable targets.

Genetic drivers of lung cancer

The lab utilises a multitude of strategies to identify critical pathways required to promote lung tumorigeneis. These include high-throughput bioinformatics and structural modelling, siRNA screening, and precision genome editing to establish a functional genomic approach to identify novel drivers. Utilising bioinformatics we identify novel kinases enriched for functional mutations or amplifications in lung cancer to hone in on activated enzymes that can serve as drug targets. We then assess the structural consequences of a subset of mutations in the respective kinases, where crystal structures are available, to determine if the mutations are likely to increase or decrease catalytic activity. These approaches have been successful in identifying kinases with activating mutations in lung cancer, 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 this results 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.

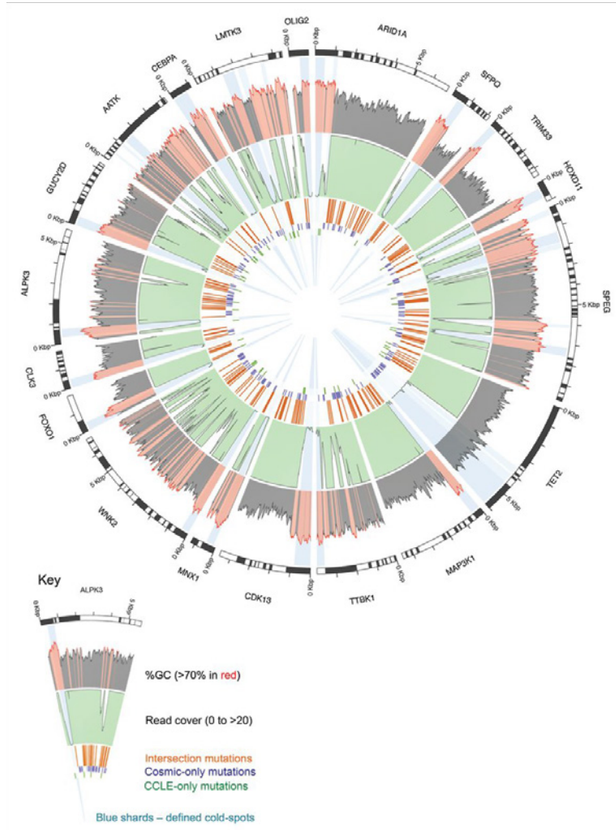

Figure 1
The 20 largest cold-spots detected in cancer census or kinase gene transcripts (of those that were sequenced by both COSMIC and CCLE hybrid capture) using CCLE whole-exome sequencing data. All but one of these cold-spots was located in a high GC-content area and resulted in no mutations being detected by either institute. The *TET2* cold spot was not located in high-GC content areas and contained mutations detected by COSMIC, indicating that this cold spot was not present in the COSMIC data. The outer shaded grey plot shows the GC content at each base (calculated as 50 bp either side) with GC content over 70% shaded in red. The middle light green plot shows sequencing read coverage with white troughs representing poor read coverage. The inner three rings record the position of mutations found by both institutes (orange), COSMIC-only (violet), and CCLE (green). Light blue shards show cold-spots over 100 bp in length with the top 20 shaded darker. Data were plotted using a combination of Circos and custom scripts.

Exploiting 'cold-spots' to elucidate novel drivers of cancer

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. The main reason for these discrepancies is inadequate sequencing of GC-rich areas of the exome, or cold-spots (Figure 1). We mapped over 400 regions of consistently inadequate sequencing in known cancer-causing genes and kinases in which neither institute found mutations. We demonstrated, using a newly identified PAK4 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. Going forward we will continue to mine these cold-spot regions of the exome to elucidate novel drivers of lung cancer, missed by cancer genome consortium studies.

PKCs in cancer

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. Crispr/Cas genome editing was used to verify the importance of PKC LOF mutations, and correcting these mutations with genome editing suppressed tumour growth in vivo consistent with a tumour suppressive role for PKCs in cancer. Lastly, germ line loss-of-function mutations in PKC delta are associated with development of genetic disorders associated with hallmarks of cancer including increases in proliferation and suppression of cell death in immune cells (mainly B cells). Specifically, LOF mutations have been identified in lymphoproliferative syndromes and we elucidated a causal LOF mutation in PKC delta in Juvenile Systemic Lupus Erythematous patients that promoted proliferation and suppressed cell death in B cells acquired from the patients. In summary these data provide compelling evidence that PKCs in general are tumour suppressors and PKC inhibitors should not be used to treat cancer patients with mutations in this family of kinases.

MLKs 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 if inhibition of the MLKs can be exploited to suppress tumorigenesis for specific types of cancer, including lung cancer.

Publications listed on page 57

STEM CELL BIOLOGY

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Understanding how blood cells are generated has clear implications for the treatment of blood diseases. Such knowledge could potentially lead to defining new conditions to amplify haematopoietic stem cells (HSCs) or could translate into new methods to produce HSCs, or other types of blood cells, from human embryonic stem (ES) cells or induced pluripotent stem (iPS) cells.

As most key transcription factors (TFs) regulating early haematopoietic development have also been implicated in various types of leukaemias, elucidating their function during normal development could result in a better understanding of their roles during abnormal haematopoiesis in leukaemia. In particular, the genes encoding the AML1/RUNX1 transcription factor and its cofactor CBFβ are master regulators of blood development and are frequently rearranged or mutated in human leukaemias such as acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL). Similarly, the transcriptional co-activator MOZ that is involved in three independent myeloid chromosomal translocations fusing *MOZ* to the partner genes *CBP*, *P300* or *TIF2* in human leukaemia is a critical regulator of blood development. Our group studies, in particular, the function of RUNX1 and MOZ in haematopoietic development and maintenance in order to better understand how alterations of these functions lead to leukaemogenesis.

RUNX1 regulates cell adhesion and migration prior to blood emergence

The earliest site of blood cell development in the mouse embryo is the yolk sac where blood islands, consisting of haematopoietic cells surrounded by a layer of angioblasts, develop at approximately day 7.5 of gestation. The parallel development of these two lineages in close association provided the basis for the hypothesis that they arise from a common precursor, a cell called the haemangioblast. A conflicting theory however associates the first haematopoietic cells to a phenotypically differentiated endothelial cell with haematopoietic potential, i.e. a haemogenic endothelium. Support for the haemangioblast concept was initially provided by the identification during embryonic stem (ES) cells differentiation of a clonal precursor, the blast colony-forming cell (BL-CFC), which gives rise (after 4 days of culture) to blast colonies with both endothelial, smooth muscle and haematopoietic potential. We recently established a new model of haematopoietic development. We demonstrated that haematopoietic cells are generated from the haemangioblast through the formation of a haemogenic endothelium intermediate. During this process, the haemogenic endothelial cells first cluster and then lose their endothelial identity, altering their flat, adherent appearance into the characteristic round shape of mobile haematopoietic precursor cells.

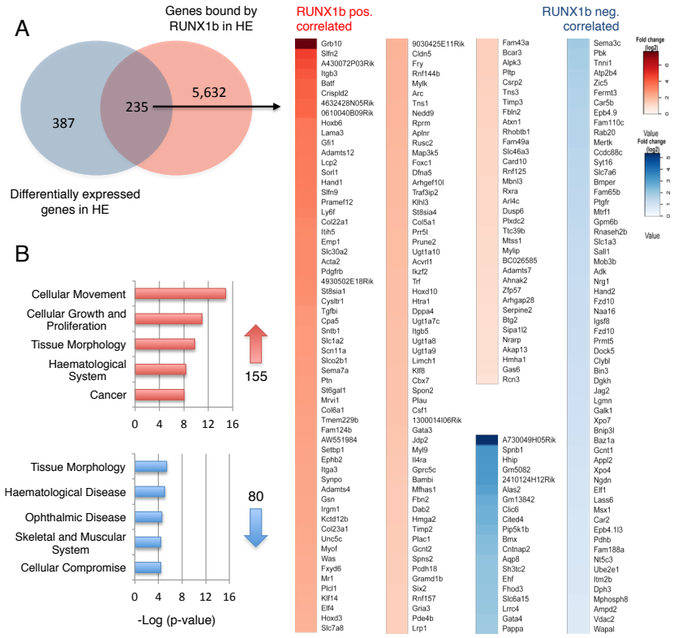

Figure 2
RUNX1b binds to and positively regulates genes associated with cell adhesion, cell migration and ECM interaction.
(**A**) Venn diagram showing the overlap between RUNX1-bound genes in haemogenic endothelium (HE), as determined by DamID-seq, and RUNX1b-dependent differentially expressed genes in haemogenic endothelium. Heatmap depiction of the 235 genes that are both bound and regulated by RUNX1b in haemogenic endothelium. RUNX1b- bound and positively correlated genes are depicted in red and RUNX1b-bound and negatively correlated genes are depicted in blue. All genes are ordered from higher to lower expression. (**B**) IPA on RUNX1b-bound and positively correlated genes (top panel) and on RUNX1b-bound and negatively correlated genes (bottom panel).

The identification of the earliest transcriptional programme regulated by RUNX1 would be key to understanding the onset of haematopoiesis. Previous studies aiming to reveal the initial RUNX1 programme have however been largely hampered by the fact that the haemogenic endothelium represents a rare, transient subset of endothelial cells with low endogenous RUNX1 expression. In addition, the RUNX1-dependent transition to haematopoiesis takes place rapidly, making it difficult to distinguish between immediate effects of RUNX1 in haemogenic endothelium cells and later direct or indirect effects in committed blood progenitors. To overcome these caveats, we coupled DamID (DNA adenine methyltransferase identification) with high-throughput sequencing, to map RUNX1 binding sites in haemogenic endothelium. This alternative to ChIP relies on the deposition of "methylation tags" around the binding sites of RUNX1 by the *E.coli* DNA adenine methyltransferase (Dam) (Figure 1). The stability of the methylation marks and the alleviation of the need for antibodies, make this technique ideal for binding site analysis of low expressed DNA-binding proteins in small populations. Compared to ChIP-seq, DamID-seq generated more control peaks (untethered Dam vs ChIP IgG or input control) and wider peaks overall, rendering existing ChIP-seq analysis packages unsuitable. We therefore developed a new TF specific DamID Peak Identification Pipeline (DamID-PIP) in collaboration with Dr Crispin Miller (CRUK Manchester Institute). Integration of the haemogenic endothelium specific RUNX1-DamID binding profile with matching transcriptome data (Figure 2) revealed that RUNX1 binds to and up-regulates the expression of genes involved in cell adhesion and migration, including components of the integrin signalling pathway. This suggests that at this early stage of haematopoietic development, RUNX1 organises the formation of haemogenic endothelium clusters required for the release of blood progenitors. Overall, this study provides the first comprehensive genome-wide RUNX1 target profiling in the early haemogenic endothelium and demonstrates that RUNX1 acts in a stage-specific fashion by activating adhesion and migration associated genes prior to the emergence of haematopoietic cells and the down-regulation of the endothelial programme. Outside of the haematopoietic context, this endothelial-epithelial RUNX1 signature in haemogenic endothelium might also reflect the emerging role of RUNX1 in epithelial-based tumour formation and progression. In particular the RUNX1 targets that associate with cell migration in haemogenic endothelium may represent important regulators of the potential metastatic role of RUNX in solid tumours.

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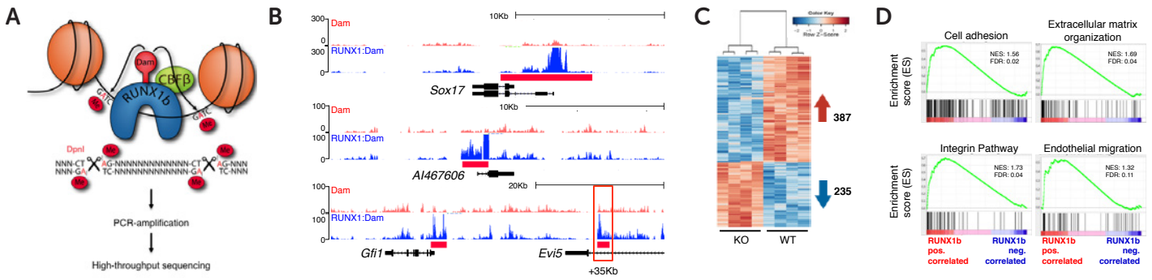

Figure 1
Identification of genes bound by RUNX1 and identification of genes differentially expressed in the absence of RUNX1 in Haemogenic Endothelium.
(**A**) Schematic representation of the RUNX1b DamID system. Top. The RUNX1b::Dam fusion binds RUNX1 binding sites and the Dam methylates adenines within nearby GATC sequences. The methylated fragments are isolated using the methylation specific enzyme DpnI, amplified by PCR, subjected to high throughput sequencing and the sequences analysed with the DamID Peak Identification Pipeline. Non-specific binding is controlled by sequencing of untethered Dam samples. (**B**) Validation of detection of RUNX1 binding at some known transcriptional targets. Raw sequencing read data from RUNX1b:Dam and control Dam haemogenic endothelium samples. Red bars indicate the RUNX1b:Dam peaks as identified by the DamID-PIP pipeline. RUNX1b::Dam binds to the Sox17 and AI467606 promoter regions and the +35Kb enhancer element of Gfi1, which are all known RUNX1 targets. (**C** and **D**) Genes differentially expressed between Runx1 WT and Runx1 KO haemogenic endothelium. (**C**) Heat maps and (**D**) GSEA analyses.

STEM CELL HAEMATOPOIESIS

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The recent advances in the generation, propagation and differentiation of pluripotent stem cells offer great promise in the field of regenerative medicine. Both embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) provide limitless sources of self-renewing cells endowed with the capacity to generate tissue-specific cell populations that could be used in the clinic.

These in vitro-derived cells could be used to regenerate a damaged haematopoietic system or to modulate the repair of other tissues or the immune response. One major obstacle to the realisation of this potential however, remains a lack of specific and efficient protocols for the differentiation of these pluripotent stem cells to the desired populations usable for therapeutic applications.

While stem cell-based regenerative medicine is still a goal far ahead, outstanding progress has been made in the successful generation and engraftment of ESC-derived lineages such as dopamine neurons or cardiomyocytes. In contrast, progress in the derivation of haematopoietic cells able to engraft in vivo have been rather modest. To date, the most successful in vitro derivation of haematopoietic cells able to repopulate mouse models has relied on the ectopic expression of transcription factors such as HOXB4, CDX4, LHX2 or RUNX1a. However, very few reports have documented the in vitro generation of haematopoietic repopulating potential from unmanipulated ESCs. One study reported the efficient in vitro derivation of haematopoietic repopulating cells from mouse ESCs differentiated in serum-supplemented culture conditions. However, this approach has not been pursued suggesting that serum-dependent conditions could not be easily reproduced. The use of high serum concentration and/or stroma cell lines to support the generation of repopulating haematopoietic cells derived from human ESCs has also shown promising results but to date no follow-up studies have further validated or extended these differentiation protocols.

The in vitro differentiation of ESCs has been widely used by our group and others as a model system to dissect and understand the early events of haematopoietic specification both in term of molecular mechanisms and cellular steps. The careful dissection of this in vitro programme has demonstrated that, similar to in vivo development, blood cells are generated from mesodermal haemangioblast precursors through a haemogenic endothelium intermediate and that the same network of transcription factors orchestrates both in vivo and in vitro processes. Detailed studies into the generation of primitive erythroid, myeloid and lymphoid progenitors have suggested a temporal emergence of these blood lineages in vitro, reflecting their sequential emergence in vivo during embryonic development. This has led to the concept that, similar to the in vivo situation, repopulating activity might emerge at late stages of the haematopoietic programme during ESC differentiation and that the emergence of lymphoid potential marks the establishment of the definitive programme. However to date, attempts to derive in vivo repopulating haematopoietic cells from late stages of ESC differentiation have been unsuccessful.

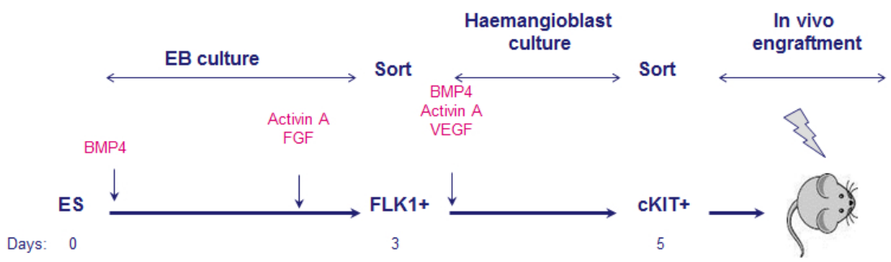

Figure 1
Schematic to illustrate the experimental strategy used to generate in vivo repopulating haematopoietic progenitors from in vitro differentiating ES cells.

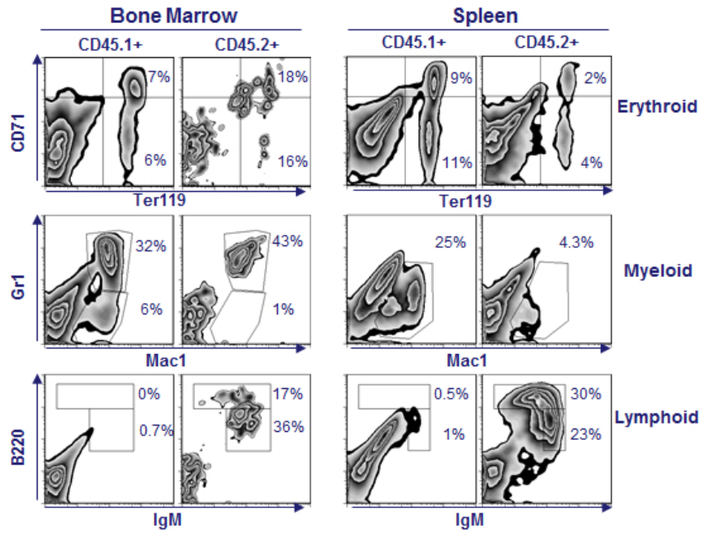

Figure 2
Long-term multilineage contribution of in vitro-derived haematopoietic progenitors. Flow cytometric analysis for the indicated markers of cells derived from the bone marrow (left panels) and spleen (right panels) 22 weeks post engraftment. Recipient-derived cells express CD45.1 while donor-derived cells express CD45.2.

Exploring the first step of haematopoietic specification

To revisit this long-standing challenge, we took an alternative approach and explored the very first step of haematopoietic specification from the mesoderm. We hypothesised that multi-lineage progenitors with in vivo repopulating ability might be specified very early upon commitment of mesoderm to the blood programme and might be difficult to maintain as such in the presence of serum or haematopoietic cytokines.

Based on our previous work, we first evaluated the minimal growth factors required for the optimal specification of haemangioblast to haemogenic endothelium in serum-free culture. Next, we evaluated the biological characteristics of the haemogenic endothelial population generated in these culture conditions through multi-parameter flow cytometry, clonogenic replating assays and gene expression analysis. Altogether, these experiments revealed that FLK1$^+$ mesodermal progenitors grown for two days in the sequential presence of BMP4, Activin A, FGF followed by BMP4, Activin A and VEGF gave rise to a population of cKIT$^+$ cells presenting a haemogenic endothelium immuno-phenotype and endowed with the capacity to generate erythroid, myeloid and both T and B lymphoid cells. Interestingly, these findings revealed the concomitant emergence of erythroid, myeloid and lymphoid programmes during ESC differentiation when cultured in the absence of serum or haematopoietic cytokines. In contrast to previously held concepts, these data suggest that during ESC differentiation all haematopoietic programmes are unravelled simultaneously from the mesoderm, in the absence of cues restricting the coordinated emergence of each lineage as is normally observed during embryogenesis.

Testing the engraftment capability of the haemogenic endothelium population

Given the in vitro multi-lineage capacity of this haemogenic endothelium population, we next investigated whether this population contained cells able to engraft in vivo by injection into sub-lethally irradiated recipient mice (Figure 1). The detection and persistence of a small donor-derived population was observed in several mice over a 16-week period. Analysis of lineage contribution in bone marrow and spleen at 22 weeks post-engraftment revealed the presence of CD45.2$^+$ donor-derived cells expressing cell surface markers characteristic of erythroid (Ter119, CD71), myeloid (Gr1, Mac1) and lymphoid (IgM, B220) lineages (Figure 2). The repopulating activity observed upon engraftment of haemogenic endothelium isolated from day-2 culture was reproducible but low in term of chimerism level and frequency of mouse repopulated. We therefore explored whether higher repopulation activity could be achieved by changing the timing of cell isolation during the course of FLK1 differentiation to haematopoiesis. FLK1$^+$ cells or cells isolated from day-3 culture were unable to engraft in vivo, while in contrast day-1 cKIT$^+$ cells gave rise to reproducible engraftment capability, with an overall higher level of chimerism than cells derived from day-2 culture.

Collectively, our findings demonstrate that haemogenic endothelium isolated from ESCs differentiated in serum-free culture with restricted temporal exposure to specific growth factors are able to confer long-term multi-lineage engraftment in vivo, and that this repopulating ability emerges rapidly upon mesoderm specification and is very transient. This study establishes a first critical step toward the generation of in vitro-derived repopulating haematopoietic cells that might be usable for therapeutic applications.

Publications listed on page 58

SYSTEMS ONCOLOGY

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In addition to the cancer cells, solid tumours also contain a range of "normal" cells, such as immune cells, endothelial cells and fibroblasts, which together form the tumour stroma. There has been a general appreciation that understanding the role of these infiltrating cells on tumour progression and response to therapy is needed in order to develop more efficient treatments. In the Systems Oncology group we aim to determine how tumour cells signal with cells in the stroma and specifically how signals from the tumour cells can co-opt the stromal cells to promote tumour progression and decrease therapeutic response. Delineating such signals may lead to the development of novel therapeutic strategies.

Pancreatic Ductal Adenocarcinoma (PDAC) has a dismal prognosis with a median survival below six months and an average five-year survival below 5%. This is due to the aggressive nature of the cancer, a lack of effective therapy and late diagnosis. The most frequent occurring genetic mutations have been identified with activating mutations in the oncogene KRAS, inactivation of the tumour suppressor CDKN2A in more than 90% of all tumours, and loss of TP53 and SMAD4 function occurring in 50-60% of all cases.
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.

Analysing signalling through the interrogation of post-translational modifications

To define how tumour and stromal cells signal to each other 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., 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., 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. As such, this approach has dramatically improved our ability to interrogate how reciprocal cellular signalling networks develop between tumour and stromal cells. We are currently utilising this technology to map which signals are exchanged between tumour and stromal cells in pancreatic cancer. In parallel, we have developed phenotypic assays to monitor the effect of tumour-stroma signalling and are currently conducting a loss of function genetic screen to identify components that are required for stromal co-option. Integration of the biochemical signalling network with a functional readout allows us to identify regulated and functionally important signals in a cell specific manner. Recently this has led to the identification of novel regulatory mechanisms whereby tumour cells communicate with stromal cells and we are currently evaluating these as putative therapeutic targets.

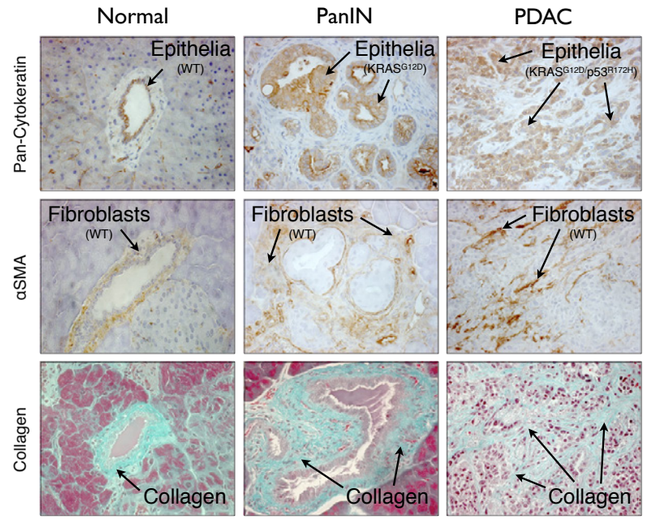

Figure 1
Pancreatic Ductal Adenocarcinoma (PDAC) is characterised by extensive stromal reaction and desmoplasia. Immunohistochemistry for epithelia (pan-cytokeratin), activated fibroblasts (alpha smooth muscle actin, αSMA) and collagen (Massons Trichrome) shown on pancreatic tissue isolated from a genetically engineered mouse model of pancreatic cancer. Shown is normal wild type (WT), KRas expressing early stages pancreatic ductal neoplasia (KC) or KRas/P53R172H expressing PDAC. Noteworthy, the epithelia loses its structure progressively as disease develops alongside an extensive fibroblast activation and collagen deposition.

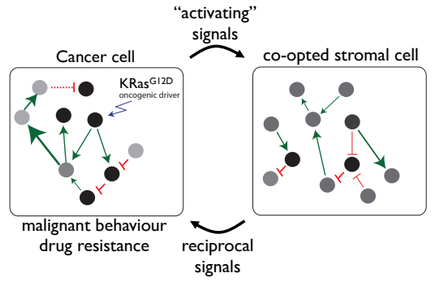

Figure 2
Working model of reciprocal tumour-stroma signalling. Expression of mutant KRas in tumour cells leads to changes in their cellular signalling network alongside increased secretion of paracrine acting growth factors, cytokines and morphogens. Neighbouring genetically normal stromal cells respond 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.

Development of a semi-automated platform for the enrichment of phosphorylated peptides

Protein post-translational modification (PTM) constitutes a major regulatory mechanism of cellular signalling and controls protein stability, activity, subcellular localisation and protein-protein interactions. Protein phosphorylation constitutes a critical PTM, which is underscored by the frequent changes to protein kinase and phosphatase activity in cancer. To study protein phosphorylation as a regulatory mechanism in tumour-stroma signalling, we have developed a robust semi-automated platform for the enrichment of phosphorylated peptides (Tape et al., 2014). This platform allows the simultaneous processing of 96 samples in less than one hour and thus facilitates experiments where multiple conditions can be evaluated at high fidelity. Utilising this platform we have analysed how mutant KRAS alters the cellular signalling network. Although mutant KRAS has been widely studied, there is no comprehensive map of the widespread changes occurring in cells expressing the mutant protein. As such we compared the effect of expressing the frequently occurring mutated $Kras^{G12D}$ across multiple pancreatic cancer cell lines and conditions, allowing us to pinpoint the core regulatory network that is controlled across multiple conditions.

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., 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 level of expression.

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TRANSCRIPTIONAL NETWORKS IN LUNG CANCER

www.cruk.manchester.ac.uk/Research/CRUK-MI-Groups/Transcriptional-Networks-in-Lung-Cancer/Home

Group Leader
Michela Garofalo[1]

Postdoctoral Fellow
Srivatsava Naidu[1]

Scientific Officer
Peter Magee[1]

[1]joined in 2014

Our group focuses on the identification of the causes, involving non-coding RNAs, behind lung cancer development and resistance to chemotherapy. The most well-known non-coding RNAs are microRNAs (miRNAs), single stranded RNAs of 19–25 nucleotides in length, that negatively regulate gene expression by translational inhibition or degradation of mRNA targets. MicroRNA dysregulation has been found to be involved in several processes including proliferation, apoptosis and cancer. Our goal is to identify mechanisms of miRNA dysregulation and generate new microRNA delivery systems to restore normal gene networks in vitro and in vivo, improving the efficacy of chemotherapy for lung cancer cure.

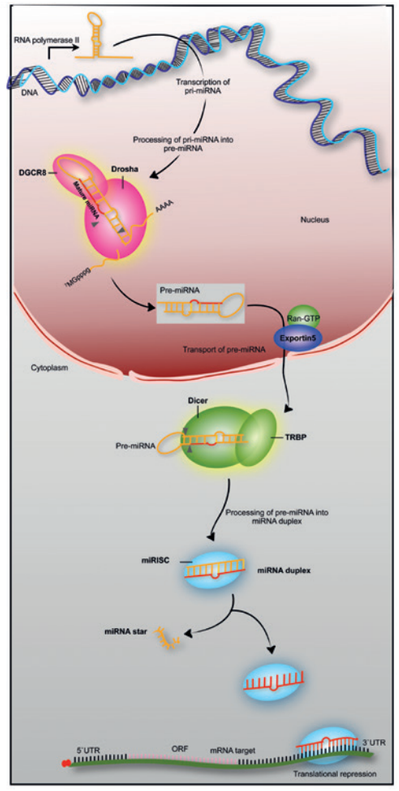
MicroRNA biogenesis and function

MicroRNAs or miRNAs are short (20–24-nucleotides) non-coding RNAs that regulate gene expression at the post-transcriptional level by binding to the 3'-untranslated regions (3'UTRs) or the open reading frames of target genes, leading to the degradation of target mRNA or repression of mRNA translation. miRNAs are transcribed as long primary transcripts characterised by hairpin structures (pri-miRNAs) whose maturation occurs through sequential processing events (Gregory 2004) (Figure. 1). The mature miRNAs are incorporated into a complex named RISC (RNA-induced silencing complex), which contains Argonaute proteins. The function of the miR is to guide the RISC to complementary or partially complementary target sites located in the 3' UTRs of mRNAs target inducing mRNA degradation or a block to translation, respectively. The mechanism of action of microRNAs has revolutionised the concept of gene expression regulation, because we now know that mRNA levels in a cell do not strictly correlate with protein expression (Bartel 2004). miRNAs are predicted to regulate a total of ~60% of human genes. A single miRNA can act on different mRNA targets whereas multiple miRNAs can regulate single mRNA molecules (Bartel 2004). As more miRNAs are unravelled and their role identified, it becomes clear that the involvement of these molecules in cancer is much more extensive than initially thought (Kasinsky and Slack 2001). The most striking evidence of the involvement of miRNAs in cancer is the alteration of miRNA expression in malignant cells compared to the normal counterparts (Calin and Croce 2006). The specific miRNA signature, called miRNome, characterises the malignant state and defines the clinicopathological features of the tumours (e.g. stage, grade, aggressiveness, proliferation index). Several high-throughput technologies revealed that miRNA stratification can be easily used to classify tumours and predict patient outcome. Because a single miRNA can target multiple pathways, miRNA-based anticancer therapies are being developed, either alone or combined with chemotherapy to improve the response and increase cure rates.

MicroRNAs and chemoresistance

Lung cancer still represents a very deadly disease in strong need of new, effective therapeutic approaches. The long-term survival for patients with advanced high-grade lung cancer has been limited by the frequent occurrence of resistance to chemotherapeutic drugs. In this context, TNF-related apoptosis-inducing ligand (TRAIL) may represent an alternative therapeutic molecule for this type of cancer. Several TRAIL inhibitors have entered clinical trials and seem to be effective in a small fraction of lung cancer patients (Lorusso et al., 2012; Herbst 2010). However, as with other molecularly targeted agents, resistance is likely to develop. Acquired apoptosis resistance is detrimental not only because it dampens the anticancer activity of the drugs but also because it promotes cancer progression and metastasis (Malhi 2006). The molecular mechanisms underlying the resistant TRAIL phenotype is still unclear and little is known regarding how lung cancer can acquire resistance to TRAIL. Therefore, it is fundamental to identify biomarkers to predict the response to the drug and to improve its therapeutic efficacy using drug combinations that not only synergise with TRAIL but that might also overcome resistance as it arises. To this end, we generated TRAIL-resistant cells (H460R and H292R) by exposing H460 and H292 sensitive cells (H460S and H292S) to stepwise increases in TRAIL concentrations over a period of six months to select cells capable of growing at high concentrations of TRAIL. MicroRNA expression profile in H460R versus H460S revealed dysregulation of a set of miRNAs (Jeon et al., under review). We found that these miRNAs are transcriptionally regulated by NF-kB and modulate important tumour suppressor genes involved in the TRAIL pathway. A combination of TRAIL and NF-KB inhibitors has shown, in vitro and in vivo, increased apoptosis and reduced cell proliferation in TRAIL resistant cells compared to treatments involving TRAIL or NF-KB inhibitors alone. The results not only suggest that combinatory treatment of TRAIL and NF-KB inhibitors could be effective in overcoming TRAIL resistance in NSCLC but also that, in the near future, the delivery and modulation of specific microRNAs could improve the response of lung cancer patients to TRAIL.

Figure 1
miRNA biogenesis and function. The primary miRNA (pri-miRNA) is transcribed by the RNA pol II from its genomic location and cleaved by the microprocessor complex, which comprises Drosha and DGCR8. The resulting precursor miRNA (pre-miRNA) is transported by exportin 5 to the cytoplasm where it is further processed into a double strand mature miRNA by Dicer and its cofactors. One strand of this duplex is degraded, whereas the other strand accumulates as the mature microRNA, which binds and guides the protein effector complex, formed by the RNA-induced silencing complex (RISC) and miRgonaute, to messenger RNA targets inducing either block of translation or mRNA degradation.

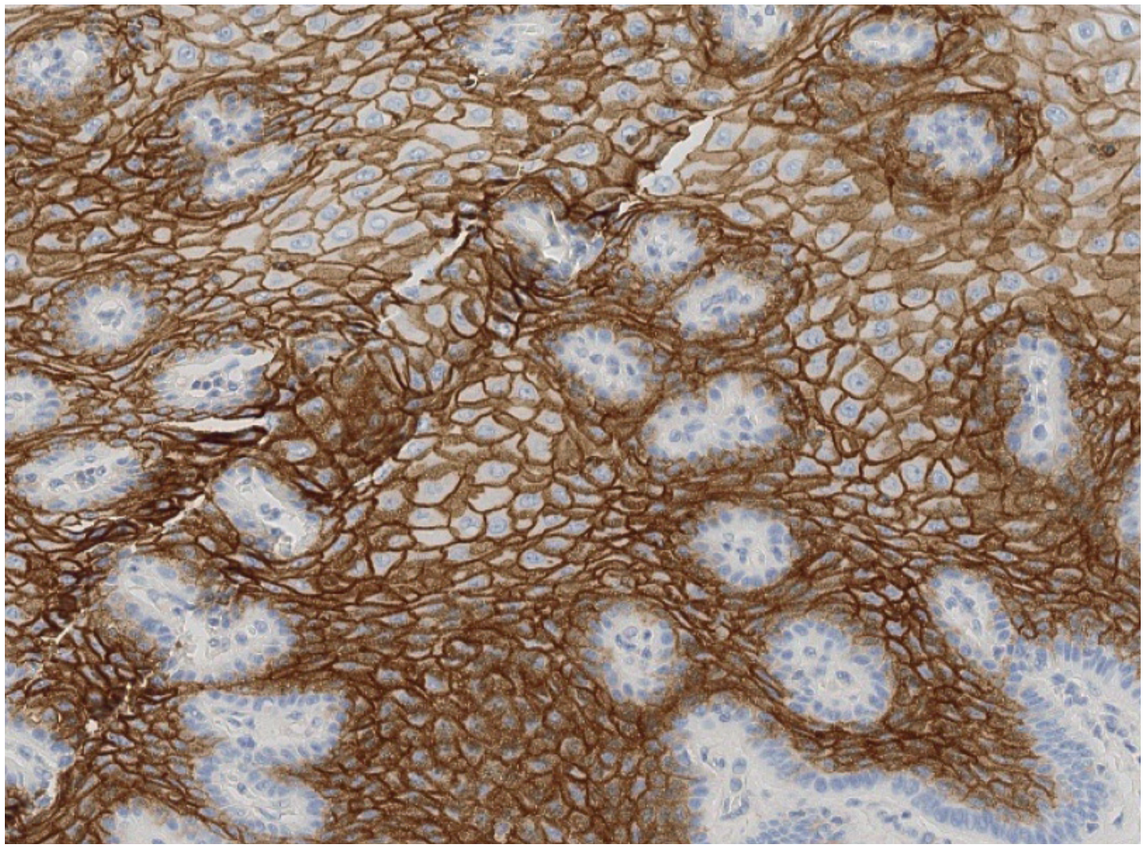


Our future plans involve the study of TKR-regulated-microRNAs. TKRs have shown a crucial role in several tumours, including lung cancer (Garofalo 2012). Currently, the epidermal growth factor receptor (EGFR) inhibitors represent the standard of care for patients with locally advanced or metastatic non-small cell lung cancer (NSCLC) harbouring activating EGFR mutations. Other genetic abnormalities have been reported in several small distinct subsets of NSCLC. Among these rare genetic changes, anaplastic lymphoma kinase (ALK) gene rearrangements result in the abnormal expression and activation of this tyrosine kinase. These rearrangements occur in 2-5% of NSCLC. Crizotinib, a first-in-class dual ALK and c-MET inhibitor, has been shown to be particularly effective against ALK positive NSCLC, showing dramatic and prolonged responses with low toxicity. However, resistance to crizotinib inevitably emerges and the mechanisms of such resistance are unknown. In this context we plan to investigate the role of non-coding RNAs in crizotinib resistance in vitro and in vivo. Plasma microRNAs will be assessed using microfluidic array technology in a screening cohort of healthy controls and crizotinib sensitive and resistant patients as noninvasive biomarkers to predict the response to the drug.

MicroRNAs and the microenvironment

The communication between the tumour cells and the surrounding cells—the microenvironment—helps drive the process of tumour progression. Two of the key hallmarks of cancer, angiogenesis and metastasis, are dependent on the surrounding microenvironment. Exosomes function as mediators of intercellular communication and contain multiple functional molecules including microRNAs. We plan to study how exosome-released microRNAs influence the communication between normal and malignant cells to induce lung tumorigenesis. Networks of pro-metastatic regulators involved in angiogenesis, collagen remodelling and proteolysis will be analysed as targets of the secreted microRNAs. Understanding molecular signalling in the tumour microenvironment may provide new mechanistic rationales for optimising current cancer therapies and the development of future novel therapeutic modalities.

Publications listed on page 58



Sections of oesophageal tissue stained with a Ly6D antibody.

Image supplied by Esther Baena (Prostate Oncobiology).

CANCER RESEARCH UK MANCHESTER INSTITUTE

RESEARCH SERVICES

RESEARCH SERVICES

www.cruk.manchester.ac.uk/Research/

Chief Laboratory Officer
Stuart Pepper

As you will see in the sections below, 2014 has been a very exciting year for the core facilities. They have experienced significant growth both in terms of increasing the number of staff in some of the teams, and purchasing a raft of new equipment. Much of this equipment has been bought with £8.7m of funding from the UK Research Partnership Investment Fund.

Chief Laboratory Officer
Stuart Pepper

A key success was becoming the first site worldwide to receive a new Phenix high content screening platform. The initial testing was very successful and the instrument is now in regular use. Histology has also benefitted from the addition of new automated staining equipment. Towards the end of the year we have also made some major purchases in the area of mass spectrometry and in vivo imaging; over the next few months this will lead to further expansion of the applications supported by the core facilities.

Advanced Imaging and Flow Cytometry

Steve Bagley, Jeff Barry (Deputy Manager, Flow Cytometry), Toni Banyard[1], Helen Bradley, Helen Carlin[1], Mike Hughes[2], Abi Johnson, Kang Zeng

[1]joined in 2014 [2]left in 2014

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 to guide scientists by helping to apply these techniques to their research. 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 millions of cells across multiple conditions.

Over the year the facility has experienced tremendous growth in data output, method development, introduction of new equipment, and importantly development of the team. This year the facility has provided over 5000 hours of microscopy, 9500 hours cytometry, 2500 hours of high content screening, and 9000 histology slides have been imaged whilst over 3000 hours of image analysis has been performed.

A large proportion of the output of the facility requires the ability to assay across several cell types, under different conditions and often from different patients thus high throughput (HTS) and high content screening (HCS) is an essential function of the laboratory. Over the last two years, new systems have been introduced to automate both tissue and single cell data acquisition, processing, analysis and informatics. This year a new system has been introduced at the 'cutting edge' of equipment design, the PerkinElmer Opera Phenix. This was installed as a beta test, the first system placed in a laboratory outside of the manufacturer's own labs, and presents to the Institute new methods of screening under confocal illumination for both fixed and live cells, in two- or three-dimensional cell culture. The system is capable of the rapid imaging of four discrete markers per cell, across several fields of view per well, and over hundreds of discrete experiments; for example using automation to assess fifty 384 multi-well plates would represent a data run of around 700,000 images (around 21 million cells) and would take fourteen hours to complete, after which automated image analysis is performed on a per well and on a per cell basis. In conjunction with this equipment, a small-molecule compound library and acoustic dispensing is being introduced (in association with the Molecular Biology Core Facility and Drug Discovery Unit) to aid in the identification

of phenotypic changes within the cell. Working alongside Scientific Computing, a cloud based image database and analysis system has been installed in order to allow research access to the HCS data in the lab or office.

Flow cytometry has shown marked growth as the equipment that was introduced a year ago, to allow the sorting and analysis of translational data, has become a routine procedure. In 2014 around 5,500 hours of cell sorting, 4000 hours of analysis and 1000 hours of data processing was performed. Staffing in Cytometry underwent a change this year as a long-standing member of the team retired; consequently a new member of staff was taken on as a replacement and another was appointed to expand the facility's capability further. A training programme has been initiated with our new team members and with pre-existing staff as the demands upon the facility become more complex.

A large part of the facility's success has been working closely with the research groups and the other facilities within the Institute, such as Histology, where we have been developing new visualisation methods for multiplexing of tissue. The whole slide or algorithm-directed imaging methods provide the ability to perform analysis across multiple tissues, thus stratified medicine regimes or the detection of rare cells can be carried out across multiple patients in an automated fashion. The requirement for histological analysis has shown a marked increase across the research groups hence in the coming year another member of staff is to be appointed to help support this work.

An innovation this year has been the introduction of a cloud based management system for the researchers to book time on the equipment. As the work load and complexity of the research intensifies, demands for the equipment and training also increase, which the new system manages along with asset management, risk assessment, hand-over of samples, document control and billing. This software has now been rolled out across all of the facilities.

Over the next year a series of new developments are being planned in response to the demands of the research groups, which include analysis of data for large-scale high content screens, cytometry high throughput analysis and new methods of in vivo visualisation.

Biological Mass Spectrometry Facility

Duncan Smith, Yvonne Connolly, John Griffiths

The facility's role is to enhance research groups' cancer research capabilities by enabling cutting edge proteomic workflows. The facility has key strengths in project design, routine service provision, data interpretation and R&D by way of collaborative project support to simultaneously enhance research output and the organic growth of novel workflows designed to answer previously intractable biological questions. The goal of the facility is to maximise CRUK MI's output by delivering world class quality service with an evolving portfolio of workflows designed to lead in this area of technical expertise.

We have further developed our abilities to quantitatively profile both ubiquitin and SUMO modifications simultaneously utilising chemical derivitisation and data independent acquisition. This approach allows cancer researchers to probe an entire complex sample for the presence and alteration in abundance of these

critical modifications (Griffiths et al., 2014). We are now interested in extending this approach to facilitate the simultaneous profiling of many other modifications within the same experiment as this will allow us to gain a critical insight into the post translational modification events that underpin the advantages cancer cells gain over their normal counterparts.

In the area of global protein profiling, we have built an exceptionally rapid and reproducible method that significantly increases the sensitivity to detecting real biological differences in protein abundance in cancer research projects (Griffiths et al., 2014). This year has seen the retirement of two of our Mass Spectrometers after many years of productivity. The facility and CRUK MI is incredibly fortunate to have benefitted from a £1M investment in new Mass Spectrometers. The Thermo Orbitrap Fusion and ABSciex 6600 arrived in the Institute in late December 2014 and will be installed early in 2015. Both these premium instruments offer huge improvements in speed, sensitivity, resolution and workflow flexibility over the instruments they replace. The facility's capabilities to support CRUK MI's research output will be revolutionised by this investment and we are very much looking forward to an exciting, challenging and successful 2015.

Biological Resources Unit

Team Leaders: Kim Acton (Transgenic Unit), **Lisa Doar** (Experimental Unit)

In 2014, the capacity of animal research services at the Institute doubled, providing improved and expanded services to the increasing number of research groups. All animal research is conducted in mice and in full compliance with regulatory requirements and our high ethical standards. During the year, the facility has been subject to inspection by the Home Office Animals in Science Regulation Unit and the Health and Safety Executive, with positive outcomes. The constitution and remit of the Institute's Animal Welfare and Ethical Review Body has been revised and improved in line with the revised legislation, and with an increasing focus on the 3Rs (Replacement, Reduction and Refinement) of animal research.

Transgenic Services

The transfer of the transgenic mouse breeding function to the University Incubator Building continued during 2014 and will complete in 2015. The move has required the rederivation of over 100 existing lines plus 80 new lines that have been bred during 2014. Stock has been maintained at the Paterson Building facility until sufficient is available to supply from the Incubator, placing a significant demand on resources; however, the transfer has been successful with minimal interruption to research. Currently, the Transgenic Unit breeds more than 180 lines of genetically altered mice for the Institute's scientists and provides expertise in the import and export of strains.

Experimental Services

The Experimental Unit has continued the expansion of services started last year and now supports double the research capacity provided in 2013. Space has been a limiting factor that will be eased with the completion of the transfer of the breeding function in 2015; this will allow a redesign and refurbishment of the Paterson Building facility, a key feature of which will be a new in vivo imaging facility. The Experimental team has continued to provide professional animal technology advice and support to the Institute's scientists, guided further by expertise from the Royal Veterinary College veterinarians. Noted 3R achievements during the year comprise improvements in surgical practice including intra-femoral techniques and blood microsampling, as well as an improved animal health screening programme.

Histology

Garry Ashton, Caron Abbey, Michelle Greenhalgh (MCRC, Tissue Biobank)
David Millard, Joanna Molenda[1], Deepti Wilks (Haematological Malignancy Biobank)

[1]joined in 2014

Following the laboratory refurbishment in 2013, enabling better use of existing space, additional laboratory space was secured in 2014 and refurbished. This now houses two new immunohistochemistry/in situ hybridisation platforms. Both the Leica Bond RX and the Ventana Discovery platforms allow us to continue to offer a high throughput, routine, troubleshooting and antibody validation services in line with current demand. In addition the flexibility of both platforms has allowed us to develop, automate and offer in situ hybridisation as a routine service, thus allowing the researcher to obtain gene expression information in the context of tissue/cell morphology. The technique can be used for fixed tissues, cells and circulating tumour cells (CTCs). Both chromogenic (CISH) and fluorescence (FISH) in situ hybridisation can be performed either in a single or multiplex format. This had proved very successful and is currently being employed in several research groups' projects.

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 this will allow us to study tumour heterogeneity from a different angle that complements efforts in understanding the genetic/molecular heterogeneity of tumours. Like ISH, this again has been automated and is now offered as a service and is currently being used by several groups. Automation has resulted in improved standardisation of this technically demanding area.

The joint position between the Histology and the Advanced Imaging facilities, focusing on the numerical analysis of histological data in tissue biomarker expression studies, is continuing to prove very successful. As the expansion of tissue biomarkers continues to gather pace, the construction of tissue microarrays continues to grow. This has resulted in a large valuable resource now being used by several groups.

The year has seen developments in laser capture microdissection and macrodissection. The extraction of both RNA and DNA, sufficient in quantity and quality for NGS from relatively small amounts of material, is now performed routinely. Improved methods for the microdissection of samples from both frozen and FFPE samples based on immunophenotype have continued. DNA and RNA from frozen human and mouse GEM, PDX and xenograft models have been used to correlate to various clinical subtypes of melanoma.

The unit continues to process FFPE and frozen samples for the MCRC Biobank. To date, samples from over 6700 patients have been collected. Blood, bone marrow and plasma (at various disease status time points) from over 400 haematological malignancy patients has also been collected. To date over 90 research projects have been approved. Samples have also been released for method validation studies.

Specific examples of our work include IHC for Tiam1, Huwe1 and c-Met, showing a positive correlation between Huwe1 and c-Met levels and an inverse correlation between Tiam1 and Huwe1 levels in human lung tumours. This demonstrated a regulatory mechanism of Tiam1 by Huwe1 that was first identified in vitro. This work was recently published in Cell Reports by the Cell Signalling group.

The drug discovery unit are using IHC in order to determine if there is a significant population of patients who are deficient in a particular protein (XRCC1). If a deficient patient population is observed, the search for a partner (synthetic lethal) protein which can be targeted with a drug in order to have a therapeutic effect can be explored.

The Signalling in Cancer Networks group are using IHC / ISH to reveal molecular/genomic events that occur during lung carcinogenesis. Following gene expression analysis of premalignant and invasive squamous lesions, ISH and IHC are being used to help validate the expression data and to give valuable insights into the heterogeneous expression pattern of these genes within squamous lung tumours.

The Molecular Oncology group are using a fluorescence-based co-detection strategy to simultaneously study the expression of two macrophage markers in tumour material from melanoma patients before treatment with targeted therapy and following the development of resistance. The application of

an additional, melanoma-specific marker allows us to study the spatial resolution of the immune cells within the tissue context. An additional advantage of this co-staining strategy is that optimal use is made of the limited material that we have available. To further maximise the information gained from a single section, we are currently optimising the colorimetric co-detection of two additional immune cell markers following the fluorescent read-out.

The Stem Cell Biology group are using the Histology facility to investigate the cellular and molecular mechanisms of blood cell development. This has allowed the endothelial to haematopoietic transition (EHT) process to be studied in more depth. Cryotomy, laser dissection and IHC have all been used. Given that changes have been observed in protein expression via immunofluoresence, the additional use of in situ hybridisation to provide information about the mRNA expression pattern of the gene of interest is now being used to further evaluate what is happening and combine it with the data already available from FACS. These techniques are also being incorporated into the Systems Oncology group who study pancreatic ductal adenocarcinoma, which is a heterogeneous disease with multiple different cells infiltrating the tumour microenvironment. In addition, we are also incorporating multiplexing to allow multiple targets to be analysed simultaneously. This is particularly important for validating genes expressed in neighbouring cell-types. Data analysis is performed with the PerkinElmer Vectra, which allows quantification of the relative level of expression.

With the adoption of sophisticated labelling techniques and the availability of new platforms/technologies, histology and molecular pathology are at very exciting point, placing the unit at the forefront of biomarker analysis in oncology. The addition of new platforms, the lab expansion and additional recruitment has allowed the unit to focus primarily on its development within these areas.

Laboratory Services

Mark Craven, Tony Dawson, Antony Griffin, Corinne Hand, John Higgins, Christine Whitehurst

During 2014, the department has continued to support the Research Groups and Service Groups within the Paterson Building and at The University of Manchester's Wolfson Molecular Imaging Centre.

Our main role continues to be to provide 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 can adapt to the needs of a laboratory's users. Using our industrial glasswashers and autoclaves we can sterilise bulk pipette tips and microtubes. We can also sterilise items sent up from the labs.

The department make daily visits to laboratory areas to remove dirty glassware and also makes visits to check and top up supplies of clean glassware and plastics. Our improved Clean Room now supplies bacteriological media and buffers to the Paterson Building and will also supply these items to the new MCRC building when it opens in 2015.

The Lab Services department also performs other tasks to support the building. We ensure all taps are run as part of the Legionella testing programme each month. We maintain the Institute's film developer and are responsible for the provision of clean lab coats. The department also checks and replenishes the assorted First Aid supplies across the building and oversees the monthly Pipette Clinics.

Molecular Biology Core Facility and Cancer Research UK Microarray Service

Stuart Pepper, Chris Clark, Toni Grady[1], Yvonne Hey, Nelson Iley[2], Gill Newton, Leanne Wardleworth, John Weightman

[1]joined in 2014 [2]left in 2014

The HiSeq 2500 is being heavily used for a range of sequence projects including exome sequencing, RNA sequencing and ChIP based sequencing. Managing this workload on one instrument has proved to be quite challenging so we were especially pleased when we were able to add a new NextSeq platform to the facility this year. The NextSeq offers greater flexibility for run scheduling and should help to maintain quick turnaround times, even when demand is high.

For expression profiling by quantitative PCR (qPCR) the facility has two LifeTech 7900 instruments which have proved to be reliable workhorses over many years. Last year we were also able to add the Fluidigm platform which allows efficient processing of very large qPCR projects. This year we have further extended support by adding the Fluidigm C1 module to support expression profiling in single cells. By combining cell sorting techniques with the C1 module it is possible to look at expression heterogeneity within defined populations.

The facility also gained a pyrosequencing platform for quantitative analysis of sequence variants. Pyrosequencing differs considerably from traditional Sanger sequencing in that the reads are much shorter, but the readout is quantitative. This makes the Pyromark an excellent platform for detection of mutations in mixed populations of cells. The system is also suitable for detection of the methylation status of targeted sequences.

The routine screening services have continued to run efficiently throughout the year. Cell line authentication is well established in the Institute and all cell lines in use in the building can now be regularly authenticated by Short Tandem Repeat (STR) profiling. The facility has considerable experience in this technique and is able to offer a full service including interpretation of results. In conjunction with regular mycoplasma screening, researchers can be very confident in the cell lines that are used in the Institute.

During this year we have seen a sudden drop in demand for the Affymetrix Microarray service. For over a decade this service has had consistent demand and has supported over 500 projects for CRUK researchers. Now we are observing that groups are moving to Next Generation Sequencing for expression profiling work which has impacted on demand for the service. In June, a decision was made to close this service at the end of 2014 as it was no longer a viable option. Thank you to all the regular users who supported this service – it has been a real pleasure working with so many people across the whole organisation over the last twelve years.

Scientific Computing

Wei Xing, Zhi Cheng Wang, Christopher Smowton[1]

[1]joined in 2014

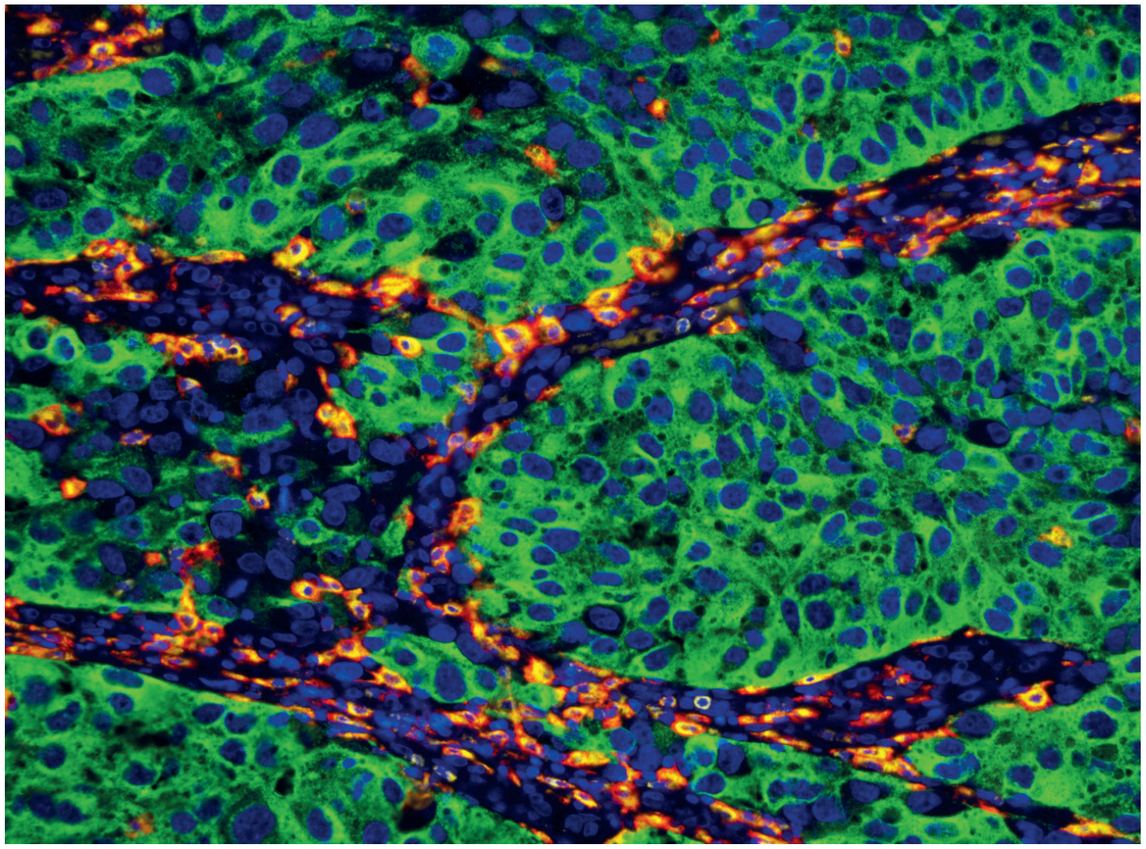
The Scientific Computing team was established in April 2014 in order to provide High Performance Computing (HPC) services to CRUK MI. Initial work involved the implementation of a new HPC system, "Troodon", which provides 628 CPU cores, 7TB RAM, and 700TB storage.

Troodon has since been expanded to 1058 CPU cores, 24TB RAM, and 3PB storage space, and provides HPC facilities on site that are principally used in the analysis of deep sequencing, proteomics and imaging data.

The SciCom team has also been exploring other architectures and has recently deployed both GPU and Intel PHI based CPU accelerators with a view to supporting a variety of use-cases including genome alignment, matrix algebra and molecular dynamics.

A dedicated HPC cluster and secure storage space has been built for downloading and processing "controlled" data including that from TCGA. The Scientific Computing team also helped the Advanced Imaging and Flow Cytometry team to setup its Columbus image analyses server and provide 200TB storage space for imaging data.

A new dedicated machine room with an advanced water-cooling system is currently under construction. Upon completion, during 2015, it will become the new home for all of the Institute's HPC. It will host a total of 2048 CPU cores and 6PB storage space.



Malignant melanoma tissue fluorescently co-stained for the melanoma-specific marker HMB45 (green) and macrophage markers CD68 (yellow) and CD163 (red).

Image supplied by Gabriela Gremel (Molecular Oncology).

CANCER RESEARCH UK MANCHESTER INSTITUTE

PUBLICATIONS AND ADMINISTRATION

RESEARCH PUBLICATIONS

Karim Labib[1]
Cell Cycle

[1]Now at University of Dundee

Refereed Research Papers

Maric, M., Maculins, T., De Piccoli, G., and Labib, K. (2014)
Cdc48 and a ubiquitin ligase drive disassembly of the CMG helicase at the end of DNA replication. ***Science*** *346*, 1253596.

Simon, A.C., Zhou, J.C., Perera, R.L., van Deursen, F., Evrin, C., Ivanova, M.E., Kilkenny, M.L., Renault, L., Kjaer, S., Matak-Vinkovic, D., Labib, K., Costa, A., and Pellegrini, L. (2014)
A Ctf4 trimer couples the CMG helicase to DNA polymerase alpha in the eukaryotic replisome. ***Nature*** *510*, 293-297.

Iain Hagan (page 12)
Cell Division

Refereed Research Papers

Carpy, A., Patel, A., Tay, Y.D., Hagan, I.M., and Macek, B. (2014)
Nic1 Inactivation Enables Stable Isotope Labeling with 13C6 15N4-Arginine in *Schizosaccharomyces pombe*. ***Molecular & Cellular Proteomics*** : doi: 10.1074/mcp.O114.045302.

Fennessy, D., Grallert, A., Krapp, A., Cokoja, A., Bridge, A.J., Petersen, J., Patel, A., Tallada, V.A., Boke, E., Hodgson, B., Simanis, V., and Hagan, I.M. (2014)
Extending the *Schizosaccharomyces pombe* molecular genetic toolbox. ***PLoS One*** *9*, e97683.

Nic Jones (page 14)
Cell Regulation

Refereed Research Papers

Gozdecka, M., Lyons, S., Kondo, S., Taylor, J., Li, Y., Walczynski, J., Thiel, G., Breitwieser, W., and Jones, N. (2014)
JNK Suppresses Tumor Formation via a Gene-Expression Program Mediated by ATF2. ***Cell Rep*** *9*, 1361-1374.

Leong, H.S., Dawson, K., Wirth, C., Li, Y., Connolly, Y., Smith, D.L., Wilkinson, C.R., and Miller, C.J. (2014)
A global non-coding RNA system modulates fission yeast protein levels in response to stress. ***Nat Commun*** *5*, 3947.

Marusiak, A.A., Edwards, Z.C., Hugo, W., Trotter, E.W., Girotti, M.R., Stephenson, N.L., Kong, X., Gartside, M.G., Fawdar, S., Hudson, A., Breitwieser, W., Hayward, N.K., Marais, R., Lo, R.S., and Brognard, J. (2014)
Mixed lineage kinases activate MEK independently of RAF to mediate resistance to RAF inhibitors. ***Nat Commun*** *5*:3901.

Angeliki Malliri (page 16)
Cell Signalling

Refereed Research Papers

Vaughan, L., Tan, C.T., Chapman, A., Nonaka, D., Mack, N.A., Smith, D., Booton, R., Hurlstone, A.F., and Malliri, A. (2014)
HUWE1 Ubiquitylates and Degrades the RAC Activator TIAM1 Promoting Cell-Cell Adhesion Disassembly, Migration, and Invasion. ***Cell Rep*** Epub 2014 Dec 24.

Caroline Dive (page 18)
Clinical and Experimental Pharmacology

Refereed Research Papers

Aung, K.L., Donald, E., Ellison, G., Bujac, S., Fletcher, L., Cantarini, M., Brady, G., Orr, M., Clack, G., Ranson, M., Dive, C., and Hughes, A. (2014)
Analytical validation of BRAF mutation testing from circulating free DNA using the amplification refractory mutation testing system. ***J Mol Diagn*** *16*, 343-349.

Backen, A., Renehan, A.G., Clamp, A.R., Berzuini, C., Zhou, C., Oza, A., Bannoo, S., Scherer, S.J., Banks, R.E., Dive, C., and Jayson, G.C. (2014)
The combination of circulating Ang1 and Tie2 levels predicts progression-free survival advantage in bevacizumab-treated patients with ovarian cancer. ***Clin Cancer Res*** *20*, 4549-4558.

Cove-Smith, L., Woodhouse, N., Hargreaves, A., Kirk, J., Smith, S., Price, S.A., Galvin, M., Betts, C.J., Brocklehurst, S., Backen, A., Radford, J., Linton, K., Roberts, R.A., Schmitt, M., Dive, C., Tugwood, J.D., Hockings, P.D., and Mellor, H.R. (2014)
An integrated characterization of serological, pathological, and functional events in doxorubicin-induced cardiotoxicity. ***Toxicological Sciences*** *140*, 3-15.

Cummings, J., Sloane, R., Morris, K., Zhou, C., Lancashire, M., Moore, D., Elliot, T., Clarke, N., and Dive, C. (2014)
Optimisation of an immunohistochemistry method for the determination of androgen receptor expression levels in circulating tumour cells. ***BMC Cancer*** *14*, 226.

Hodgkinson, C.L., Morrow, C.J., Li, Y., Metcalf, R.L., Rothwell, D.G., Trapani, F., Polanski, R., Burt, D.J., Simpson, K.L., Morris, K., Pepper, S.D., Nonaka, D., Greystoke, A., Kelly, P., Bola, B., Krebs, M.G., Antonello, J., Ayub, M., Faulkner, S., Priest, L., Carter, L., Tate, C., Miller, C.J., Blackhall, F., Brady, G., and Dive, C. (2014)
Tumorigenicity and genetic profiling of circulating tumor cells in small-cell lung cancer. ***Nature Medicine*** *20*, 897-903.

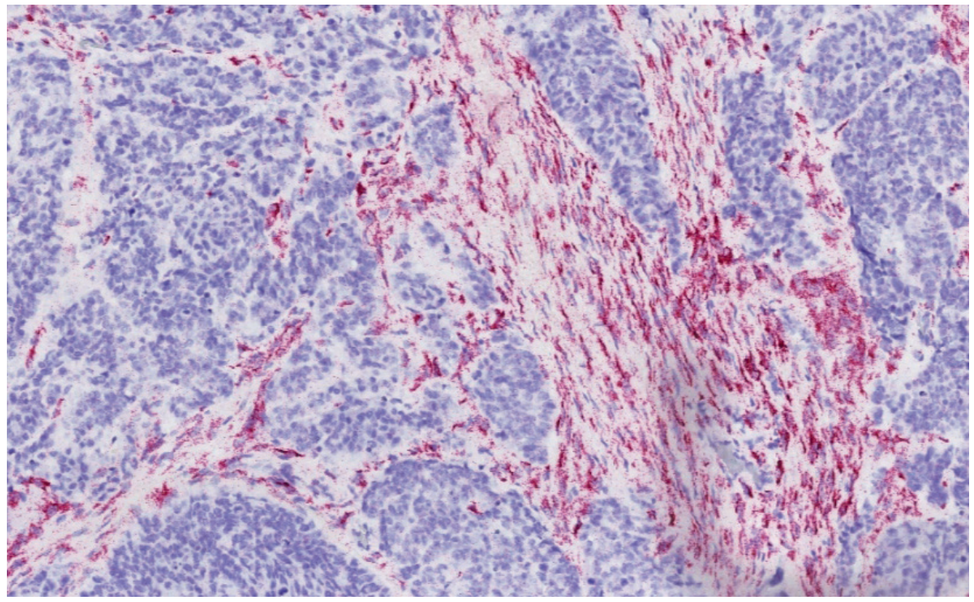
Jamal-Hanjani, M., Hackshaw, A., Ngai, Y., Shaw, J., Dive, C., Quezada, S., Middleton, G., de Bruin, E., Le Quesne, J., Shafi, S., Falzon, M., Horswell, S., Blackhall, F., Khan, I., Janes, S., Nicolson, M., Lawrence, D., Forster, M., Fennell, D., Lee, S.M., Lester, J., Kerr, K., Muller, S., Iles, N., Smith, S., Murugaesu, N., Mitter, R., Salm, M., Stuart, A., Matthews, N., Adams, H., Ahmad, T., Attanoos, R., Bennett, J., Birkbak, N.J., Booton, R., Brady, G., Buchan, K., Capitano, A., Chetty, M., Cobbold, M., Crosbie, P., Davies, H., Denison, A., Djearman, M., Goldman, J., Haswell, T., Joseph, L., Kornaszewska, M., Krebs, M., Langman, G., MacKenzie, M., Millar, J., Morgan, B., Naidu, B., Nonaka, D., Peggs, K., Pritchard, C., Remmen, H., Rowan, A., Shah, R., Smith, E., Summers, Y., Taylor, M., Veeriah, S., Waller, D., Wilcox, B., Wilcox, M., Woolhouse, I., McGranahan, N., and Swanton, C. (2014)
Tracking genomic cancer evolution for precision medicine: the lung TRACERx study. ***PLoS Biology*** *12*, e1001906.

Morris, K.L., Tugwood, J.D., Khoja, L., Lancashire, M., Sloane, R., Burt, D., Shenjere, P., Zhou, C., Hodgson, C., Ohtomo, T., Katoh, A., Ishiguro, T., Valle, J.W., and Dive, C. (2014)
Circulating biomarkers in hepatocellular carcinoma. ***Cancer Chemother Pharmacol*** *74*, 323-332.

Potter, D.S., Kelly, P., Denneny, O., Juvin, V., Stephens, L.R., Dive, C., and Morrow, C.J. (2014)
BMX acts downstream of PI3K to promote colorectal cancer cell survival and pathway inhibition sensitizes to the BH3 mimetic ABT-737. ***Neoplasia*** *16*, 147-157.

Rothwell, D.G., Li, Y., Ayub, M., Tate, C., Newton, G., Hey, Y., Carter, L., Faulkner, S., Moro, M., Pepper, S., Miller, C., Blackhall, F., Bertolini, G., Roz, L., Dive, C., and Brady, G. (2014)
Evaluation and validation of a robust single cell RNA-amplification protocol through transcriptional profiling of enriched lung cancer initiating cells. ***BMC Genomics*** *15*, 1129.

Tait, C., Moore, D., Hodgson, C., Brown, M., Morris, T., Growcott, J., Malone, M., Hughes, A., Renehan, A., Clarke, N.W., and Dive, C. (2014)
Quantification of skeletal metastases in castrate-resistant prostate cancer predicts progression-free and overall survival. ***BJU Int***. 114(6b):E70-3.



Human lung squamous cell carcinoma stained with chromogenic in-situ hybridisation targeting mRNA from the gene lumican (red), which accumulates in the cellular component of the tumours' interstitial matrix. Counterstained with haematoxylin. *Image supplied by Andre Koper (Signalling Networks in Cancer).*

Zhou, C., Walker, M.J., Williamson, A.J., Pierce, A., Berzuini, C., Dive, C., and Whetton, A.D. (2014)
A hierarchical statistical modeling approach to analyze proteomic isobaric tag for relative and absolute quantitation data. ***Bioinformatics*** *30,* 549-558.

Other publications

Castle, J., Shaker, H., Morris, K., Tugwood, J.D., and Kirwan, C.C. (2014)
The significance of circulating tumour cells in breast cancer: A review. ***Breast*** *23,* 552-560.

Khoja, L., Lorigan, P., Dive, C., Keilholz, U., and Fusi, A. (2014)
Circulating tumor cells as tumor biomarkers in melanoma: detection methods and clinical relevance. ***Ann Oncol***. Epub 2014 Jun 6.

Krebs, M.G., Metcalf, R.L., Carter, L., Brady, G., Blackhall, F.H., and Dive, C. (2014)
Molecular analysis of circulating tumour cells-biology and biomarkers. ***Nat Rev Clin Oncol*** *11,* 129-144.

Donald Ogilvie (Page 22)

Drug Discovery

Refereed Research Papers

Atkin, J., Halova, L., Ferguson, J., Hitchin, J.R., Lichawska-Cieslar, A., Jordan, A.M., Pines, J., Wellbrock, C., and Petersen, J. (2014)
Torin1-mediated TOR kinase inhibition reduces Wee1 levels and advances mitotic commitment in fission yeast and HeLa cells. ***J Cell Sci*** *127,* 1346-1356.

Wiseman, D.H., Small, H.F., Wilks, D.P., Waddell, I.D., Dennis, M.W., Ogilvie, D.J., and Somervaille, T.C. (2014)
Elevated plasma 2-hydroxyglutarate in acute myeloid leukaemia: association with the IDH1 SNP rs11554137 and severe renal impairment. ***Br J Haematol*** *166,* 145-148.

Other publications

Jordan, A.M., Waddell, I.D., and Ogilvie, D.J. (2014)
Rethinking 'academic' drug discovery: the Manchester Institute perspective. ***Drug Discov Today***. doi: 10.1016/j.drudis.2014.12.011.

Mould, D.P., McGonagle, A.E., Wiseman, D.H., Williams, E.L., and Jordan, A.M. (2014)
Reversible Inhibitors of LSD1 as Therapeutic Agents in Acute Myeloid Leukemia: Clinical Significance and Progress to Date. ***Medicinal Research Reviews***. doi: 10.1002/med.21334.

Nullin Divecha[1]

Inositide Laboratory

[1]Now at University of Southampton

Refereed Research Papers

Gelato, K.A., Tauber, M., Ong, M.S., Winter, S., Hiragami-Hamada, K., Sindlinger, J., Lemak, A., Bultsma, Y., Houliston, S., Schwarzer, D., Divecha, N., Arrowsmith, C.H., and Fischle, W. (2014)
Accessibility of different histone H3-binding domains of UHRF1 is allosterically regulated by phosphatidylinositol 5-phosphate. ***Mol Cell*** *54,* 905-919.

Jones, D.R., Keune, W.J., Anderson, K.E., Stephens, L.R., Hawkins, P.T., and Divecha, N. (2014)
The hexosamine biosynthesis pathway and O-GlcNAcylation maintain insulin-stimulated PI3K-PKB phosphorylation and tumour cell growth after short-term glucose deprivation. ***FEBS J*** *281,* 3591-3608.

Jude, J.G., Spencer, G.J., Huang, X., Somerville, T.D., Jones, D.R., Divecha, N., and Somervaille, T.C. (2014)
A targeted knockdown screen of genes coding for phosphoinositide modulators identifies PIP4K2A as required for acute myeloid leukemia cell proliferation and survival. ***Oncogene***. doi: 10.1038/onc.2014.77.

Tim Somervaille (page 24)

Leukaemia Biology

Refereed Research Papers

Jude, J.G., Spencer, G.J., Huang, X., Somerville, T.D., Jones, D.R., Divecha, N., and Somervaille, T.C. (2014)
A targeted knockdown screen of genes coding for phosphoinositide modulators identifies PIP4K2A as required for acute myeloid leukemia cell proliferation and survival. ***Oncogene***. doi: 10.1038/onc.2014.77.

Wiseman, D.H., Small, H.F., Wilks, D.P., Waddell, I.D., Dennis, M.W., Ogilvie, D.J., and Somervaille, T.C. (2014)
Elevated plasma 2-hydroxyglutarate in acute myeloid leukaemia: association with the IDH1 SNP rs11554137 and severe renal impairment. ***Br J Haematol*** *166,* 145-148.

Other publications

Mould, D.P., McGonagle, A.E., Wiseman, D.H., Williams, E.L., and Jordan, A.M. (2014)
Reversible Inhibitors of LSD1 as Therapeutic Agents in Acute Myeloid Leukemia: Clinical Significance and Progress to Date. ***Medicinal Research Reviews***. doi: 10.1002/med.21334.

Richard Marais (page 26)

Molecular Oncology

Refereed Research Papers

Escuin-Ordinas, H., Atefi, M., Fu, Y., Cass, A., Ng, C., Huang, R.R., Yashar, S., Comin-Anduix, B., Avramis, E., Cochran, A.J., Marais, R., Lo, R.S., Graeber, T.G., Herschman, H.R., and Ribas, A. (2014)
COX-2 inhibition prevents the appearance of cutaneous squamous cell carcinomas accelerated by BRAF inhibitors. ***Molecular Oncology*** *8,* 250-260.

Gentien, D., Kosmider, O., Nguyen-Khac, F., Albaud, B., Rapinat, A., Dumont, A.G., Damm, F., Popova, T., Marais, R., Fontenay, M., Roman-Roman, S., Bernard, O.A., and Stern, M.H. (2014)
A common alternative splicing signature is associated with SF3B1 mutations in malignancies from different cell lineages. ***Leukemia*** *28,* 1355-1357.

Furney, S.J., Turajlic, S., Stamp, G., Thomas, J.M., Hayes, A., Strauss, D., Gavrielides, M., Xing, W., Gore, M., Larkin, J., and Marais, R. (2014)
The mutational burden of acral melanoma revealed by whole-genome sequencing and comparative analysis. ***Pigment Cell Melanoma Res*** *27,* 835-838.

Marusiak, A.A., Edwards, Z.C., Hugo, W., Trotter, E.W., Girotti, M.R., Stephenson, N.L., Kong, X., Gartside, M.G., Fawdar, S., Hudson, A., Breitwieser, W., Hayward, N.K., Marais, R., Lo, R.S., and Brognard, J. (2014)
Mixed lineage kinases activate MEK independently of RAF to mediate resistance to RAF inhibitors. ***Nat Commun*** 5:3901.

Orgaz, J.L., Pandya, P., Dalmeida, R., Karagiannis, P., Sanchez-Laorden, B., Viros, A., Albrengues, J., Nestle, F.O., Ridley, A.J., Gaggioli, C., Marais, R., Karagiannis, S.N., and Sanz-Moreno, V. (2014)
Diverse matrix metalloproteinase functions regulate cancer amoeboid migration. ***Nat Commun*** *5,* 4255.

Pedersen, M., Viros, A., Cook, M., and Marais, R. (2014)
(G12D) NRAS and kinase-dead BRAF cooperate to drive naevogenesis and melanomagenesis. ***Pigment Cell Melanoma Res*** *27,* 1162-1166.

Sanchez-Laorden, B., Viros, A., Girotti, M.R., Pedersen, M., Saturno, G., Zambon, A., Niculescu-Duvaz, D., Turajlic, S., Hayes, A., Gore, M., Larkin, J., Lorigan, P., Cook, M., Springer, C., and Marais, R. (2014)
BRAF inhibitors induce metastasis in RAS mutant or inhibitor-resistant melanoma cells by reactivating MEK and ERK signaling. ***Sci Signal*** *7,* ra30.

Smith, M.P., Sanchez-Laorden, B., O'Brien, K., Brunton, H., Ferguson, J., Young, H., Dhomen, N., Flaherty, K.T., Frederick, D.T., Cooper, Z.A., Wargo, J.A., Marais, R., and Wellbrock, C. (2014)
The Immune Microenvironment Confers Resistance to MAPK Pathway Inhibitors through Macrophage-Derived TNFalpha. ***Cancer Discov*** *4,* 1214-1229.

Turajlic, S., Furney, S.J., Stamp, G., Rana, S., Ricken, G., Oduko, Y., Saturno, G., Springer, C., Hayes, A., Gore, M., Larkin, J., and Marais, R. (2014)
Whole-genome sequencing reveals complex mechanisms of intrinsic resistance to BRAF inhibition. ***Ann Oncol*** *25*, 959-967.

Viros, A., Sanchez-Laorden, B., Pedersen, M., Furney, S.J., Rae, J., Hogan, K., Ejiama, S., Girotti, M.R., Cook, M., Dhomen, N., and Marais, R. (2014)
Ultraviolet radiation accelerates BRAF-driven melanomagenesis by targeting TP53. ***Nature*** *511*, 478-482.

Other publications

Ascierto, P.A., Grimaldi, A.M., Anderson, A., Bifulco, C., Cochran, A., Garbe, C., Eggermont, A.M., Faries, M., Ferrone, S., Gershenwald, J.E., Gajewski, T.F., Halaban, R., Hodi, F., Kefford, R., Kirkwood, J.M., Larkin, J., Leachman, S., Maio, M., Marais, R., Masucci, G., Melero, I., Palmieri, G., Puzanov, I., Ribas, A., Saenger, Y., Schilling, B., Seliger, B., Stroncek, D., Sullivan, R., Testori, A., Wang, E., Ciliberto, G., Mozzillo, N., Marincola, F.M., and Thurin, M. (2014)
Future perspectives in melanoma research: meeting report from the inverted question markMelanoma Bridge inverted question mark, Napoli, December 5th-8th 2013. J ***Transl Med*** *12*, 277.

Girotti, M.R., Saturno, G., Lorigan, P., and Marais, R. (2014)
No longer an untreatable disease: how targeted and immunotherapies have changed the management of melanoma patients. ***Molecular Oncology*** *8*, 1140-1158.

Viros, A., and Marais, R. (2014)
Hooked on UVR. ***Pigment Cell Melanoma Res*** *27*, 1009-1010.

Crispin Miller (page 30)

RNA Biology

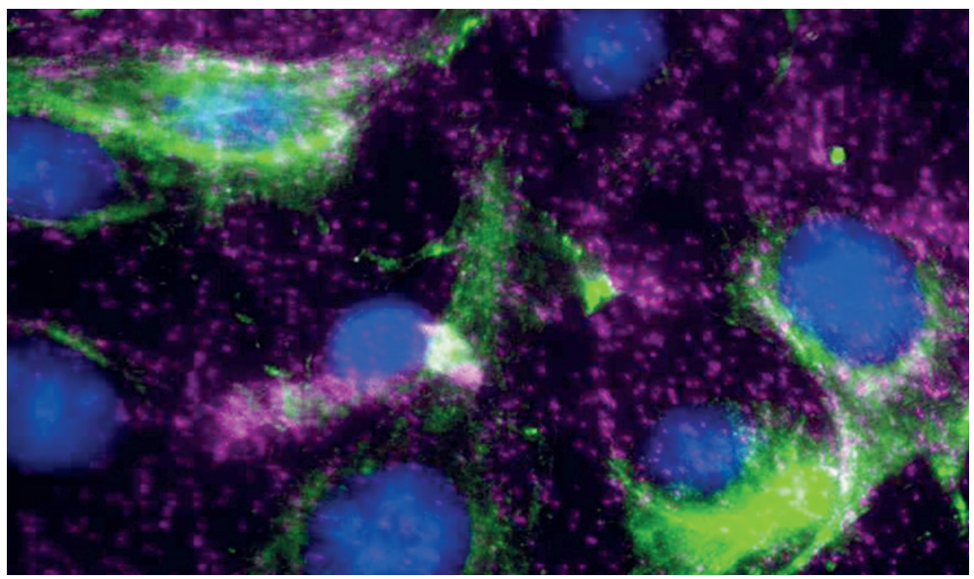
Refereed Research Papers

Bjorner, S., Fitzpatrick, P.A., Li, Y., Allred, C., Howell, A., Ringberg, A., Olsson, H., Miller, C.J., Axelson, H., and Landberg, G. (2014)
Epithelial and stromal microRNA signatures of columnar cell hyperplasia linking Let-7c to precancerous and cancerous breast cancer cell proliferation. ***PLoS One*** *9*, e105099.

Gozdecka, M., Lyons, S., Kondo, S., Taylor, J., Li, Y., Walczynski, J., Thiel, G., Breitwieser, W., and Jones, N. (2014)
JNK Suppresses Tumor Formation via a Gene-Expression Program Mediated by ATF2. ***Cell Rep*** *9*, 1361-1374.

Hall, J.S., Iype, R., Senra, J., Taylor, J., Armenoult, L., Oguejiofor, K., Li, Y., Stratford, I., Stern, P.L., O'Connor, M.J., Miller, C.J., and West, C.M. (2014)
Investigation of radiosensitivity gene signatures in cancer cell lines. ***PLoS One*** *9*, e86329.

Hodgkinson, C.L., Morrow, C.J., Li, Y., Metcalf, R.L., Rothwell, D.G., Trapani, F., Polanski, R., Burt, D.J., Simpson, K.L., Morris, K., Pepper, S.D., Nonaka, D., Greystoke, A., Kelly, P., Bola, B., Krebs, M.G., Antonello, J., Ayub, M., Faulkner, S., Priest, L.,



Accumulation of ubiquitylated TIAM1 at cell-cell adhesions in the presence of the cellular scattering factor HGF. Cells are stained with DAPI (blue) to mark the nuclei and pink dots represent ubiquitylated TIAM1. *Image supplied by Lynsey Vaughan (Cell Signalling).*

Carter, L., Tate, C., Miller, C.J., Blackhall, F., Brady, G., and Dive, C. (2014)
Tumorigenicity and genetic profiling of circulating tumor cells in small-cell lung cancer. ***Nature Medicine*** *20*, 897-903.

Hudson, A.M., Yates, T., Li, Y., Trotter, E.W., Fawdar, S., Chapman, P., Lorigan, P., Biankin, A., Miller, C.J., and Brognard, J. (2014)
Discrepancies in Cancer Genomic Sequencing Highlight Opportunities for Driver Mutation Discovery. ***Cancer Res***. 74(22):6390-6.

Leong, H.S., Dawson, K., Wirth, C., Li, Y., Connolly, Y., Smith, D.L., Wilkinson, C.R., and Miller, C.J. (2014)
A global non-coding RNA system modulates fission yeast protein levels in response to stress. ***Nat Commun*** *5*, 3947.

Lie-A-Ling, M., Marinopoulou, E., Li, Y., Patel, R., Stefanska, M., Bonifer, C., Miller, C., Kouskoff, V., and Lacaud, G. (2014)
RUNX1 positively regulates a cell adhesion and migration program in murine hemogenic endothelium prior to blood emergence. ***Blood*** *124*, e11-20.

Rothwell, D.G., Li, Y., Ayub, M., Tate, C., Newton, G., Hey, Y., Carter, L., Faulkner, S., Moro, M., Pepper, S., Miller, C., Blackhall, F., Bertolini, G., Roz, L., Dive, C., and Brady, G. (2014)
Evaluation and validation of a robust single cell RNA-amplification protocol through transcriptional profiling of enriched lung cancer initiating cells. ***BMC Genomics*** *15*, 1129.

John Brognard (page 32)

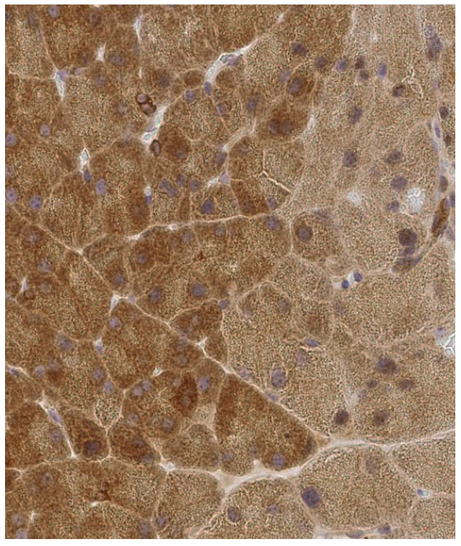
Signalling Networks in Cancer

Refereed Research Papers

Hudson, A.M., Yates, T., Li, Y., Trotter, E.W., Fawdar, S., Chapman, P., Lorigan, P., Biankin, A., Miller, C.J., and Brognard, J. (2014)
Discrepancies in Cancer Genomic Sequencing Highlight Opportunities for Driver Mutation Discovery. ***Cancer Res***. Nov 15;74(22):6390-6. doi: 10.1158/0008-5472.

Marusiak, A.A., Edwards, Z.C., Hugo, W., Trotter, E.W., Girotti, M.R., Stephenson, N.L., Kong, X., Gartside, M.G., Fawdar, S., Hudson, A., Breitwieser, W., Hayward, N.K., Marais, R., Lo, R.S., and Brognard, J. (2014)
Mixed lineage kinases activate MEK independently of RAF to mediate resistance to RAF inhibitors. ***Nat Commun*** *5*:3901.

Reyes, G., Niederst, M., Cohen-Katsenelson, K., Stender, J.D., Kunkel, M.T., Chen, M., Brognard, J., Sierecki, E., Gao, T., Nowak, D.G., Trotman, L.C., Glass, C.K., and Newton, A.C. (2014)



Section of kidney stained for Tiam1. *Images supplied by Lynsey Vaughan (Cell Signalling).*

Pleckstrin homology domain leucine-rich repeat protein phosphatases set the amplitude of receptor tyrosine kinase output. ***Proc Natl Acad Sci U S A*** *111*, E3957-3965.

Other Publications

Belot A, Brognard J, Crow YJ, Bonnefoy N. (2014)
Author Reply. ***Arthritis Rheum***. 66(1):229-30.

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)

Newton AC, GaoT, Brognard J
Compositions and Methods for Treating Diseases Associated with PHLPP. US Patent Pending 60/667,709, filed: March 31, 2006. (WO/2006/105490)

Georges Lacaud (page 34)

Stem Cell Biology

Refereed Research Papers

Batta, K., Florkowska, M., Kouskoff, V., and Lacaud, G. (2014)
Direct reprogramming of murine fibroblasts to hematopoietic progenitor cells. ***Cell Rep*** *9*, 1871-1884.

Lie-A-Ling, M., Marinopoulou, E., Li, Y., Patel, R., Stefanska, M., Bonifer, C., Miller, C., Kouskoff, V., and Lacaud, G. (2014)
RUNX1 positively regulates a cell adhesion and migration program in murine hemogenic endothelium prior to blood emergence. ***Blood*** *124*, e11-20.

Perez-Campo, F.M., Costa, G., Lie-A-Ling, M., Stifani, S., Kouskoff, V., and Lacaud, G. (2014)
MOZ-mediated repression of p16(INK) (4) (a) is critical for the self-renewal of neural and hematopoietic stem cells. ***Stem Cells*** *32*, 1591-1601.

Other Publications

Fleury, M, Perez-Campo, F, Costa, Lacaud, G, Kouskoff, V (2014)
Epigenetic and Transcriptional Mechanisms Regulating the Development of the Haematopoietic System in Mammals. Springer Book Series: ***Epigenetics and Human Health***, XIII, p 67-93.ISBN 978-3-642-45198-0

Stefanska, M, Kouskoff, V, Lacaud, G (2014)
Embryonic Stem Cell Differentiation: A Model System to Study Embryonic Haematopoiesis. ***Pluripotent Stem Cell Biology - Advances in Mechanisms, Methods and Models***, ISBN 978-953-51-1590-8.

Valerie Kouskoff (page 36)

Stem Cell Haematopoiesis

Refereed Research Papers

Batta, K., Florkowska, M., Kouskoff, V., and Lacaud, G. (2014)
Direct reprogramming of murine fibroblasts to hematopoietic progenitor cells. ***Cell Rep*** 9, 1871-1884.

Lie-A-Ling, M., Marinopoulou, E., Li, Y., Patel, R., Stefanska, M., Bonifer, C., Miller, C., Kouskoff, V., and Lacaud, G. (2014)
RUNX1 positively regulates a cell adhesion and migration program in murine hemogenic endothelium prior to blood emergence. ***Blood*** *124*, e11-20.

Perez-Campo, F.M., Costa, G., Lie-A-Ling, M., Stifani, S., Kouskoff, V., and Lacaud, G. (2014)
MOZ-mediated repression of p16(INK) (4) (a) is critical for the self-renewal of neural and hematopoietic stem cells. ***Stem Cells*** *32*, 1591-1601.

Other Publications

Fleury, M, Perez-Campo, F, Costa, Lacaud, G, Kouskoff, V (2014)
Epigenetic and Transcriptional Mechanisms Regulating the Development of the Haematopoietic System in Mammals. Springer Book Series: ***Epigenetics and Human Health***, XIII, p 67-93.ISBN 978-3-642-45198-0

Stefanska, M, Kouskoff, V, Lacaud, G (2014)
Embryonic Stem Cell Differentiation: A Model System to Study Embryonic Haematopoiesis. ***Pluripotent Stem Cell Biology - Advances in Mechanisms, Methods and Models***, ISBN 978-953-51-1590-8.

Claus Jorgensen (page 38)

Systems Oncology

Refereed Research Papers

Tape, C.J., Norrie, I.C., Worboys, J.D., Lim, L., Lauffenburger, D.A., and Jorgensen, C. (2014)
Cell-specific labeling enzymes for analysis of cell-cell communication in continuous co-culture. ***Molecular & Cellular Proteomics*** *13*, 1866-1876.

Tape, C.J., Worboys, J.D., Sinclair, J., Gourlay, R., Vogt, J., McMahon, K.M., Trost, M., Lauffenburger, D.A., Lamont, D.J., and Jorgensen, C. (2014)
Reproducible Automated Phosphopeptide Enrichment Using Magnetic TiO2 and Ti-IMAC. ***Anal Chem*** *86*, 10296-10302.

Worboys, J.D., Sinclair, J., Yuan, Y., and Jorgensen, C. (2014)
Systematic evaluation of quantotypic peptides for targeted analysis of the human kinome. ***Nat Methods*** *11*, 1041-1044.

Michela Garofalo (page 40)

Transcriptional Networks in Lung Cancer

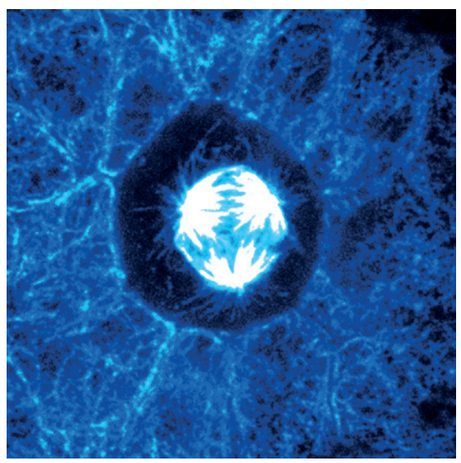
Other Publications

Di Leva G, Garofalo M (2014)
Non-coding RNAs and Cancer: ***Oncogenes and Cancer***. Yahwardiah Siregar (Ed.)

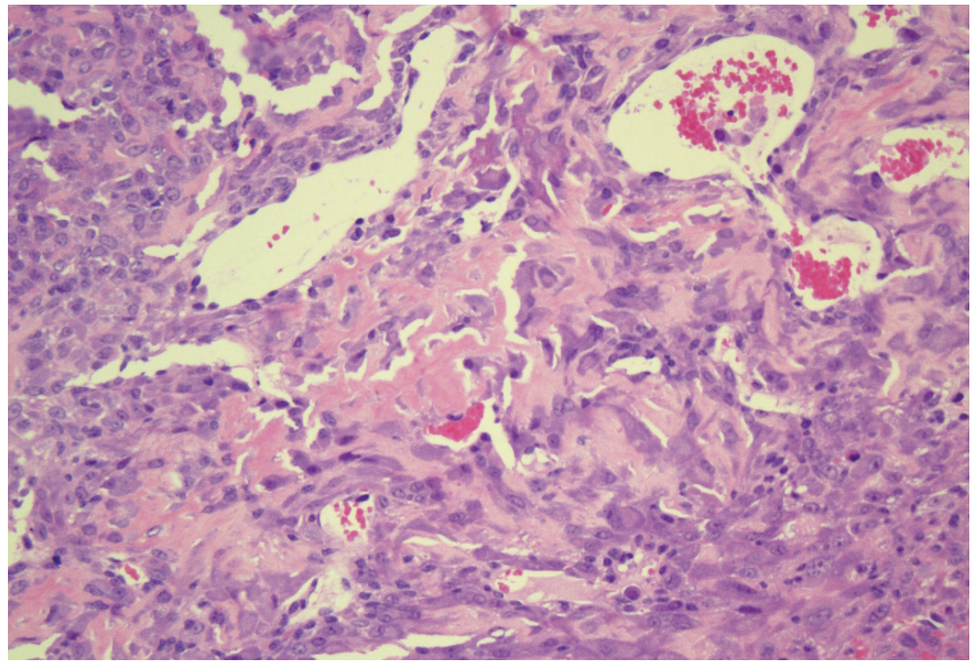
Di Leva G, Garofalo M (2014)
MicroRNAs and solid tumors: ***MicroRNAs, Key Regulators of Oncogenesis***. Babashah, Sadegh (Ed.)

Joshi P, Middleton J, Jeon YJ, Garofalo M. (2014)
MicroRNAs in lung cancer. ***World J Methodol*** 4(2):59-72.

Volinia S, Nuovo G, Drusco A, Costinean S, Abujarour R, Desponts C, Garofalo M, Baffa R,



Mitotic MDCK II cells in 2D– one of which is abnormal and tripolar. *Image supplied by Andrew Porter (Cell Signalling).*



Osteogenic sarcoma developing in a mouse model. *Image supplied by Amaya Viros (Molecular Oncology).*

Aeqilan R, Maharry K, Sana ME, Di Leva G, Gasparini P, Dama P, Marchesini J, Galasso M, Manfrini M, Zerbinati C, Corrà F, Wise T, Wojcik SE, Previati M, Pichiorri F, Zanesi N, Alder H, Palatini J, Huebner KF, Shapiro CL, Negrini M, Vecchione A, Rosenberg AL, Croce CM, Garzon R. (2014)
Pluripotent stem cell miRNAs and metastasis in invasive breast cancer. ***J Natl Cancer Inst*** 106(12):dju324

Additional Institute Publications

Boname, J.M., Bloor, S., Wandel, M.P., Nathan, J.A., Antrobus, R., Dingwell, K.S., Thurston, T.L., Smith, D.L., Smith, J.C., Randow, F., and Lehner, P.J. (2014)
Cleavage by signal peptide peptidase is required for the degradation of selected tail-anchored proteins. ***J Cell Biol*** *205*, 847-862.

Cheadle, E.J., Sheard, V., Rothwell, D.G., Bridgeman, J.S., Ashton, G., Hanson, V., Mansoor, A.W., Hawkins, R.E., and Gilham, D.E. (2014)
Differential role of Th1 and Th2 cytokines in autotoxicity driven by CD19-specific second-generation chimeric antigen receptor T cells in a mouse model. ***Journal of Immunology*** *192*, 3654-3665.

Chicooree, N., Unwin, R.D., and Griffiths, J.R. (2014)
The application of targeted mass spectrometry-based strategies to the detection and localization of post-translational modifications. ***Mass Spectrometry Reviews***. doi: 10.1002/mas.21421.

Griffiths, J.R., Chicooree, N., Connolly, Y., Neffling, M., Lane, C.S., Knapman, T., and Smith, D.L. (2014)
Mass spectral enhanced detection of Ubls using SWATH acquisition: MEDUSA-simultaneous quantification of SUMO and ubiquitin-derived isopeptides. ***J Am Soc Mass Spectrom*** *25*, 767-777.

Jongjitwimol, J., Feng, M., Zhou, L., Wilkinson, O., Small, L., Baldock, R., Taylor, D.L., Smith, D., Bowler, L.D., Morley, S.J., and Watts, F.Z. (2014)
The S. pombe translation initiation factor eIF4G is Sumoylated and associates with the SUMO protease Ulp2. ***PLoS One*** *9*, e94182.

Lamb, R., Harrison, H., Hulit, J., Smith, D.L., Lisanti, M.P., and Sotgia, F. (2014)
Mitochondria as new therapeutic targets for eradicating cancer stem cells: Quantitative proteomics and functional validation via MCT1/2 inhibition. ***Oncotarget*** *5*, 11029-11037.

THESES

Kyaw Aung

Kyaw Aung
CEP

The utility of mutations in circulating free DNA as potential predictive biomarkers for mechanism based therapeutics in cancer treatment

Marija Maric

Marija Maric
Cell Cycle

The mechanism of CMG helicase disassembly during the completion of eukaryotic chromosome replication

Eva Barkauskaite

Eva Barkauskaite
DNA Damage Response

The structure and the catalytic mechanism of Poly(ADP-Ribose) Glycohydrolases (PARGS)

Elli Marinopoulou

Elli Marinopoulou
Stem Cell Biology

The transcriptional programme controlled by RUNX1 during blood development

Sara Cuvertino

Sara Cuvertino
Stem Cell Haematopoiesis

A potential role for SOX7 in leukaemogenesis

Francisa Marti Marti

Francisa Marti Marti
CEP

Qualification of a panel of angiogenesis biomarkers in patients with colorectal cancer

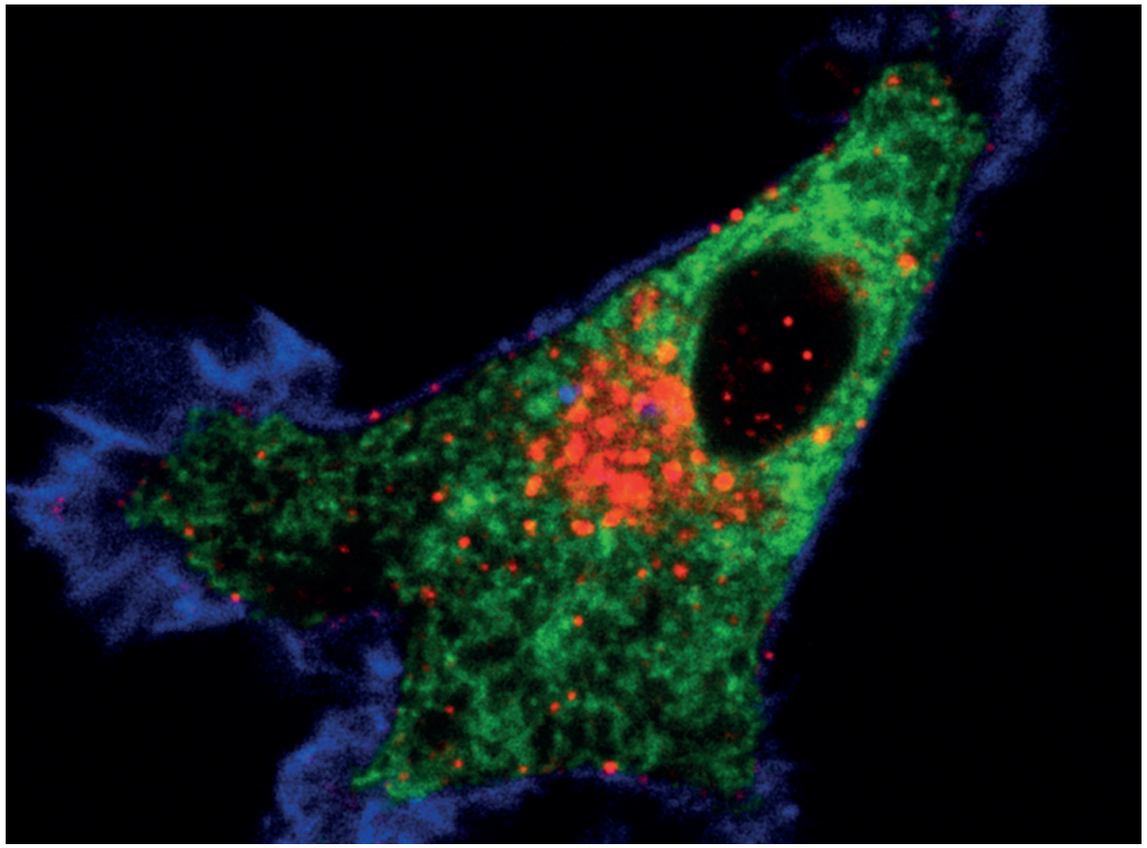
Rebecca Foulger

Rebecca Foulger
Inositide Laboratory

Investigating the production and function of oxidative stress induced PtdIns5P

Hadir Marei

Hadir Marei
Cell Signalling
Selection of Rac1 binding partners by Guanine Nucleotide exchange factors



A cultured breast cancer cell labelled with an ER marker (green) and an endosome marker (red). Actin is stained blue.

Image supplied by Haoran Tang (Molecular Oncology).

EXTERNAL SEMINAR SPEAKERS 2014

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. In its sixth year, The Breakthrough Breast Cancer 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.

David Adams
The Wellcome Trust Sanger Institute

Catherine Alix-Panabières
University Medical Center of Montpellier

Kurt Anderson
CRUK Beatson Institute

Alberto Bardelli
University of Torino

David Barford
MRC Laboratory of Molecular Biology

Eduard Batlle
Institute for Research in Biomedicine (IRB Barcelona)

Salvador Aznar Benitah
Institute for Research in Biomedicine (IRB Barcelona)

Jan Cools
VIB Centre of the Biology of Disease

Vincenzo D'Angiolella
University of Oxford

Patrick Eyers
Institute of Integrative Biology, University of Liverpool

Simón Méndez Ferrer
Centro Nacional de Investigaciones Cardiovasculares

Brian Gabrielli
The University of Queensland Diamantina Institute

Holger Gerhardt
CRUK London Research Institute

Nick Gilbert
MRC Human Genetics Unit MRC IGMM

Olaf Heidenreich
Northern Institute of Cancer Research

Shane Herbert
University of Manchester

Paul Huang
Institute of Cancer Research, London

Peter Kuhn
The Scripps Research Institute

Tony Ng
King's College London & University College London

Rick Pearson
Peter MacCallum Cancer Centre

Jordan Raff
Sir William Dunn School of Pathology

Emanuela Romano
University Hospital of Lausanne

Charles Rudin
Memorial Sloan Kettering Cancer Center

Tomas Stopka
Institute of Molecular Genetics ASCR

Kjetil Tasken
The Biotechnology Centre of Oslo

Simon Wilkinson
Edinburgh Cancer Research Centre

Santiago Zelenay
CRUK London Research Institute

Eric Lam
Imperial College

Michael Lewis
Baylor College of Medicine

Val Speirs
University of Leeds

Leonie Young
Breast Cancer Ireland

Breakthrough Breast Cancer Research Unit Seminar Series 2014

Carlos Caldas
CRUK Cambridge Institute

Judith Campisi
Buck Institute for Research on Aging

Jason Carroll
CRUK Cambridge Institute

Mitch Dowsett
Royal Marsden Hospital

Montse Garcia-Closes
Institute of Cancer Research

Mien-Chie Hung
The University of Texas, M.D. Anderson Cancer Center

POSTGRADUATE EDUCATION

www.cruk.manchester.ac.uk/education

Julie Edwards
Postgraduate
Education Manager

Ian Waddell
Postgraduate Tutor

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 talented students and clinical research fellows the opportunity to study for cancer-related PhD or MD degrees. 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 students in the past eight years have found employment after graduation; half of these are in American or European laboratories, while 20% continue to progress in their clinical careers in the NHS. Students leave the CRUK MI with excellent career prospects across the world.

In 2014, we welcomed five graduate students, including one Clinical Research Fellow 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, eight of the Institute's publications had students as first authors in journals as diverse as *Science, British Journal of Haematology and Oncology.* During the course of this year, a total of five PhD students and three Clinical Fellows were awarded their PhDs.

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 the course of their studies via 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 that are so fundamental to the majority of careers in science and elsewhere. Graduate training is monitored by an Education Committee, which features the Institute's Group Leaders, the Chief Operating Officer and student representatives (see below). A main supervisor and a second or co-supervisor is nominated for each student, who is 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, while 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 all of these external seminars. The speakers are internationally renowned scientists and we consider it essential that our students are exposed to outstanding work 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 that the students attend. 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 annual CRUK Manchester Institute Colloquium, held 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 2014, Danish Memon from the RNA Biology Group was the recipient of the Lizzy Hitchman Prize for his work describing the global transcriptomic changes in response to hypoxia. From analysis of gene expression data, Danish has identified a novel non-coding transcript called HINCR1 (Hypoxia Induced Non Coding RNA 1) which is prognostic of survival in colorectal cancer patients. With the help of collaboration with CEP group, Danish and Keren Dawson (Senior Scientific Officer in the RNA Biology group) are now investigating the mechanism of action of HINCR1 in hypoxia.

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 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 over a two-day period in early January.

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

Fellowships in Clinical Pharmacology Research

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

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

Education Committee 2014

The Education Committee (EC) acts for postgraduate students based within CRUK core-funded research groups and consists of Group Leaders, the Chief Operating Officer, the Postgraduate Tutor and the Postgraduate Education Manager of CRUK MI.

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 of the PhD programme. Such assessments help not only to monitor progress, but also help to develop performance and presentations skills.

Valerie Kouskoff
(Chair, Education Committee)

Julie Edwards
Angeliki Malliri
Richard Marais - Ex-Officio Member
Donald Ogilvie
Tim Somervaille
Ian Waddell - Postgraduate Tutor
Caroline Wilkinson

Student Representatives

Danish Memon (until September)
Alekh Thapa
Emma Williams (from September)

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 Estates and Logistics, fall under the leadership of Margaret Lowe while Stuart Pepper oversees IT as well as 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 helped our three new Junior Group Leaders with the necessary arrangements to establish their groups and start their research programmes. Other important projects include major health and safety related audits, an overhaul of the Institute's induction process for new starters and the development of a new intranet by our web developer Tom Bolton, which will be launched in 2015. A number of team members have been involved with various operational preparations in readiness for the opening of the MCRC Building in 2015.

Director's Office and Administration Services

Ruth Perkins, Ekram Aidaros

In addition to providing administrative support to the Director, the department has assisted with the organisation of several events over the course of the year, including the Institute Colloquium, quinquennial reviews and a public engagement event to mark the launch of the Belfast-Manchester Movember Centre of Excellence. Administrative support is provided for the external seminar series, which has been a great success in 2014. We aim to provide a varied programme of national and international speakers, serving to foster collaboration and encourage positive interaction within the wider scientific community. Details can be found at www.cruk.manchester.ac.uk/seminars.

Estates

Steve Alcock, Graham Hooley, Lewis Parkinson, Tony Woollam

The Estates team has enjoyed a busy year in 2014 with several significant challenges having been met.

They have completed a number of small schemes including reconfiguring an area to accommodate a new laboratory for the Histology facility. In addition, they have converted two offices into laboratory accommodation for the Advanced Imaging facility to house the gated STED microscope.

The Estates team endeavours to identify sustainable solutions when putting new schemes together to help reduce the Institute's carbon footprint whenever possible. One of the major users of electrical power in the building is the chiller in the Biological Resources Unit, which runs continuously. The team has put in place a restrictive controls' schedule which has reduced the electrical usage by over 30% while maintaining its desired function.

Many legislative requirements such as Legionella best practices and fire alarm testing have been attended to throughout the year and team members performed relevant courses to improve their skills and keep their knowledge up to date with current working practices and changing legislation. Two such courses were *Authorised Person* refresher training for Piped medical gases and a mechanical to electrical conversion course.

Finance & Purchasing

Margaret Lowe, Neepa Begum, David Jenkins, Denise Owen, Muhammad Raja, Debbie Trunkfield

The Institute Finance Team supports the Director with the management of the Institute's £21m 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 University upgraded the Oracle system in 2013. This has involved close liaison with the Project Team to ensure as little disruption as possible within the Institute. There have inevitably been a few minor issues but the effect on the scientists has been minimal.

The Institute has been successful in securing several new external grant awards that were activated in 2014 and 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 and administering existing grants.

Towards the end of 2012, we were advised that an application to UK Research Partnership Investment Fund (UKRPIF) had been successful. This has resulted in the purchase of several significant pieces of equipment during 2014.

Health & Safety

Colin Gleeson

In late 2103, a survey of airborne allergen levels in the Biological Resources Unit (BRU) revealed that only the cage cleaning area exhibited levels of concern. All the other areas were below the limits of detection. Subsequently in January 2014, with BRU management approval, procedural changes were made in the cage cleaning area to try and minimise exposure to laboratory animal allergens (LAA). After six months the area was re-surveyed. This revealed that the airborne allergen levels had greatly reduced and that the procedural changes made were effective at enhancing control and reducing the risk of exposure to LAA.

In February, the BRU was inspected by the Health and Safety Executive Biological Agents Unit Regulatory Compliance Officer. The inspection included a wide scope review of our paperwork and procedures in the unit. This was followed by an inspection of the facility. It was pleasing that the Inspector found no issues of concern and could not make any recommendations to improve procedures. No actions of any sort resulted from this inspection.

A building-wide fire risk assessment and asbestos survey was undertaken in conjunction with the University's Estates team. This harmonised the procedures and paperwork with current University systems. The fire risk assessment revealed that some remedial work was necessary to improve fire safety; this work has almost been completed. Some areas of the building were found to contain asbestos; work to remove it is underway.

In summer, hands-on fire extinguisher training sessions were organised. Some 55 members of staff received training and put out fires using a range of extinguishers. Once again this received very positive feedback from the participants and forms an important part of fire safety training.

Also in the summer, a Portable Appliance Testing (PAT) programme was undertaken across the building. Some 10000 items were tested over a period of several weeks. This helps to ensure the electrical safety of our plug-in electrical equipment. As usual, our fume cupboards and safety cabinets underwent

statutory-required annual testing and examination to ensure they perform effectively.

Towards the end of the year a series of compliance exercises were run and these included checks on stocks held of chemical weapons, desensitised explosives, dangerous pathogens and toxins, Euratom materials and radioactive waste disposal records. The building-wide data was fed back to the University Compliance Officer.

Human Resources

Rachel Powell, Laura Jones, Julie Jarratt, David Stanier[1]

[1] joint with administration

Over the past year, the HR Department has continued to successfully deliver a high quality proactive service to the Institute. 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 2014, 68 individuals were successfully appointed who will complement and enhance the work of the Institute. This is an increase of 35% compared to last year, and includes the appointment of four Junior Group Leaders which emphasises the continued growth and development of CRUK MI. Recruitment of new groups will continue into 2015 in line with the Institute's strategic objectives.

The department has supported the Institute through the restructuring of several core facilities to ensure that they are fit for purpose due to the expansion of CRUK MI and the new MCRC building. We have continued our joint partnership working with the union which has resulted in the revision of many policies such as the Recruitment and Selection Policy, Flexible Working Policy, and the Probation Policy.

Next year, we will be developing and launching a new in house online performance review system and working towards the Athena Swan accreditation.

Information Technology

Malik Pervez[1], Hong Mach, Brian Poole, Steve Royle, Matthew Young

[1] left in 2014

The CRUK Manchester Institute IT team has continued to provide a wide range of IT services to the high standard expected by our service users, who now number in excess of 400 researchers and service providers.

During 2014, we have built a closer working relationship with the University of Manchester's IT Services team to plan network connectivity into the new MCRC building and the startup and ongoing support of its new wave of cancer researchers. In anticipation of this increase in service users we have also been developing, and have now implemented, new systems to facilitate the automated setup of new desktops and mobile devices.

The technology we use and depend on every day is expanding and developing at a phenomenal rate, and this year has seen huge growth in the use of mobile devices – phones, phablets and tablets. In particular, 2014 has seen significant growth in the use of, and our support provision for, Apple products. We now offer a full support service for Apple OSX & IOS users.

We are currently hosting a new High Performance Computing cluster prior to its planned move into a purpose built facility. We are also working closely with the Scientific Computing team and advising on datacentre and network design relating to their new facility.

Planning commenced in 2014 for significant IT infrastructure upgrades in 2015. This is to replace legacy network and server infrastructure.

Logistics

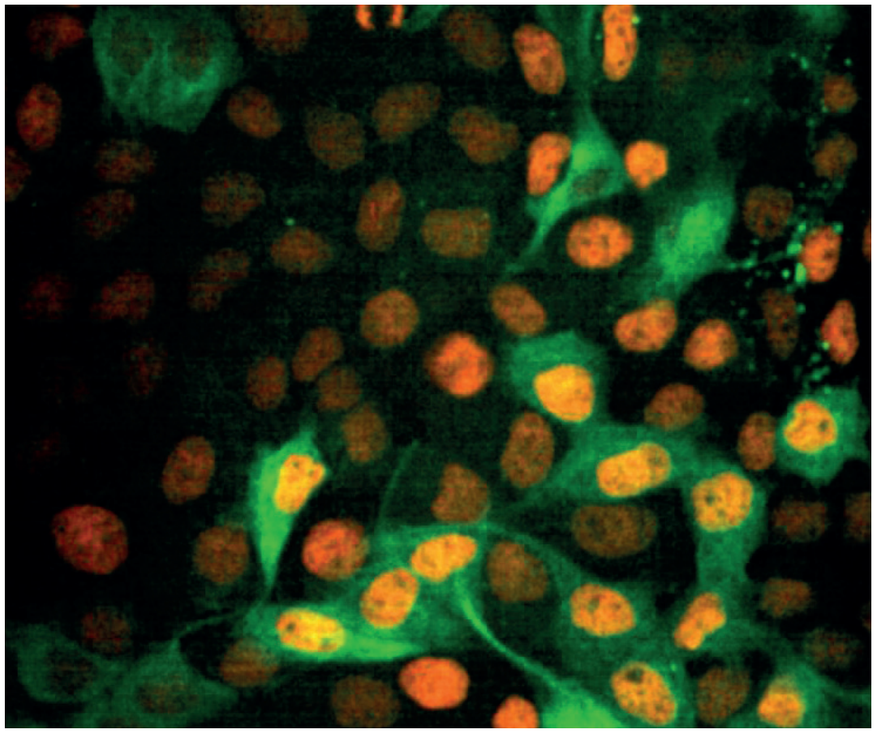
Maurice Cowell[1], Andrew Lloyd, Michael Alcock, Edward Fitzroy, Sedia Fofana, Stephen Keane, Jonathan Lloyd

[1] left in 2014

The past year has seen a change of team personal with the retirement of Maurice Cowell and with the arrival of Eddie Fitzroy and Michael Alcock.

The team has continued to provide 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 including cardboard, plastic bottles, tin cans, wooden crates and pallets, ink toners, and scrap metal which reduces the amount of material going to land fill.

Some larger liquid nitrogen vessels are now in operation, increasing the usage of nitrogen and its transportation to the laboratories. During 2014, the Institute used approximately 2000 litres of liquid nitrogen in storage vessels each



Live image obtained using the Opera Phenix of MDCK cells expressing GFP-Tubulin and RFP-Histone-H2B. *Image supplied by Andrew Porter (Cell Signalling).*

week. The Logistics team also provides a dry ice service with deliveries taking place twice a week. Gas cylinders are also monitored and replaced as necessary and in June, all of the Institute's gas manifolds were replaced, during which we were able to combine some of the manifolds which proved to be more cost effective as we were able to cut down on our cylinder holding stock, thus decreasing the rental charges.

Researchers can order central stores stock items via the intranet which can be collected or distributed by the Logistics team. We currently stock over 100 stores items from tissue culture reagents to cleaning products. Included in this system are the enzymes and media stored in the Institute freezers (Sigma, Invitrogen, Roche, Promega, New England Biolabs, Fisher 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. In 2014, we managed to save £12,000 on precast gels. We have also 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. Logistics also look after the Institute's "Shred It" service and water coolers.

Scientific Operations

Caroline Wilkinson, Tom Bolton[1], Gillian Campbell

[1] joint with MCRC

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 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 treble including successful applications to the MRC, the Roy Castle Lung Cancer Foundation, The Lung Cancer Research Foundation and the Moulton Charitable Foundation.

A major undertaking this year has been to produce, in-house, a new intranet to serve occupants of both the Paterson and MCRC buildings. This is due to be launched in early 2015.

Cancer Research Technology

Martyn Bottomley

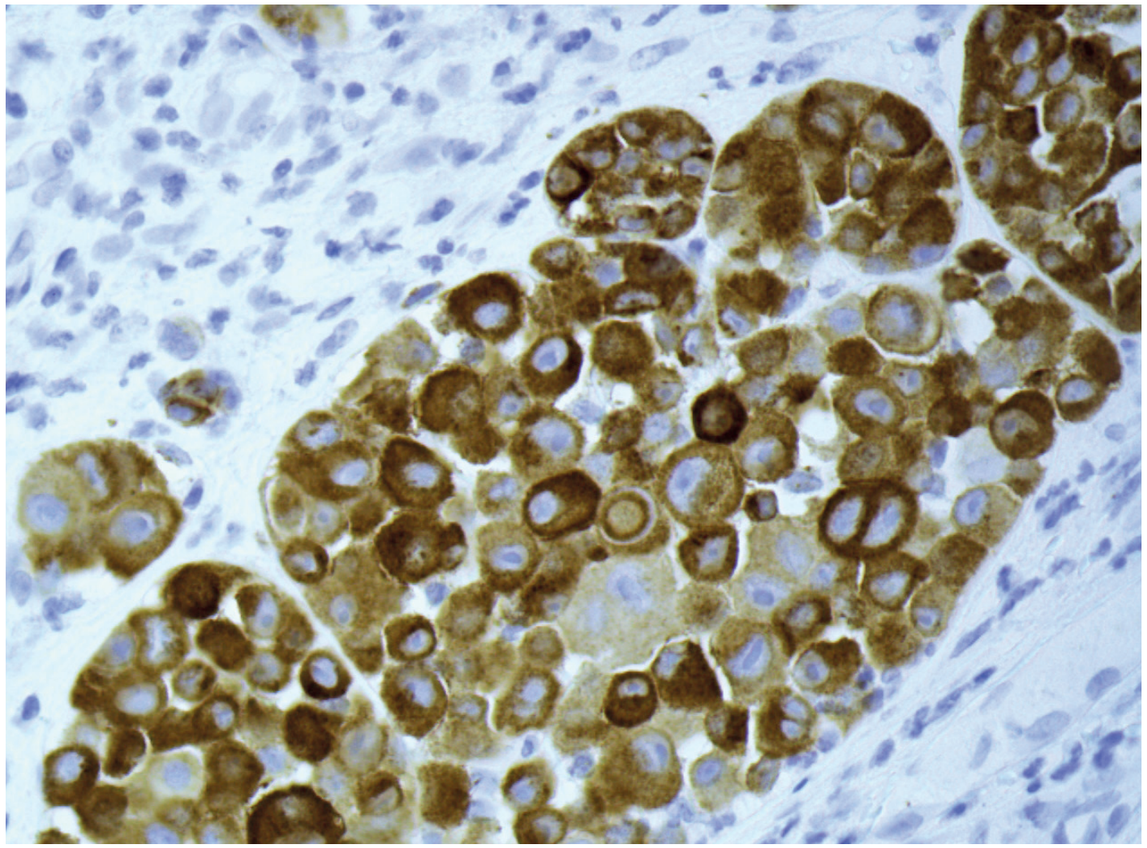
Cancer Research Technology (CRT) is a specialist oncology-focused development and commercialisation company wholly owned by Cancer Research UK. CRT aims to maximise patient benefit from publicly funded research worldwide by advancing research discoveries into development with pharmaceutical and biotechnology parties.

At CRT we bridge the fundamental 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 now also have access to the CRT Pioneer Fund; this £70m fund has been established with Cancer Research Technology (CRT), 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). Our relationship with the Cancer Research UK Manchester Institute reflects the specific requirements of the scientist, Cancer Research UK Manchester Institute, Cancer Research UK and the individual project. To effectively facilitate these requirements and interactions, 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 molecules drug therapies to satisfy the unmet clinical needs of cancer patients. In the year to date we have negotiated additional funding for Drug Discovery Unit to accelerate the development of RET inhibitors and fund early clinical trials of potential drugs developed through collaboration. We have also facilitated the filing 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.



Melanoma cells from a human patient derived xenograft, stained with Hmb45, surrounded by unstained mouse stroma cells.

Image supplied by Amaya Viros (Molecular Oncology).

CANCER RESEARCH UK'S RESEARCH ENGAGEMENT

Cancer Research UK's Research Engagement Manager

Hannah Leaton[1]

[1]joined in 2014

Public engagement remained an important part of the Institute's work during 2014. We welcomed a record number of visitors for lab tours, and our researchers brought their science to life for thousands of people at external events.

Over 1300 people visited the Institute for lab tours in 2014, including school children, Cancer Research UK Ambassadors, local businesses and fundraising committees. Lab tours help to increase knowledge of our research, whilst inspiring and motivating supporters. They are also a fantastic opportunity to thank supporters, and to show them that we're spending the money wisely.

More than 50 researchers were involved in our Institute Open Day, which became a bi-annual event due to unprecedented demand. Guests learnt about the Institute's recent successes, our important research into cancers of unmet need and our ambitious plans for the future. Their visits also included hands-on science activities, lab tours and citizen science demos.

One supporter said "I have had such an incredible day. It has been so humbling to meet such passionate and enthusiastic people, and I will definitely be continuing my support of Cancer Research UK". Another commented, "The lab tours were an inspiration! It was an incredible opportunity to see real research in action. Thank you!"

Away from the Institute, our scientists have enjoyed using creative and collaborative ways to engage the public with their research. Following the success of our involvement in last year's event, we returned to Science Spectacular, a huge family fun science fair run by Manchester Museum during Manchester Science Festival. We helped more than 300 children make cells from balloons in order to teach them about how their bodies work. Also during the Manchester Science Festival, we organised a public debate to celebrate the launch of the new Belfast-Manchester Movember Centre of Excellence, in partnership with Prostate Cancer UK. Members of the public had the chance to meet our world class scientists and to discover what we're doing to change the face of men's health.

We also took part in the 50th Annual Bollington Science Festival, where more than 200 children and their parents extracted DNA from strawberries and learnt about targeted therapies through fun hands-on activities.

Other highlights of the year's public engagement activities include our participation in an After Hours event run at Manchester Museum, a walking tour through Manchester's proud history of cancer research that was organised as part of Manchester Histories Festival, and Professor Caroline Dive's involvement in a local Pint of Science Festival.

Left: Dr Allan Jordan presenting dedicated fundraisers with a Certificate of Appreciation.
Right: Dr Caroline Wilkinson discussing the future of cancer research at a local networking event.

Left: A supporter enjoying loading gels and extracting strawberry DNA on lab tours.
Middle: Getting 'hands-on' with science at one of the Institute Open Days.
Right: A supporter looking at their own cheek cells under a microscope during Manchester Science Festival.

We also held a successful patient engagement event, which gave local patients and their families the chance to have their say about the future of Cancer Research UK.

Several labs have taken part in 'virtual' engagement activities, enabling us to share our science with an even wider audience. To mark the launch of a new Cancer Research UK brand campaign, Dr Jonathan Tugwood and Dr Allan Jordan were filmed for a nationwide advert explaining why they believe we will beat cancer sooner. Professor Caroline Dive also stepped in front of the camera to explain her research into lung cancer in 60 seconds, and a number of researchers from Drug Discovery, Cell Signalling and Cell Regulation filmed videos for Cancer Research UK supporters.

As ever, Institute staff members have been working hard to raise funds for charity. A team of 23 brave members of the Institute took on the challenge of walking or running 40 miles from Keswick to Barrow in the Lake District National Park, whilst a number of staff members grew moustaches and took part in lunchtime yoga sessions in support of Movember. We had plants sales, a group 'make-up selfie' and supported Stand Up to Cancer with the return of the Science Cakes competition and David Jenkins' lucky dip. Congratulations must also go to the team of scientists from the Institute who took part in this year's Stockport Relay for Life, walking alongside survivors and local supporters for 24 hours straight. Despite the rain, our team of Relayers enjoyed being able to share our science with the public.

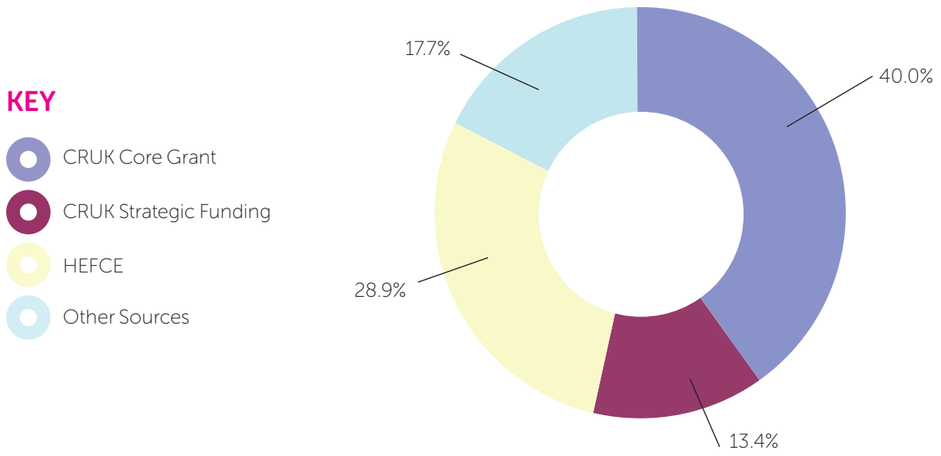
In 2015 we hope to build on our successes. In addition to our programme of lab tours, we hope to reach new audiences with more public events, and are looking forward to holding a debate about the future of treating lung cancer in the early part of the year. Excitingly, we also plan to expand our virtual engagement activity. All staff involved in public engagement at the Institute agree that it is both important and rewarding to meet with supporters and the public, sharing the success that their support has enabled us to achieve and showing them where we hope to deliver breakthroughs in the future.

Left: The Relay for Life team of scientists from the Institute.
Right: Volunteers at Manchester Science festival.

ACKNOWLEDGEMENT FOR FUNDING OF THE CANCER RESEARCH UK MANCHESTER INSTITUTE

The total funding of the CRUK Manchester Institute for 2014 was £26m. The major source of this funding was awarded by Cancer Research UK (CR-UK) via a core grant of £10.4m plus additional strategic funding of £3.5m. This funding enables the various scientific groups to carry out their research and supports the various research services.

CRUK MANCHESTER INSTITUTE FUNDING 2014



The infrastructure of the CRUK Manchester Institute is funded by HEFCE generated income at a cost of £2.4m. An additional £5.1m from the UK Research Partnership Investment Fund (UKRPIF) was used to enhance the technological platforms in the core research facilities.

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:

- AstraZeneca
- BBSRC
- The Christie Hospital NHS Foundation Trust
- European Commission
- John Swallow Fellowship
- Kay Kendall Leukaemia Fund
- Leukaemia & Lymphoma Research Fund
- Lung Cancer Research Foundation
- Medical Research Council
- Menarini Biomarkers Singapore
- Pancreatic Cancer Research Fund
- Parsortix
- Prostate Cancer UK
- Roy Castle Lung Cancer Foundation
- Wellcome Trust
- Worldwide Cancer Research

We are immensely grateful to everyone who provides funds for our research.

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 very 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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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).
Registered address: Angel Building, 407 St John Street, London, EC1V 4AD.

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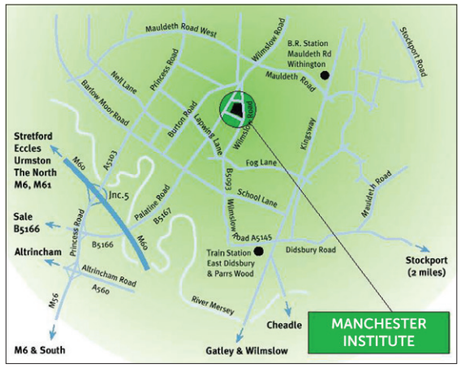

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