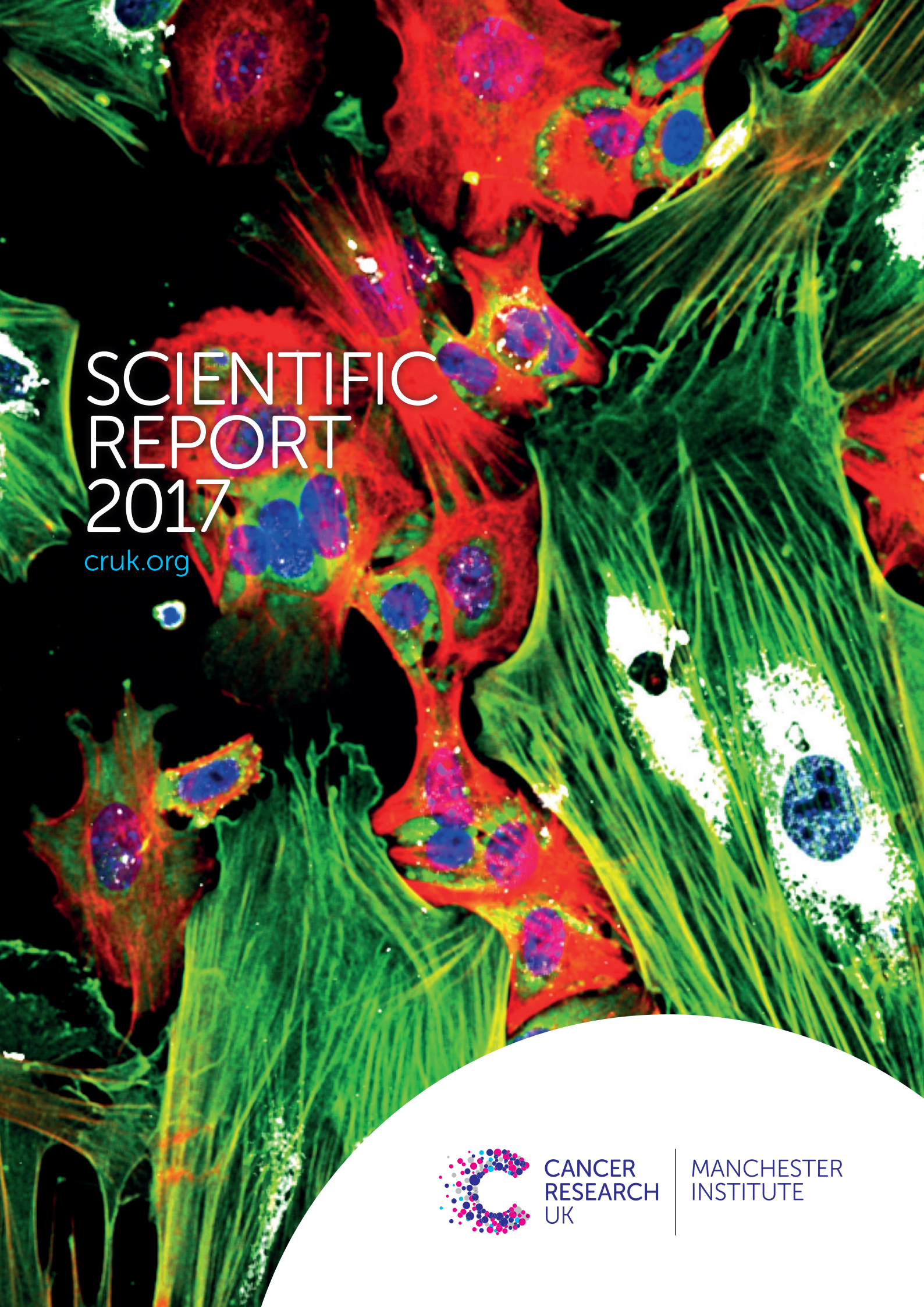

SCIENTIFIC REPORT 2017

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COVER IMAGE

Confocal photomicrograph of PyMT mouse breast cancer cells (Red) and mouse breast cancer associated fibroblasts (Green). Cells were labelled with total actin (Red), Smooth Muscle Actin (Green), Type 1 Collagen (Grey) and DAPI (Blue).

Image supplied by Haoran Tang (Molecular Oncology)

SCIENTIFIC REPORT 2017

MANCHESTER INSTITUTE

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The University of Manchester

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CONTENTS

The Cancer Research UK Manchester Institute is temporarily located at Alderley Park in Cheshire until we return to our original site in Withington. Some research groups and staff remain in the MCRC Building.

The Manchester Cancer Research Centre Building

DIRECTOR'S INTRODUCTION

Professor
Richard Marais

Director of the Cancer Research
UK Manchester Institute

The past year has been defined by two main events; the Paterson Building fire and the Institute's Quinquennial Review. On April 26th 2017, a fire broke out on the roof of the Paterson Building, which housed the majority of the CRUK Manchester Institute alongside other University of Manchester cancer researchers. Thankfully, everyone evacuated safely and no-one was hurt. However, the fire resulted in significant damage to the building's roof and plant infrastructure and the fire-fighting measures required resulted in substantial water damage throughout the building.

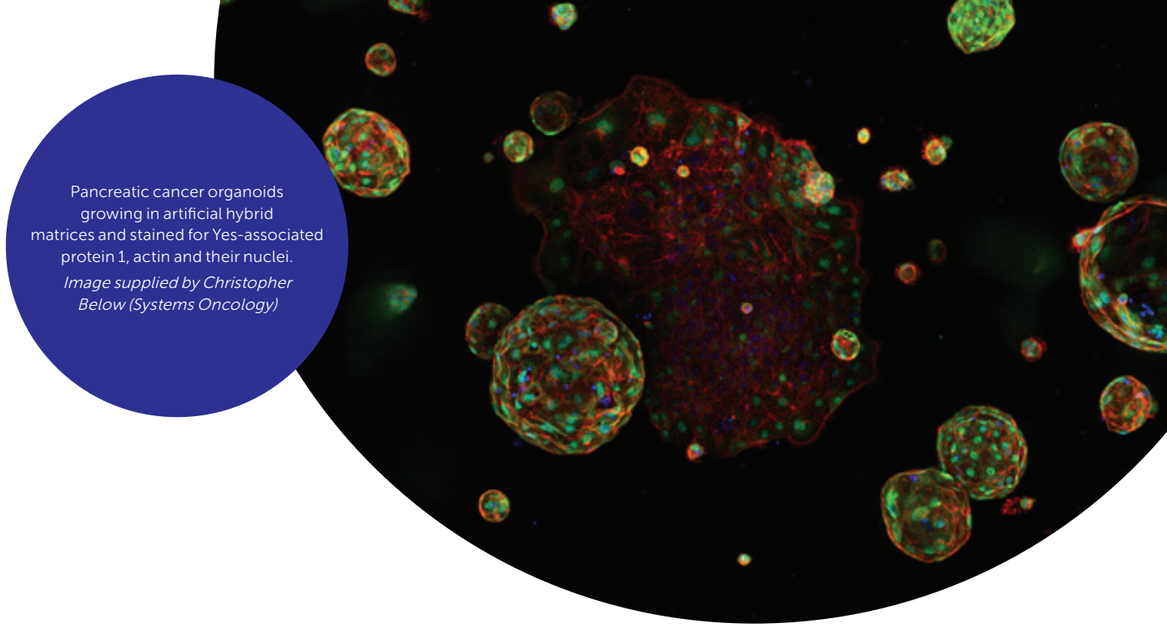
The Greater Manchester Fire and Rescue Service were outstanding, not only in tackling the blaze but also by helping with our business continuity needs; even while fighting the fire, they allowed us to move our mouse colony to The University of Manchester's main animal facility. They also helped us to recover our low temperature storage systems, including the MCRC Biobank and many of the -80°C freezers and liquid nitrogen storage tanks, thus preserving the majority of our most valuable research collections and clinical trial samples.

The remainder of the year was dominated by our efforts to clear the Paterson Building and re-house our scientists and staff. We received a great deal of help to get everyone up and running in the aftermath of the fire and I would like to thank colleagues at The University of Manchester, AstraZeneca, Alderley Park and at other institutes further afield who accommodated our staff for various periods over the last year. The recovery efforts also involved checking all of our scientific equipment and an assessment of our losses. Overall the relocation and recovery has been an enormous project for a number of the Institute's staff who, together with colleagues at The University of Manchester, have led these efforts.

Various options regarding the future of the Paterson Building have been considered since the fire with a decision being made by the Christie, The University of Manchester and Cancer Research UK to take the opportunity presented by this disaster to build a state of the art cancer research facility. The new building will host scientists and clinicians thus allowing us to build on the critical partnerships that are pivotal to discovery, translational and clinical cancer research. Replacing the Paterson Building is likely to take some time so for the intervening period, the Institute has relocated to Alderley Park. We have received a great deal of help from many organisations during this time. I would like to thank everyone who has facilitated our move.

Nine weeks after the fire, the Institute underwent its Quinquennial Review during which our progress during the past five years and our future plans were reviewed by an international panel of experts. While it was highly challenging to prepare for this event at this difficult time, I took the view that it was important to continue, because it allowed us to seek the valuable advice of the panel. It also enabled us to receive endorsement for our future strategic plan from the panel and CRUK, and to receive continued support from our local partners, The University of Manchester and the Christie NHS Foundation Trust. I was delighted with the panel's recognition of the progress that we have made in the last five years and their support for our ambitions for the next quinquennium.

Despite the disruption, there are many successes to celebrate. Highlights from 2017 include a study published in *Cancer Cell* by



Pancreatic cancer organoids growing in artificial hybrid matrices and stained for Yes-associated protein 1, actin and their nuclei.
Image supplied by Christopher Below (Systems Oncology)

Angeliki Malliri's Cell Signalling group describing a tumour suppressor role for TIAM1, an activator of the small GTPase RAC1, in colorectal cancer. The Cell Division team discovered a novel signalling dialogue, between components involved in mitotic entry and exit, which controls the commitment to mitosis. My own group reported that Lysyl oxidase (LOX) promotes tumour progression by trapping the epidermal growth factor receptor at the cell surface, and working with Caroline Springer, the new Director of the Drug Discovery Unit, we have discovered new LOX small molecule inhibitors. In another study we showed that the presence of circulating tumour DNA is predictive of relapse and overall survival in high-risk stage II/III melanoma patients who have undergone surgery with curative intent.

Claus Jørgensen was promoted to a Senior Group Leader following a highly successful Senior Appointments' process. There was further success for Claus who was awarded a European Research Council (ERC) accelerator grant to further his work on the role of the tumour microenvironment in pancreatic cancer development. Isabel Romero-Camarero received a Kay Kendall Leukaemia Fund Junior Research Fellowship for her research into haematological malignancies in the Leukaemia Biology group, led by Tim Somervaille.

A few weeks after the fire, the Clinical and Experimental Pharmacology group set up an early detection laboratory at Alderley Park to process blood samples collected from participants in the Manchester Community Lung Health Check Pilot. The study aims to assess the use of Circulating Tumour Cells (CTCs) and circulating DNA (ctDNA) in the early diagnosis of lung cancer. The rest of the group moved into Alderley Park in August, also setting up the new tumour immunology and inflammation monitoring (TIIML) team. In the immediate aftermath of the fire, members of the TARGET team moved to St Mary's Hospital so that they could continue to ctDNA profile over 100 Phase I patients for stratification into molecularly matched clinical trials.

In the last year, Institute staff won 30 prizes and awards and I am delighted by this recognition of our recent success including the wonderful news that our Deputy Director Caroline Dive was awarded a CBE in the Queen's New Year's Honours list. At the NCRI meeting in Liverpool, Santiago Zelenay was awarded the Cancer Research UK Future Leaders' Prize while Caroline Dive was part of the team to win Cancer Research UK's Translational Cancer Research Prize for 2017. The prize was awarded to the Tumour Heterogeneity Team, led by Charles Swanton from The Francis Crick Institute, for its work to understand genomic complexity and heterogeneity in solid tumours, and to track tumour evolution through time and treatment including the pioneering TRACERx Lung Study. On a personal note, I was delighted to be honoured by the ARC Foundation and the Society for Melanoma Research. Lisa Doar who heads our Biological Resources Unit's Experimental Facility won the Janet Wood Innovation Award for her invention of "the mouse swing" – a cage enrichment tool to enhance the environment for laboratory mice.

Each year, the Institute bestows its own award, the Dexter Prize for Young Scientists. The Prize is named after Mike Dexter, a former Director of the Paterson Institute for Cancer Research (the former name of the Institute) and in 2017 it was awarded to Melanie Galvin. Melanie is a Scientific Officer with the Clinical and Experimental Pharmacology group and has been awarded the prize for her extremely valuable contributions to the in vivo programme in CEP, together with the external recognition of her work in the 3Rs (the replacement, reduction and refinement of use of animals in research) from the Institute of Animal Technology.

We are continuing to recruit new Group Leaders and early career fellows and welcomed four new arrivals during the second half of the year. Rob Bristow moved to Manchester from Toronto where he was previously a Clinician-Scientist and Professor in the Departments of Radiation Oncology and Medical Biophysics at the University of Toronto and, a Senior Scientist at the Princess Margaret Cancer Centre. He has taken up the position of Director of the Manchester Cancer Research Centre and Chief Clinical Academic Officer at the Christie NHS Foundation Trust. Rob is also a Senior Group Leader at CRUK MI, where he will focus on the genomics of prostate cancer progression and cancer treatment response. Just prior to the fire, Donald Ogilvie retired from the Institute having established and led the Drug Discovery Unit since 2009. We thank Donald for his hard work and contributions to the Institute over the last few years and wish him all the best for his retirement. Caroline Springer was recruited as his successor and became the Director of the Drug Discovery Unit (DDU) in the autumn joining us from The Institute of Cancer Research in London where she has developed a number of exciting drug discovery programmes. Patricia Muller joined us as an Institute Fellow in July 2017 from the MRC Toxicology Unit at Leicester. She is studying how mutant p53 protein drives more aggressive tumours that are more metastatic and chemoresistant. Her aim is to develop novel therapeutic strategies to target mutant p53 expressing tumours. Maximiliano Portal joined the Institute in September 2017 from the University of Strasbourg. He is establishing the Cell Plasticity and Epigenetics group with the aim of unravelling the role that non-coding RNA molecules play in the genesis and propagation of non-genetic information through the acquisition of drug-tolerance in cancer relevant settings.

Former Institute Director Nic Jones wound down his laboratory at CRUK MI this year. He also stepped down from his role as Director of the Manchester Cancer Research Centre having led this valuable partnership since its inception in 2006. In more recent years he had held the post of Chief Scientist of CRUK and his contributions to developing cancer research in Manchester are immense. In recognition of his work, he was honoured by The University of Manchester with its highest award, the Medal of Honour. Fortunately we shall still benefit from Nic's expertise as he is continuing to work closely with the University advising on major strategic initiatives and research awards including the new cancer research centre that will replace the Paterson Building.

Engaging with the supporters who fund our research is incredibly important and despite the fire, the past year offered many opportunities to engage with the general public and to communicate the progress that we are making. Our scientists and staff participated in two Institute Open Days, several lab tours and engaged with over 8,000 people at more than 30 external events including the Manchester Science Festival and the Relay For Life in Stockport. This year saw a new introduction to the CRUK MI events calendar with The Late Lab, which provided an opportunity for our researchers to engage with CRUK volunteers from across the North West.

There is much to be excited about in 2018. Our groups continue to publish exciting science and we shall continue to settle into our new environment at Alderley Park while striving to retain our clinical links with the Christie NHS Foundation Trust. These links are critical to our mission of improving patient care through translational research. We shall work with our Christie colleagues, and with colleagues at The University of Manchester and CRUK on plans for the new cancer research centre. Later in the year, the Society of Melanoma Research will hold its annual Congress in Manchester, allowing us once again to showcase the strength and capabilities of Manchester and its excellent cancer research community.

RESEARCH HIGHLIGHTS

In this section we highlight some research publications from 2017 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.

Diamantopoulou Z, White G, Fadlullah MZH, Dreger M, Pickering K, Maltas J, Ashton G, MacLeod R, Baillie GS, Kouskoff V, Lacaud G, Murray GI, Sansom OJ, Hurlstone AFL, Malliri A.
TIAM1 antagonizes TAZ/YAP both in the destruction complex in the cytoplasm and in the nucleus to inhibit invasion of intestinal epithelial cells.
Cancer Cell 2017; 31(5):621-634.e6.

Colorectal cancer (CRC) is one of the leading causes of cancer mortality worldwide. Colorectal carcinogenesis typically commences with inactivation of the tumour suppressor APC, resulting in constitutive activation of the canonical WNT pathway that causes stabilisation and nuclear translocation of the transcription factors β-catenin, YAP and TAZ and subsequent activation of transcription of genes involved in CRC initiation and progression. In this study the Cell Signalling group found that TIAM1, an activator of the small GTPase RAC1, is a component of the cytoplasmic APC complex known as the destruction complex. TIAM1 in the destruction complex promotes TAZ degradation by enhancing its interaction with the ubiquitin-ligase βTrCP. They further showed that when the canonical WNT pathway is activated and the destruction complex inactivated, TIAM1, along with TAZ/YAP, accumulates and translocates to the nucleus. In the nucleus, however, TIAM1 continues to antagonise nuclear TAZ/YAP despite constitutive WNT signalling. Nuclear TIAM1 suppresses TAZ/YAP interaction with TEAD transcription factors, inhibiting expression of TAZ/YAP target genes implicated in epithelial-mesenchymal transition, cell migration, and invasion, and consequently suppresses CRC cell migration and invasion. Importantly, nuclear TIAM1 is downregulated in advanced colorectal cancer, and low nuclear TIAM1 predicts poor prognosis. In summary, this study demonstrates that in CRC, nuclear TIAM1 suppresses tumour progression by regulating YAP/TAZ activity and highlights the prognostic significance of TIAM1.

Chan KY, Alonso-Nuñez M, Grallert A, Tanaka K, Connolly Y, Smith DL, Hagan IM.
Dialogue between centrosomal entrance and exit scaffold pathways regulates mitotic commitment.
Journal of Cell Biology 2017; 216(9):2795-2812.

In addition to organising microtubules, centrosomes appear to act as signalling hubs for a number of pathways that regulate progression through the cell division cycle. However, the challenges of interrogating signalling pathways in human cells have meant that the molecular basis for the reported phenomena remains obscure. The Cell Division group have therefore been using fission yeast as a model system in which to define the molecular mechanisms by which signalling events on the, centrosome equivalent, spindle pole body (SPB) can regulate cell division. Two neighbouring SPB scaffold proteins have been linked to cell cycle control: signalling events on Cut12 control commitment to division while events on Sid4 control the events of exit from division. This paper now describes how a novel signalling cascade operates from Sid4 to influence the ability of Cut12 to promote commitment to division. Thus, rather than acting as two independent signalling platforms, controlling distinct cell cycle transitions, outputs from one platform impact upon the output from the neighbouring platform. Such dialogue now provides a conceptual framework for understanding why centrosomes should be used as signalling platforms as it shows how a variety of inputs into distinct networks on neighbouring scaffolds can produce a single, coherent output to determine cell fate.

Tang H, Leung L, Saturno G, Viros A, Smith D, Di Leva G, Morrison E, Niculescu-Duvaz D, Lopes F, Johnson L, Dhomen N, Springer C, Marais R.
Lysyl oxidase drives tumour progression by trapping EGF receptors at the cell surface.
Nature Communications 2017; 8:14909.

Tumours arise from mutated normal cells that have gained the ability to continuously proliferate. Although normal tissue architecture initially prevents the spreading of tumour cells, proliferating cells eventually compromise and remodel the tissue's original structures. This process of tumour microenvironment remodelling is critical for tumour cell proliferation and invasion. Lysyl oxidase (LOX) is a secreted amine oxidase that remodels the tumour microenvironment by cross-linking the extracellular matrix. High LOX expression correlates to poor outcome in colon, breast, pancreas, prostate and lung cancers. However, the mechanisms whereby LOX supports tumour progression are only partially understood. In this study, the Molecular Oncology group reveal that LOX facilitates cell surface expression of epidermal growth factor receptor (EGFR) to drive tumour cell proliferation. Specifically, LOX secreted by cultured tumour cells suppresses TGFβ1 signalling through an extracellular TGFβ1 degrading serine protease HTRA1. Attenuated TGFβ1 signalling allows expression and secretion of an extracellular adaptor protein, Matrilin-2 (MATN2), which traps EGFR at the cell surface to promote EGFR activation by EGF. As a result, LOX promotes EGF driven cell proliferation via enhanced EGFR activity. In collaboration with Caroline Springer, the new Director of the Drug Discovery Unit, the team are also able to report the discovery of a new LOX small molecule inhibitor, CCT365623. By inhibiting LOX, CCT365623 is able to disrupt EGFR surface retention and block mammary tumour growth and metastasis *in vivo*. This study demonstrates that LOX functions in remodelling tumour microenvironment and regulating cell surface EGFR can be therapeutically exploited.

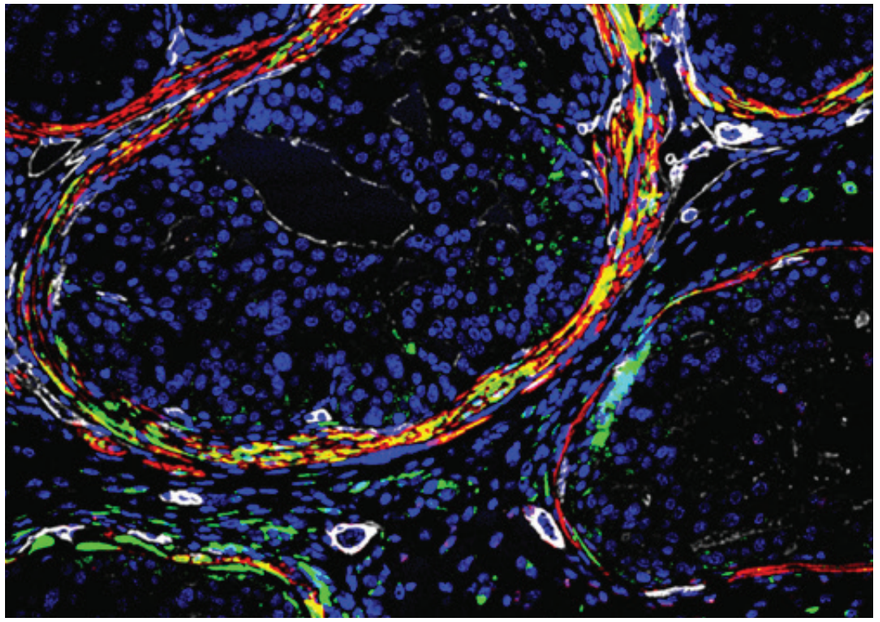
Draper JE, Sroczynska P, Leong HS, Fadlullah MZH, Miller C, Kouskoff V, Lacaud G.
Mouse RUNX1C regulates premegakaryocytic/erythroid output and maintains survival of megakaryocyte progenitors.
Blood 2017; 130(3):271-284.

The transcription factor RUNX1/AML1 is crucial for the establishment of adult haematopoiesis, haematopoietic cell fate decisions and maturation, and is frequently found translocated or mutated in leukaemia. Alongside other defects, RUNX1-deficient animals present with thrombocytopenia due to impaired megakaryocyte maturation. This defect reproduces the defect in platelet formation observed in patients suffering from an autosomal disorder caused by germline mutations or deletions in *RUNX1* - called familial platelet disorder with predisposition to acute myeloid leukaemia (FPD/AML).

Along the two major RUNX1 protein isoforms (RUNX1B and RUNX1C), RUNX1C is the most abundantly expressed isoform in adult haematopoiesis and is present in all RUNX1-expressing populations. The Stem Cell Biology group investigated the role of this isoform and observed that its deletion resulted in numerous multilineage haematopoietic defects in adults, including thrombocytopenia. To distinguish whether this was the result of a specific requirement for RUNX1C or a lower amount of total RUNX1 protein, the team generated a novel mouse line that lacks RUNX1C expression but has normal total RUNX1 levels, represented now entirely by RUNX1B. Observed in this line was a bias towards erythroid specification and away from megakaryopoiesis, similar to the defect observed upon depleting total RUNX1 in haematopoietic progenitors. In contrast, the group did not observe any impact on megakaryocytic maturation, unlike in total RUNX1-deficient mice. Instead, they detected a reduced proliferation and increased apoptosis in megakaryocyte progenitors. Altogether, the new results suggest distinct roles of RUNX1 isoforms at particular stages of megakaryocytic specification and maturation.

Mould, DP, Alli, C, Bremberg, U, Cartic, S, Jordan, A, Geitmann, M, Maiques-Diaz, A, McGonagle, AE, Somervaille, TCP, Spencer, GJ, Turlais, F, Ogilvie, D.
Development of (4-Cyanophenyl)glycine Derivatives as Reversible Inhibitors of Lysine Specific Demethylase 1.
Journal of Medicinal Chemistry 2017; 60, 7984-7999.

Lysine Specific Demthylase 1 (LSD1) is an epigenetic regulator thought to maintain leukaemic cells in a stem cell-like state, leading to both disease progression and treatment resistance. Whilst irreversible inhibitors of this enzyme are now undergoing clinical evaluation, potent, selective and reversible inhibitors have been long sought after but have been found difficult to deliver, despite considerable effort.



Confocal photomicrograph of normal mouse prostate showing stromal cells (Red), LOX (Green), blood vessels (Grey) and cell nuclei (Blue).
Image supplied by Haoran Tang (Molecular Oncology)

Such reversible compounds may offer a different therapeutic profile and offer a more useful tool to dissect the underlying role of LSD1 in cancers such as acute myeloid leukaemia and small cell lung cancer.

Applying detailed computational drug design techniques, medicinal and synthetic chemistry and a robust biological screening cascade, the Drug Discovery Unit have been able to overcome the challenges of reversible inhibitor design against this increasingly important oncology target and have delivered a unique family of highly validated and reversible inhibitors of LSD1.

These compounds have already led to several significant international collaborations, for example helping further research into the previously unrecognised role of LSD1 in breast cancer. These findings suggest that these novel compounds will allow a better understanding of LSD1 biology and may, ultimately, open the door to new classes of targeted therapeutic agents.

Edwards ZC, Trotter EW, Torres-Ayuso P, Chapman P, Wood HM, Nyswaner K, Brognard J.
Survival of head and neck cancer cells relies upon LZK kinase-mediated stabilization of mutant p53.
Cancer Research 2017; 77(18):4961-4972.

Head and neck squamous cell carcinoma (HNSCC) includes epithelial cancers of the oral and nasal cavity, larynx and pharynx, and accounts for ~350,000 deaths/year worldwide. Patients who present with early stage HNSCC are treated with surgery and/or radiotherapy and survival rates are high. However, a majority of patients present with late stage disease when local advancement to nearby tissue or lymph nodes has already occurred. After treatment, 50% of these patients will experience disease progression within two years, and five-year survival rates are under 50%. HNSCC is associated with few targetable mutations but is defined by frequent copy-number alteration, the most common of which is gain at 3q, seen in ~80% of HNSCCs. *MAP3K13* (the gene encoding LZK) resides on the 3q amplicon, and shows increased expression in HNSCC tumours and cell lines with 3q copy number gain. The Signalling Networks in Cancer group demonstrated that amplified LZK promotes proliferation in HNSCC by maintaining expression of gain-of-function mutant p53. Their data identify LZK as a novel genetic dependency in HNSCC tumours harbouring the 3q amplicon, and suggest LZK as a potential therapeutic target in HNSCC. Future studies will investigate the mechanism by which LZK stabilises mutant p53, which is present in a wide range of cancers.

Lee RJ, Gremel G, Marshall A, Myers KA, Fisher N, Dunn J, Dhomen N, Corrie PG, Middleton MR, Lorigan P, Marais R.
Circulating tumor DNA predicts survival in patients with resected high risk stage II/III melanoma.
Annals of Oncology 2017; Epub 3 November 2017

The incidence of melanoma, the most aggressive form of skin cancer, has continued to increase since 1960. Although early stage melanoma (stage I, II and resectable stage III, local lesion, local lymph nodes spread) can be cured by surgery, late stage metastatic disease (unresectable stage III and IV, lesions spread to distant organs) has generally been considered to be incurable. Notably, it has been observed that many patients with loco-regional melanoma will later succumb from distant metastases, stressing the need of biomarkers of disease progression in order to improve and/or anticipate their treatment. Unfortunately, accurate predictors of relapse for the individual have not yet been identified.

In this study, the Molecular Oncology group conducted a retrospective analysis to investigate if detection of circulating tumour DNA (ctDNA) bearing *BRAF* or *NRAS* driver mutations could predict relapse and survival in patients with resected melanoma. Specifically, they performed a relatively inexpensive and minimal invasive blood test based on droplet digital polymerase chain reaction (ddPCR) that can be carried out in 5 days using as little as 2 ml of plasma. Data obtained from the analysis of 161 stage II/III high-risk melanoma patients showed that detecting ctDNA in plasma collected within 12 weeks of curative intent surgery is indeed highly predictive of local recurrence, distant metastatic relapse and overall survival. These findings were independent of standard staging indices, demonstrating the value of this approach for melanoma. Interestingly, their results showed that the majority of the patients with detectable ctDNA relapsed within 1 year of surgery, suggesting that this approach can reveal occult metastatic disease that is not yet evident on radiological imaging. Thus, by monitoring ctDNA, we are able to identify a subgroup of high-risk patients whose treatment could start before metastasis is detected by imaging or clinical examination.

Finally, recent findings indicate that immune checkpoint inhibitors improve overall survival in stage III melanoma; however there is associated treatment toxicity in a population potentially cured by surgery alone. These observations further emphasise the need for better stratification of patients into high-risk groups and further identify early disease progression in order to target treatment for those who will benefit from it. In that regard, the team is currently developing prospective studies to validate our findings and to examine if the overall survival of these high-risk patients could be improved by using early ctDNA-guided decisions to provide treatment.

Naidu S, Shi L, Magee P, Middleton JD, Laganá A, Sahoo S, Leong HS, Galvin M, Frese K, Dive C, Guzzardo V, Fassan M, Garofalo M.
PDGFR-modulated miR-23b cluster and miR-125a-5p suppress lung tumorigenesis by targeting multiple components of KRAS and NF-kB pathways.
Scientific Reports 2017; 7(1):15441.

Both PDGF ligands and the receptors have been detected in lung cancer cells but not in normal cells and are markers of worse prognosis. In this study, the Transcriptional Networks in Lung Cancer group explored PDGFR-regulated microRNAs demonstrating that miR-23b cluster and miR-125a-5p are downregulated by increased expression of PDGFR-α or PDGFR-β in NSCLC cells. Enforced expression of miR-23b cluster and miR-125a-5p directly or indirectly decreased the expression of multiple oncogenes involved in the KRAS and NF-kB signalling, reducing cell proliferation and enhancing drug-induced apoptosis. Mechanistically, the miR-23b cluster and miR-125a-5p are transcriptionally activated by p53 and negatively regulated by NF-kB p65. In vivo delivery of these microRNAs suppressed the growth of a highly aggressive tumour derived from a patient with metastatic NSCLC and unresponsive to standard-of-care chemotherapy. These results shed new light on the mechanisms involved in lung tumorigenesis and lay the foundations to potentially develop more effective therapeutic strategies for both PDGFR- and KRAS-driven NSCLC.

CANCER RESEARCH UK MANCHESTER INSTITUTE

RESEARCH GROUPS

CANCER INFLAMMATION AND IMMUNITY

Group Leader
Santiago Zelenay

Postdoctoral Fellows
Eduardo Bonavita
Victoria Pelly

Scientific Officer
Chikkanna-Gowda CP[2]

Graduate Students
Eimear Flanagan
Charlotte Bell[1]
Christian Bromley[1]

[1]joined in 2017
[2]left in 2017

Immunotherapy constitutes the most promising pan-cancer treatment approach since the development of the first chemotherapies. Unprecedented outcomes continue to be observed in multiple malignancies including some once thought to be treatment refractory. Responses, especially complete and durable, are nevertheless only observed in a very limited subset of patients, underscoring the need for basic research to elucidate the basis for these remarkable but rare outcomes. The Cancer Inflammation and Immunity group investigates the signals and pathways regulating the establishment of tumour microenvironments that support or restrain cancer progression spontaneously or following treatment. Our overarching hypothesis is that the ability of cancer cells to form progressive tumours is invariably linked to their immunoevasive properties.

This past year has been a very important one for immunotherapy. Over a dozen cancer treatments aimed at harnessing the inherent anti-cancer activity of the immune system have been approved world wide, including some as first-line treatments. One of the most promising approaches consists of the administration of blocking antibodies against the PD-1/PD-L1 pathway to enhance and reactivate the anti-cancer function of pre-existing tumour specific T cells. In addition to their great clinical significance, these approaches have provided undisputable support to the concept that the immune system constitutes a natural barrier to cancer development and spread in many malignancies. The spontaneous recognition of tumours by tumour-specific T cells is no longer a phenomenon considered exclusive of highly immunogenic cancers such as cutaneous melanoma.

An implication from the realisation that tumours do not pass unnoticed by the immune system is that active mechanisms of immune evasion must exist. Of special interest are those mechanisms that are conserved across cancer types and act in a dominant manner, as their identification and targeting offer an attractive opportunity for therapeutic intervention, especially in combination with immune checkpoint blockade therapies. One such mechanism, on which many of our current research questions and projects are based, is the upregulation of the cyclooxygenase- (COX)-2/prostaglandin E2 (PGE_2) pathway by cancer cells. COX-2 is the rate-limiting enzyme for the production of prostaglandins, among which the inflammatory lipid PGE_2 has been long recognised for having tumour-promoting capabilities. In a proof-of-concept study we have shown that cancer cell-intrinsic PGE_2 production is essential for the ability of diverse cancer cells to establish tumours in immunocompetent, but not immunodeficient hosts, demonstrating that the main effect of tumour cell-derived PGE_2 is to allow evasion of spontaneous immunity (Zelenay et al. *Cell* 2015).

During the last year we have made significant progress in dissecting the basis for the immune-dependent elimination of tumours rendered PGE_2-incompetent by genetic ablation of COX-2 expression. We have extended this analysis to other cancer lines including models widely used in immunotherapy research and found that invariably, COX-2-deficient cancer cells, but not their parental counterparts, trigger a robust T cell response that controls their growth. In doing so, we have identified critical immune subsets which drive an inflammatory switch locally at the tumour site, setting the stage for the subsequent T cell response.

This radical change in the inflammatory response of the microenvironment of COX-2-deficient tumours is characterised by high expression of T cell-chemoattractants, factors that promote effector T cell differentiation and other hallmark mediators of anti-tumour immunity. Most of these factors have been commonly associated with better prognosis and outcome from treatment. Therefore, to determine the significance of our findings in preclinical models to human cancer we have carried out extensive in silico analyses of publicly available human cancer datasets. With major help from the Computational Biology group at the CRUK Manchester Institute, we have found that molecular profiles characteristic of tumours formed by PGE_2 competent and incompetent cancer cells can be found conserved across various human cancer types. Moreover, a gene signature derived from our preclinical models can predict cancer patient survival and outcome following treatment across several select malignancies. Part of these analyses was presented earlier this year by Eduardo Bonavita, an EMBO-funded Postdoctoral Fellow in the group, in an oral presentation at a Keystone Symposia on Inflammation-Driven Cancer.

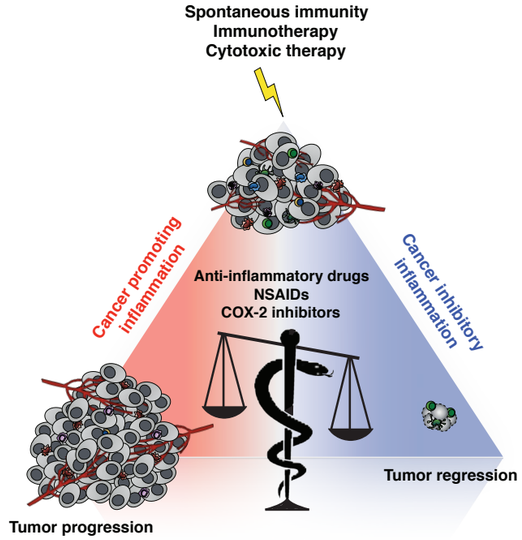


Figure 1
Spontaneous and therapy-induced alterations in the tumour microenvironment can fuel or suppress cancer growth. Working under the hypothesis that tumour malignancy and fate largely depends on the nature of the inflammatory response at the tumour site, we have made significant progress in developing ways to distinguish tumours with 'cancer-promoting' or 'cancer-inhibitory' microenvironments across different human malignancies. Also, we are further testing the hypothesis that one way to enhance the efficacy of different cancer treatment modalities is to target cancer promoting inflammation. One approach to achieve this, currently under investigation in our group, involves the use of broad-spectrum anti-inflammatory drugs such as NSAIDs or specific COX-2 inhibitors. This strategy is depicted by the snake of the Greek god of medicine Asclepius, winding around and tipping the balance towards cancer-inhibitory inflammation. Figure adapted from Eduardo Bonavita, Victoria S. Pelly and Santiago Zelenay (2018). Resolving the dark side of therapy-driven cancer cell death. J. Exp. Med. 215, 9–11. *The Journal of Experimental Medicine*, 2018.

Our analysis further revealed that our COX-2 signature outperforms previously published immune signatures in prognostic power suggesting that it could constitute a valuable biomarker of clinical outcomes. Encouraged by these results we have embarked in a new project exclusively focused on the analysis of cancer patient datasets and samples, funded by the British Research Council, to further evaluate the utility of, and simultaneously refine the COX-2 inflammatory signature as a predictive marker of overall survival and treatment response.

One major premise on which we based our research is that to elucidate mechanisms of immune evasion in cancer we need to identify what triggers cancer immunity in the first place. We are therefore continuing our efforts to decipher the very early signals and pathways that trigger the establishment of a cancer inhibitory microenvironment that precedes and supports the later T cell-mediated adaptive immune response. For this, we use and develop new versatile genetically engineered cancer models that allow us to establish cause and effect relations and pinpoint key drivers of anti-tumour immunity.

These same models are also being used for projects with direct clinical implications in which we are studying the mechanistic basis for our original observation that combinations of COX-2 inhibitors and immune checkpoint blockade antibodies synergise in promoting tumour control in several preclinical models. In these settings, the COX-2/PGE_2 pathway drives intrinsic resistance to immune checkpoint blockade. Its therapeutic inhibition, while having no significant effect in tumour progression as a monotherapy, strongly enhances the efficacy of PD-1 blockade. Our current working model is that this increase in treatment response following therapeutic inhibition of the COX-2 pathway results, like in the genetic models, from a shift in the inflammatory response. So, anti-inflammatory drugs, like NSAIDs or selective COX-2 inhibitors, rather than non-selectively suppressing inflammation at the tumour site seem to reduce tumour promoting factors but spare anti-tumour mediators, effectively shifting the balance towards cancer inhibitory inflammation (see Figure 1).

In this context we are also addressing a potential beneficial effect of anti-inflammatory drugs, and specifically of COX-2 inhibitors, in modulating the inflammatory response induced by cytotoxic therapies. In these settings, PGE_2 has been implicated in treatment failure and tumour relapse and our hypothesis is once again that most, if not all, of the detrimental effects of the COX-2/PGE_2 pathway are downstream of its potent immunosuppressive effects.

Finally, a central question that remains unanswered is how prevalent is the COX-2/PGE_2 pathway in cancer. Based on the analysis of a diverse panel of more than 40 cancer lines we have found that the pathway is often upregulated. More than half of the cell lines, obtained from a diverse range of tumour types including melanoma, breast, colorectal, liver and pancreatic cancer constitutively express COX-2 and produce significant amounts of PGE2. Many of them do not and yet they are able to establish progressive tumours in immunocompetent mice, implying that they use immune evasive mechanisms other than cell-intrinsic PGE_2 production. Using this cancer cell line collection and an approach analogous to the one employed to identify the COX-2/PGE_2 pathway, we are searching for conserved immunomodulatory and immunosuppressive factors which when targeted might enhance the efficacy of cancer treatments.

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CELL DIVISION

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The inappropriate proliferation of cancer cells can arise from unchecked cell division, a failure to engage cell death pathways, or simultaneous changes in both. Understanding the means by which the timing of cell division is set will identify novel routes by which these controls can be manipulated for therapeutic benefit.

The decision to divide is set by the integration of a multitude of inputs from diverse signalling pathways. We aim to understand how these inputs are integrated to generate a specific cell division cycle control for a particular context. Because the regulatory networks that control cell division are highly conserved, we have been using the fission yeast *Schizosaccharomyces pombe* to identify the key principles and regulatory mechanisms for cell division control, with the view of applying the lessons learnt from yeast to guide highly targeted interrogation of, the more complex context of, cell division in cancer.

In a typical cell division cycle, the G1 gap phase precedes DNA replication in S phase, before a second gap phase, G2, separates S from genome segregation in Mitosis (M phase). Growth, developmental and environmental cues regulate the G1/S and G2/M transitions to determine the rate of proliferation. Passage through these key transitions is driven by the activation of distinct CDK-Cyclin complexes. The G2/M transition is a critical safeguard of genome integrity; incomplete DNA replication or DNA damage triggers checkpoint pathways that block the G2/M transition to ensure that chromosomes are not segregated when incomplete or damaged. Once cells are in mitosis, a further checkpoint, the spindle assembly checkpoint, blocks passage through mitosis when integrity of the division apparatus has been compromised. Triggering this checkpoint through the application of spindle poisons, such as pacitaxel, can promote cell death and tumour control in the clinic.

The G2/M transition is driven by activation of the Cdk1-Cyclin B protein kinase. Wee1 related kinases phosphorylate the catalytic subunit, Cdk1, to inhibit the complex during interphase. This phosphate is removed by Cdc25 phosphatases to promote mitotic entry. Cdk1-Cyclin B activation promotes a positive feedback loop that boosts Cdc25 and inhibits Wee1 activities to ensure that mitotic commitment is a rapid and irreversible, bi-stable, switch (Figure 1). In both humans and fission yeast, this feedback control exploits polo kinase to determine the timing of mitotic entry. The checkpoint pathways that restrain division when DNA integrity is compromised enhance Wee1 and inhibit Cdc25 activities to block Cdk1-Cyclin B activation.

A number of observations support the view that the centrosome provides a specific microenvironment for the activation of Cdk1-Cyclin B to trigger the G2/M transition, with the most persuasive being the initial activation of Cdk1-Cyclin B at human centrosomes. As it is this initial trigger that determines when the cell divides, understanding how inputs from diverse signalling networks are integrated on the centrosome holds the key to understanding how the trigger decision is taken, how the checkpoint pathways influence this signalling network and how these controls may be exploited for therapeutic benefit. The potential for therapeutic benefit is considerable, as the excessive complement of centrosomes, seen in many tumour cells, is likely to alter centrosomal signalling.

Our studies of the fission yeast centrosome equivalent, the spindle pole body (SPB), provide molecular insight into how this switch may operate. We have previously found that the release of Cdk1-Cyclin B or Polo kinase activity at the SPB drives cells through the G2/M transition into division. This mechanistic insight suggests that the initial appearance of active Cdk1-Cyclin B on human centrosomes reflects a true functional relationship between centrosomal signalling and Cdk1-Cyclin B activation to drive division.

Our previous work has also defined events on the SPB scaffold Cut12 that control the timing of division. Simply blocking the recruitment of protein phosphatase 1 (PP1) to Cut12 enabled us to completely remove Cdc25 phosphatase from the cell without impacting upon viability. Blocking PP1 recruitment to Cut12 bypasses the requirement for this essential mitotic inducer because it elevates the activity of the feedback loop kinase Polo. The engagement of Polo as a Wee1 inhibitory kinase in the normal feedback loop controls in mitotic commitment provides a simple framework for understanding how elevated polo could abolish any requirement for Cdc25; if Wee1 is shut down by inappropriate elevation of Polo activity, there will be no phosphate on Cdk1 for Cdc25 to remove and so the phosphatase will not be required. In summary, the SPB acts as a catalyst to release Polo activity throughout the cell to drive the global commitment of the cell to division (Figure 1).

The influence of events on the SPB upon mitotic control is not confined to the Cut12/PP1 control over mitotic commitment as the signalling network that controls mitotic exit and cytokinesis, the septum initiation network (SIN), also relies upon anchorage to the SPB to trigger these events. This network is anchored by a second scaffolding molecule, Sid4 (Figure 2a). We recently uncovered an unanticipated cross talk between the signalling events on Sid4 and Cut12 in regulating the decision to commit to mitosis. We found that the recruitment of the DNA replication checkpoint kinase Chk2 to Sid4 expels the protein phosphatase Cdc14 from the SPB. As Cdc14 dephosphorylates sites that are phosphorylated by Cdk1-Cyclin B, the expulsion of Cdc14 by the signalling network on Cut12's neighbour, Sid4, reduces the threshold for Cdk1-Cyclin B activation at the SPB that is driven by Cut12. Thus, events on Sid4 can reduce the amplitude of signals that need to be generated by Cut12 in order to trigger division (Figure 2b).

Why are these events on the spindle pole so important? A clue comes from our previous work, and that of Janni Petersen (Flinders University, Adelaide), that has shown how Cut12/PP1/Polo signalling changes the timing of division in response to heat stress or when changes in nutrient supply alter flux through TOR signalling. Furthermore, Kathleen Gould's lab (Vanderbilt, Tenessee) has shown how Sid4 regulated events restrain mitotic progression in response to perturbation of the mitotic spindle division apparatus. Thus, events on Cut12 and Sid4 respond to distinct cell fate regulatory pathways to control progression through division (Figure 3). Such dialogue between signalling pathways on neighbouring scaffolds now provides a plausible rationale for the use of the centrosome as a signalling hub: convergence of signals from multiple pathways to a limited number of neighbouring scaffolds, clustered together on the centrosome, can integrate the inputs from the different pathways to generate a coherent signal that sets the flux through outgoing signal cascades.

Strikingly, homologues of all components of the Sid4 scaffold signalling network that we identified (NIMA kinase, Caesin kinase 1δ, Chk2 and Cdc14) associate with human centrosomes. Moreover, work by Holger Bastian's group has linked centrosomal Chk2 kinase activity to protein phosphatase 6 (PP6) activity at human centrosomes. As PP6 regulates the activity of the polo activating aurora A kinase, the parallels between our findings in yeast and analogous molecules in humans are strong. Furthermore, the definition of the means by which Chk2 can be engaged in centrosomal signalling may now pave a route to understand and exploit a long standing observation that mutation of human centrosomal scaffolds abolishes DNA checkpoint signalling.

Research Highlights:

- Signalling outputs from neighbouring scaffolding proteins on the spindle pole determine the timing of division
- The molecular basis for the recruitment of the DNA integrity checkpoint kinase Chk2 to the spindle pole

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Figure 1: The spindle pole scaffold Cut12 sets the timing of the G2/M transition
A schematic diagram shows the Cdk1-Cyclin B regulatory switch that is triggered from the spindle pole scaffold Cut12, before propagating throughout the cell, to drive the entire cell into division.

Figure 2: Signalling from the "mitotic exit" scaffold Sid4 sets the threshold for mitotic commitment signalling from the neighbouring spindle pole scaffold, Cut12
a) Sid4 anchors the septum initiation network (SIN) to one of the two anaphase B SPBs, from where SIN signalling determines the timing of mitotic exit and cytokinetic events. b) Our recent studies revealed a second role for signalling from Sid4 as a protein kinase cascade along Sid4 expelled the Cdc14 protein phosphatase from the SPB, to set the threshold for the Cut12 derived signalling events, that push the cell into division at the G2/M transition. This novel signalling relay operates independently of the Sid4 signalling that drives mitotic exit at the end of division.

Figure 3: The spindle pole as a signalling hub that integrates inputs from diverse pathways to dictate the timing of cell division
A schematic diagram summarising work from our lab and the labs of Janni Petersen (Flinders University, Adelaide) and Kathleen Gould (Vanderbilt Medical Center, Tenessee) that have revealed how inputs from different signalling networks influence the Cut12/Sid4 decision hub on the spindle pole to regulate progression into, and out of, mitosis.

Figure 1

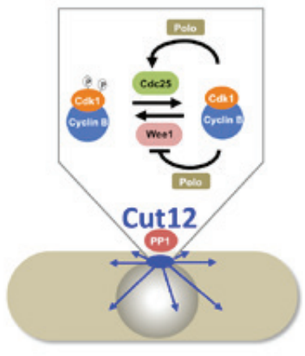


Figure 2

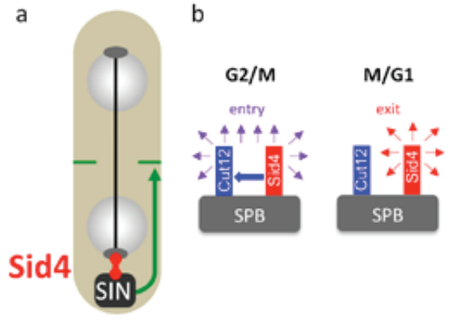
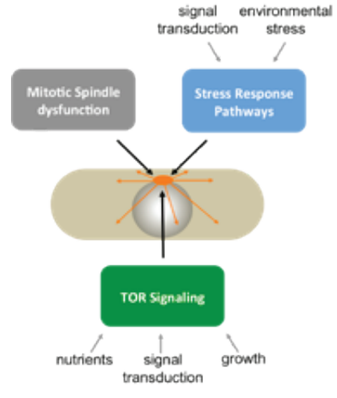


Figure 3



CELL PLASTICITY & EPIGENETICS

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Almost every model of cancer onset and progression that has been developed over the years takes a strictly deterministic standpoint that suggests the accumulation of mutations eventually triggers a cascade of events that will ultimately throw the cell into replicative disarray. However, the recent realisation that certain cancer types rely mainly on epigenetic changes rather than genetic aberrations prompts us to re-visit basic concepts in cancer biology. The ultimate goal of our lab is to shed light onto the molecular systems supporting cell plasticity with particular emphasis on the role of non-coding RNAs in the generation and propagation of epigenetic memories underlying drug-tolerance in cancer relevant settings.

It is generally accepted that tumours are subjected to a myriad of evolutionary constraints at their niche of origin and further within the ecosystems encountered while invading novel tissues. Thus, evolutionary forces shape cancer development on many levels, as progression of the disease is often correlated with the appearance of somatic mutations and the selection of genetic traits that eventually become beneficial to neoplastic growth and often prejudicial to the host. Indeed, often-acquired mutations alter growth control systems and obliterate cell death programs, ultimately granting mutated cells with replicative immortality at the expense of genetic stability. However, due to the variable nature of the selective pressure in a particular niche, stable somatic mutations arise only after recurrent encounters with a challenging force. This suggests that, though under heavy evolutionary constraints, genetic changes driving adaptation do not occur instantly and highlights the biological relevance of cancer cell plasticity during neoplastic evolution.

A striking example that brings forward the plasticity of cancer cells is their resilience when confronted with therapeutic paradigms. Indeed it is acknowledged that, in response to sustained treatment, cancer cells may acquire genetic mutations that permanently block the tumouricidal action of the administered drug.

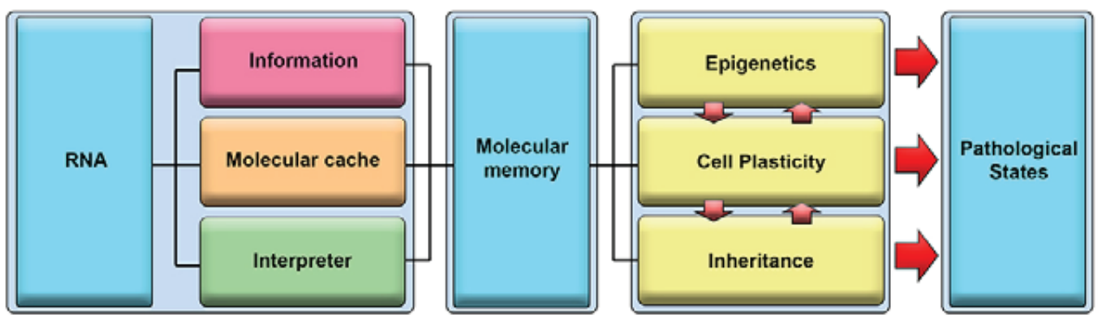


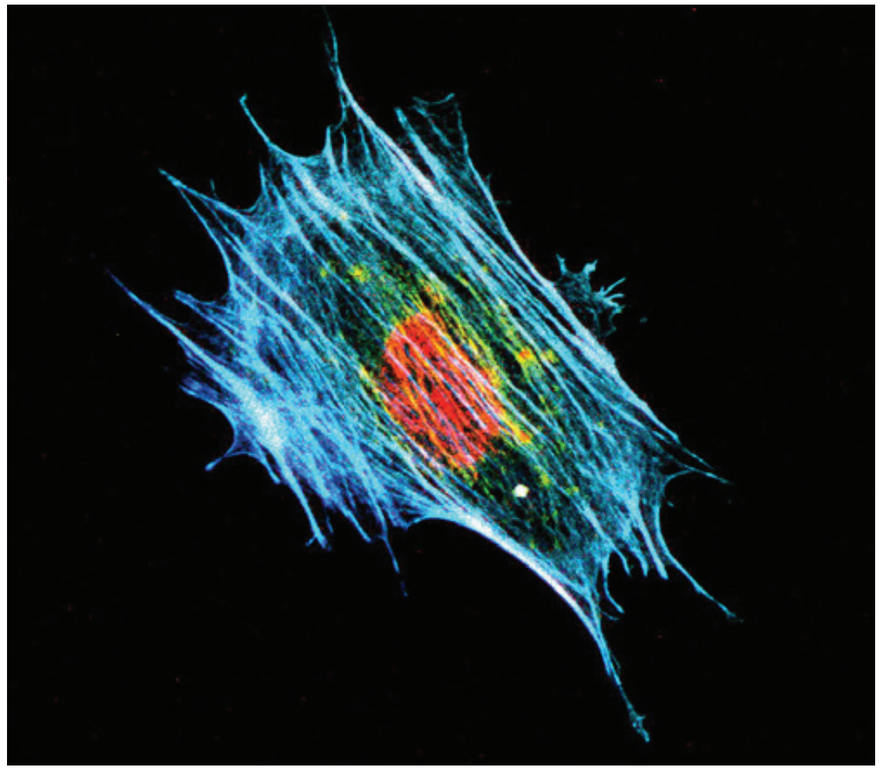
Figure 1: Conceptual flow underlying our research lines.
As RNA molecules encode genetic and epigenetic information we propose that they serve as information reservoirs and also function as genome interpreters. We hypothesise that the RNA content of a cell plays a fundamental role in the genesis, maintenance and propagation of molecular memory thus bridging cell plasticity with mechanisms ensuring the proper inheritance of epigenetically encoded traits. Ultimately, we propose that the improper acquisition of molecular memories has a profound impact in cellular homeostasis that results in the emergence of pathological states.

However, in other settings, the emergence of fully drug-resistant clones cannot be explained by genetic mechanisms and results from cells that escape the initial death challenge by "adapting" to the pernicious agent. In the latter scenario, the traits granting adaptation to treatment are reversible in nature and are readily inherited through several cell divisions. This particularity strongly suggests the existence of a non-genetically encoded temporal "memory" underlying the acquisition of "drug-tolerant" phenotypes and represents an exquisite example of the transfer of non-genetic information through cell division.

Along those lines, the ultimate goal of our lab is to unravel the network of molecular systems supporting cell plasticity and to shed light onto the core mechanisms underlying the inheritance of epigenetically encoded traits. We are currently setting up a multidisciplinary team that takes advantage of high-throughput sequencing and imaging technologies as well as classical molecular and cellular biology techniques to analyse the role of non-coding RNA molecules in the generation and propagation of epigenetic memories through subsequent cell divisions with particular interest in the acquisition of drug-tolerance in cancer relevant settings.

Actin is a major structural protein within the cell, defining the cell shape and helping control cell migration. Actin was stained with the dye phalloidin, and multiple z-stacks acquired at high magnification. Each stack is shown here in a different colour, demonstrating the diversity of actin structures throughout the cell.

Image supplied by Andrew Porter (Cell Signalling)



CELL REGULATION

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

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

The role of stress-dependent transcription in prostate cancer

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

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

Given that following radical prostatectomy, disease recurrence is caused by the presence of small metastatic lesions, we hypothesised that the expression of ATF2 targets should correlate with clinical outcome. Accordingly, we find that low expression of several ATF2 target genes strongly correlates with a high risk of recurrence. Based on these findings, we used a bioinformatics approach to derive a prognostic gene signature based solely on ATF2 target genes. The resulting prognostic index, which

Deletion of ATF2 correlates with early disease recurrence - (6% of patients have deletion)

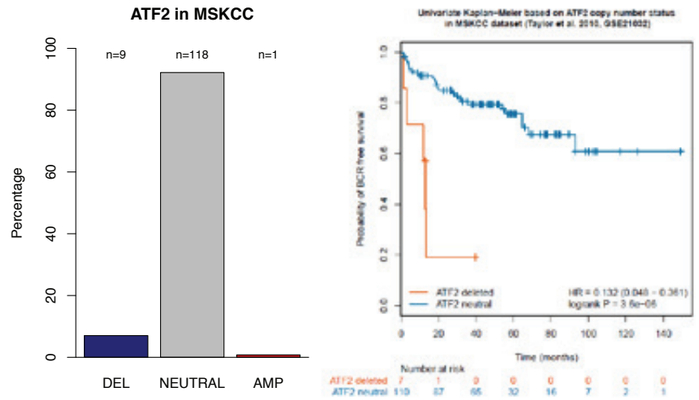


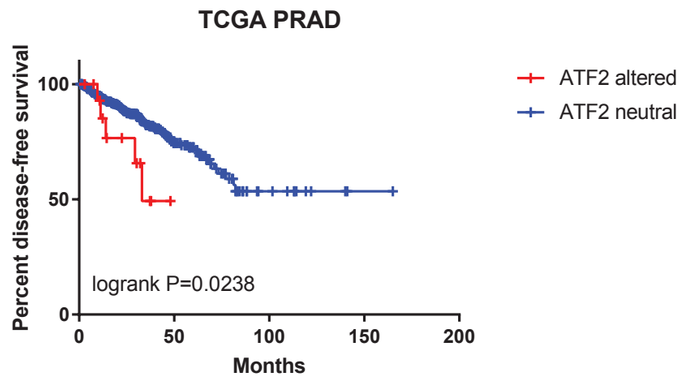
Figure 1:
Deletion of ATF2 correlates with early disease recurrence - (6% of patients have deletion)

utilises expression information from 18 ATF2 targets, is significantly associated with risk of disease recurrence in independent patient cohorts – supporting the notion that progressive disease is associated with impaired expression of stress-responsive genes.

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

Figure 2:
Deletion of ATF2 correlates with early disease recurrence (TCGA PRAD) - (3% of patients have deletion)

Deletion of ATF2 correlates with early disease recurrence (TCGA PRAD) - (3% of patients have deletion)



Genetic deletions and missense mutations involving ATF2 are relatively rare in prostate cancer. However, the paucity of these changes could be explained if loss of ATF2 was largely restricted to high-risk tumours. To examine a potential relationship between ATF2 mutation and risk in prostate cancer, we utilised copy number data from two independent patient cohorts in which the frequency of tumours harbouring homozygous loss of ATF2 was found to be 3% and 6% respectively. Kaplan-Meier analysis of patient outcome reveals that tumours lacking ATF2 are at greater risk of early recurrence than tumours retaining ATF2. Furthermore, these tumours also show a significant increase in the expression of genes involved in cellular proliferation, and higher rates of chromosomal instability. Thus, although the number of tumours lacking ATF2 is relatively low, our analysis indicates that they possess an aggressive phenotype. These observations suggest that genetic loss of ATF2 may alter the course of the disease, promoting the outgrowth of highly proliferative tumours that possess increased metastatic potential. However, this analysis of copy number data cannot be considered conclusive since neighbouring genes that are co-deleted with ATF2 could be responsible for driving an aggressive disease. To elucidate the function of ATF2 in prostate cancer, we have employed a genetically engineered mouse model in which ATF2 is deleted specifically in the prostate, along with the well-characterised tumour suppressor gene, PTEN. In this system, the deletion of PTEN leads to the development of PIN lesions (prostate intraepithelial neoplasia) which then progress to adenocarcinomas – indicated by the movement of neoplastic cells from their duct of origin into the adjacent stroma. However, progression to a fully metastatic disease is relatively slow in this model, particularly where a pure mouse background is employed, making it a good choice for examining co-operative effects from other genes. Prostate tissue from cohorts of wild-type, PTEN single knockout and PTEN:ATF2 double knockout mice has been collected for characterisation by histology. Preliminary data indicate that PTEN deletion leads to activation of the MAP kinases in PIN lesions, and it will be interesting to determine whether such signalling correlates with low rates of proliferation as our analysis of RPPA data from patient samples would suggest. Furthermore, our initial characterisation of these mice suggests that the pathological changes induced by PTEN deletion are accelerated when ATF2 is also deleted. We are currently working towards confirming our initial findings by employing a panel of immunohistochemical marker antibodies aimed at providing detailed information on proliferation and signalling.

CELL SIGNALLING

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[1]Joined in 2017
[2]Left in 2017

Mutations and overexpression in human tumours implicate the small GTPase RAC and its activators, the guanine nucleotide exchange factors (GEFs), in the formation and dissemination of cancers. Furthermore, the effects of ablating genes encoding RAC proteins or RAC GEFs in mouse, or of pharmacologically inhibiting RAC-GEF/RAC interactions, strongly suggest that targeting RAC signalling could constitute an effective cancer treatment. However, owing to multiple physiological roles of RAC and RAC functions that antagonise tumour dissemination, sustained suppression of RAC signalling could be detrimental. Given this challenge, the research of the Cell Signalling group aims to distinguish RAC-dependent effects that promote tumour growth and progression from those that antagonise tumour progression so that RAC signalling might be targeted more effectively.

RAC, a member of the family of RHO-like GTPases, 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 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 demonstrated, ubiquitylation and SUMOylation (Castillo-Lluva et al., 2013; Castillo-Lluva et al., 2010).

GEFs are typically large proteins harbouring multiple protein-protein interaction domains. Besides stimulating guanine nucleotide exchange, GEFs act as molecular scaffolds targeting active RAC to particular subcellular locations and potentially increasing the local concentration of selective effector molecules, thereby influencing downstream processes. Through influencing selectivity in RAC signalling, GEFs could therefore perform non-redundant signalling roles, which could be important for tumourigenesis. Indeed, mice deficient for the GEF *Tiam1* are resistant to the formation of skin tumours induced by chemical carcinogens that target *H-Ras*, and the few resulting tumours grow very slowly (Malliri et al., 2002). Thus TIAM1, we infer, plays a unique role in mediating RAS transformation, which the Cell Signalling group is currently elucidating, focusing recently on RAS-driven lung cancer.

Role of RAC activation in cell migration

Activation of RAC can lead to opposing migratory phenotypes - cell-cell adhesion versus motility - raising the possibility that targeting RAC in a clinical setting could exacerbate tumour progression. This calls for the identification of factors that both influence the selection of RAC-driven cellular processes as well as mediate RAC's effects. In a recent study (Marei et al., 2016), we demonstrated that two RAC GEFs, TIAM1 and P-REX1, promote RAC-driven cell-cell adhesion and RAC-driven cell migration and invasion respectively, through regulating the RAC interactome. While TIAM1 promotes the association of RAC with proteins known to be important for the formation and maintenance of cell-cell adhesions and consequently inhibits migration, we demonstrated that P-REX1 stimulates migration through enhancing the interaction between RAC and the actin-remodelling protein Flightless-1 homolog. Thus, in this study, we provided direct evidence that RAC GEFs are critical determinants of selectivity in signalling events downstream of RAC.

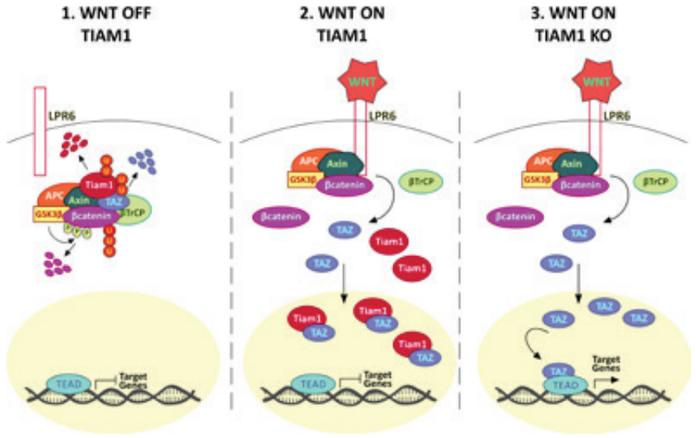

Figure 1. Model summarising the proposed mechanism of TIAM1-mediated regulation of TAZ in CRC cells.
1) WNT OFF; TIAM1 promotes TAZ degradation **2)** WNT ON; TAZ and TIAM1 accumulate, but nuclear TIAM1 blocks interaction between TAZ and TEAD **3)** WNT ON, TIAM1 absent; TAZ accumulates and can now interact with TEAD leading to expression of TAZ/TEAD target genes.

Apart from P-REX1, we have previously shown that activation of RAC by another RAC-specific GEF, STEF/TIAM2, promotes cell migration (Rooney et al., 2010). In a recent unpublished study, we established that this RAC activator is a critical regulator of the perinuclear acto-myosin cytoskeleton that is heavily implicated in determining nuclear orientation during directional cell migration. We showed that STEF localises to the nuclear membrane and through several intermediate interactions promotes apical actin filament formation or its attachment to the nuclear envelope. The apical actin filaments so formed constitute a 'nuclear cage' that restrain and compress the nucleus during front rear-polarisation. We therefore suggest that the functional role of STEF at the nuclear envelope impacts on directed cell migration, which we show is compromised in STEF depleted cells.

TIAM1 antagonises malignant progression via regulating cell-cell adhesion

Despite their slower growth, tumours arising in *Tiam1*-deficient mice progressed more frequently to malignancy (Malliri et al., 2002). Malignant progression can entail loss of cell-cell adhesion. Over-expression of activated RAC or TIAM1 promotes the formation of adherens junctions (AJs) and the accompanying induction of an epithelial-like phenotype in a number of mesenchymal cell lines (Malliri & Collard, 2003). Moreover, TIAM1 is required for both the formation as well as the maintenance of cadherin-based adhesions (Malliri et al., 2004). The oncoprotein SRC, a non-receptor tyrosine kinase, targets AJs for disassembly. Previously, we showed that SRC phosphorylates TIAM1 inducing its cleavage by Calpain and its depletion from adherens junctions. Abrogating TIAM1 phosphorylation by SRC suppressed AJ disassembly (Woodcock et al., 2009). In another study from our laboratory (Vaughan et al., 2015), we found that TIAM1 is ubiquitylated and degraded upon treatment of cells with hepatocyte growth factor (HGF), a cytokine that is abundant in cancer and promotes invasion of cancer cells. We showed that interfering with TIAM1 ubiquitylation by depleting HUWE1, the ubiquitin E3 ligase responsible for its ubiquitylation, or by mutating the ubiquitylation site retards the scattering and invasion of cells through delaying AJ disassembly.

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

In a previous study, using recombinant mouse models, we showed that TIAM1 cooperates with WNT signalling, known to drive colorectal cancer (CRC), during CRC initiation but then antagonises CRC tumour progression (Malliri et al., 2006). However, how TIAM1 influences CRC initiation and progression remained elusive. To further address the impact of TIAM1 on CRC and increase our understanding of its clinical significance, we probed a CRC tissue microarray. Intriguingly, we detected TIAM1 not only in the cytoplasm, but also in cell nuclei and showed that nuclear and cytoplasmic TIAM1 staining intensity significantly decreased with advancing Dukes stage. Thus, TIAM1 expression is negatively associated with colon cancer progression, consistent with our previous finding that TIAM1 antagonised progression of intestinal tumours in $Apc^{MIN/+}$ mice. Further analysis of these data also showed that patients with high nuclear TIAM1 had significantly better survival than those with low nuclear TIAM1. We then demonstrated that TIAM1 shuttles between the cytoplasm and nucleus antagonising the TAZ/YAP transcriptional co-activators and known effectors of WNT signalling by distinct mechanisms in the two compartments. In the cytoplasm, TIAM1 localises to the destruction complex and promotes TAZ degradation by enhancing its interaction with βTrCP. Nuclear TIAM1 suppresses TAZ/YAP interaction with TEADs, inhibiting expression of TAZ/YAP target genes implicated in epithelial-mesenchymal transition, cell migration and invasion, and consequently suppresses CRC cell migration and invasion (Figure 1. and Diamantopoulou et al., 2017). Thus, our findings showed that in CRC TIAM1 suppresses tumour progression by regulating YAP/TAZ activity.

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The Clinical and Experimental Pharmacology group has two major research goals: to discover, develop, validate and implement biomarkers to support personalised medicine for cancer patients; and to develop a patient relevant preclinical platform to discover and test novel therapies for small cell lung cancer (SCLC). Highlights this year included: (i) collection of a unique blood sample set from subjects attending a lung health check in their communities to support biomarker discovery for the early detection of lung cancer; (ii) 'roll out' of circulating tumour DNA (ctDNA) profiling to assist selection of the most appropriate trial for patients entering The Christie NHS Foundation Trust Phase I Trials Unit; (iii) establishment of a tumour immunology and inflammation monitoring biomarker capacity; and (iv) expansion of our patient derived CTC derived explant (CDX) models of SCLC and direct culture of lung cancer patients' circulating tumour cells (CTCs).

The CEP group is subdivided into eight teams; Preclinical Pharmacology (PP), Nucleic Acids Biomarkers (NAB), Cells and Proteins (CAP), Tissue Biomarkers (TB), Biomarker Bioinformatics (BBI), Quality Assurance (QA), the digital Experimental Cancer Medicine Team (digitalECMT) and CEP operations (OPS). In August 2017, we moved to new facilities in Alderley Park with physically separated Good Clinical Practice (GCP) and exploratory research laboratories, allowing us to improve our workflows for biomarker analysis to GCP. In the past 12 months, our biomarkers' portfolio supported 34 clinical trials (25 academic sponsored, 9 pharmaceutical company sponsored and 9 NIHR badged), and 16 experimental medicine studies. During 2017, we initiated several exciting new collaborations with Merck KGaA, Carrick Therapeutics and Taiho Oncology. Our QA team and CEP biomarker analysts developed an improved process for proportionate assay validation of analytical methodology for exploratory biomarker endpoints in clinical trials. The QA team maintained external links and collaborated with The Christie QA team and CRUK ECMC Quality Assurance in Translational Science (QATS) network to share knowledge, experience and practices.

Research highlights

Discovery of biomarkers for the earlier detection of lung cancer

Working closely with Dr Phil Crosbie and his clinical colleagues at Manchester University NHS Foundation Trust (MFT) we collected, processed and banked blood samples from 748 participants in the Manchester Early Detection of Lung Disease Pilot. This community-based study offered low dose CT scanning in mobile units situated in supermarket car parks across Manchester, designed to optimise participation by the 'high risk, hard to reach' subjects. Sufficient blood was collected and shipped for processing to slides for identification of CTCs and to plasma for analysis of ctDNA, microRNA and proteomic analysis. Analysis of this range of markers is designed to optimise our chances of successful identification of a diagnostic marker for early lung cancer (Figure 1).

To overcome the high technical hurdle for detection of CTCs in early stage disease, we are applying a marker-independent enrichment approach, namely HDSCA (High Definition Single Cell Analysis) developed by our collaborator Professor Peter Kuhn, University South California (USC) supported by NCI and

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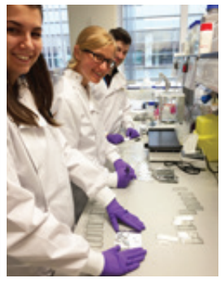


Figure 1. Collection of blood samples from subjects attending a community lung health check after their CT scan in a van. Blood samples were then couriered to our new early detection laboratories in Alderley Park for processing onto slides.

CRUK via the National Cancer Moonshot Initiative. With the tagline "no cell is left behind" the HDSCA process captures all nucleated cells onto slides, allowing staining with a flexible panel of markers and physical picking of single cells for molecular characterisation to confirm their cancer origin.

ctDNA for Phase I trial selection

Following a successful pilot feasibility study that recruited 100 patients, the TARGET Trial led by Dr Matthew Krebs, whereby tumour and ctDNA profiling assist selection of the optimal Phase I trial for Christie patients, has been extended and is now recruiting an additional 350-450 patients (Figure 2). Following the Paterson Building fire our collaborators, Dr William Newman and Dr Andrew Wallace generously provided workspace and support at the Manchester Centre for Genomic Medicine, which enabled re-establishing ctDNA mutation reporting to the TARGET Molecular Tumour Board. Subsequently, the entire ctDNA TARGET process has been re-established at the Alderley Park site and bloods from over 190 TARGET patients have now been profiled with a panel of >600 genes. The comparison of ctDNA and matched archival tumour NGS data continues to show an impressive overlap in detected mutations and increasingly ctDNA is providing current data to assist trial selection.

ctDNA analysis in SCLC

Novel bioinformatic approaches have been developed for examining genome wide copy number alteration (CNA) profiles, which have increased the sensitivity of detecting the presence of tumour DNA in plasma samples. The CNA readouts developed by NAB are based on shallow whole genome next generation sequencing (NGS) and do not require a matched germline sample or a priori knowledge of mutational hotspots. They were designed to be simple, rapid and cheap to enable their use as a first line screen prior to more extensive NGS analysis. In a pilot study of 69 SCLC patients, the CNA assay identified tumour related changes in > 85% of plasma samples and this simple blood based approach is now being extended across a range of tumour types.

The digital Experimental Cancer Medicine Team (digitalECMT)

The digital ECMT (www.digitalecmt.org.) has two primary goals: a) changing the conduct of clinical trials; and b) changing the exploration and interpretation of clinical trial data in real-time. Closely aligned with The Christie Phase I Clinical Trials Unit, this team supports translation of decision science and technology into real-world clinical trials. 2017 saw the delivery of 3 innovations:

Patient-Centred Clinical Trials A Digital Patient Design Lab was established at The Christie Phase I Clinical Trials Unit where the team have daily interactions with patients. Key programmes include: (i) Patient engagement: PROACT (Patient Reported Outcomes About Clinical Tolerability) study – first patients enrolled December 2017; (ii) Hospital-in-the-home: Nephro-oncology (creatinine monitoring) and Febrile neutropenia (neutrophil and temperature monitoring); and (iii) Wearable devices, ECG (continuous ambulatory monitoring) and Fatigue (smart devices).

Decision Science in Clinical Trials REACT (Real time Analytics for Clinical Trials), enables agile study design and an earlier understanding of the patient benefit-risk and has demonstrated value across previous sponsor studies involving over 200,000 patients. Innovations for 2017 were: (i) Oncoprint, where integrated clinical, genomic data, efficacy and exposure visualisations allow a deeper understanding of efficacy and genomic signals during an ongoing clinical trial; and (ii) Single Subject View, an integrated individual patient record visualisation with all the relevant clinical and scientific data for each patient in a trial.

digitalECMT Cloud Infrastructure Both PROACT and REACT have been transitioned to the digitalECMT cloud and organisational collaborative environments extended to include The Christie, CRUK Centre for Drug Development, Carrick Therapeutics and AstraZeneca. The digitalECMT cloud/Microsoft Azure environment significantly enhances our AI/ Machine Learning R&D capability.

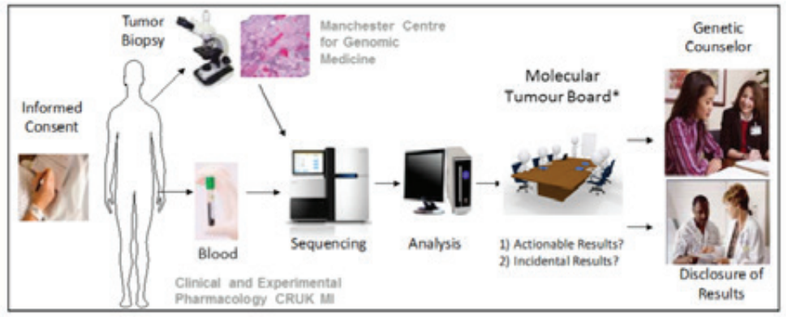


Figure 2: TARGET: Profiling tumour and plasma ctDNA to assist Phase I trial selection.

The Tumour Immunology and Inflammation Monitoring Laboratory (TIIML)

Our TIIML laboratory is being established in Alderley Park within the CAP team to develop biomarker assays to support cancer immunotherapy studies. New staff have been recruited and equipment is being GCP qualified for trial sample analysis, including a new Fortessa Flow Cytometer. Development of a core immune-oncology 'biomarker toolkit' is ongoing, including panels for lymphoid and myeloid cell phenotyping by flow cytometry, assays for circulating cytokines, analysis of PDL1 expression in CTCs and gene expression analysis from FFPE (Formalin Fixed Paraffin Embedded) tissue using the Nanostring platform. The TB Team are developing assays for studies of renal cancer and cancer of unknown primary (CUP) in both a GCP and non-GCP (exploratory) context. Specifically they are developing a multi-colour, multiplex immunofluorescence methodology to study immune cell activation and localisation and analysis of key immune markers in FFPE. The TIIML biomarker toolkit will be expanded, as required by clinical studies, to explore mechanism of action, pharmaco-dynamic effects and predictive and prognostic biomarkers for immunotherapies, including combinations with radiotherapy, chemotherapy and targeted agents. The TRIBE study (Tyrosine Kinase Inhibitor therapy in Renal Cell Carcinoma: Immune Biomarker Evaluation), with Principle Clinical Investigator Dr Fiona Thistlethwaite at the Christie Hospital, designed to evaluate the effect of the tyrosine kinase inhibitors Pazopanib and Sunitinib on the subsequent clinical response to Nivolumab, is the first clinical study that TIIML will support with first samples collected in December 2017.

Molecular analysis of lung cancer CTCs

The NAB team is continuing to build on our observation reported last year that CNA patterns identified in SCLC CTCs obtained prior to patient

Figure 3. Morphological and molecular heterogeneity among SCLC CDX models. Representative sections of SCLC tumours expressing the neuroendocrine transcriptional master regulators ASCL1 (C) or NEUROD1 (D). Expression of the epithelial marker cytokeratin (E) or the EMT marker vimentin (F). Scale bar = 50μm.

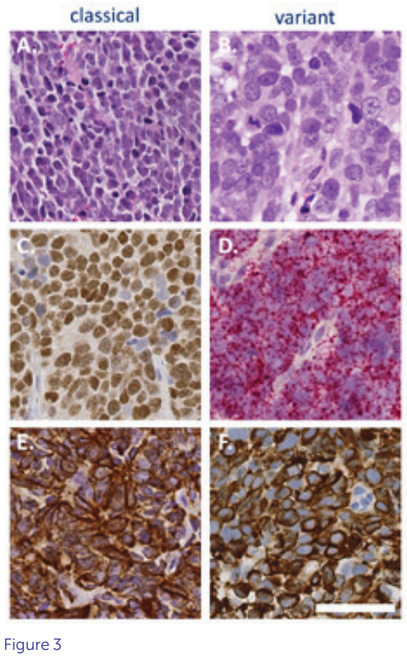


Figure 3

Figure 4. SCLC CTC ex vivo cultures. A) Light microscopy of SCLC CTC cultures depicting non-adherent cell clusters. B) RNA harvested from two to four week old SCLC CTC cultures was subjected to qRTPCR for beta2-macroglobulin reference (B2M), the pan-lymphocyte marker CD45, and the SCLC lineage marker CD56. C) CTC cultures were immunostained for pan-cytokeratin (CK) as a marker of epithelial cells and CD45/CD31 (Lin) as markers of lymphocytes and endothelial cells, as well as DNA (DAPI).

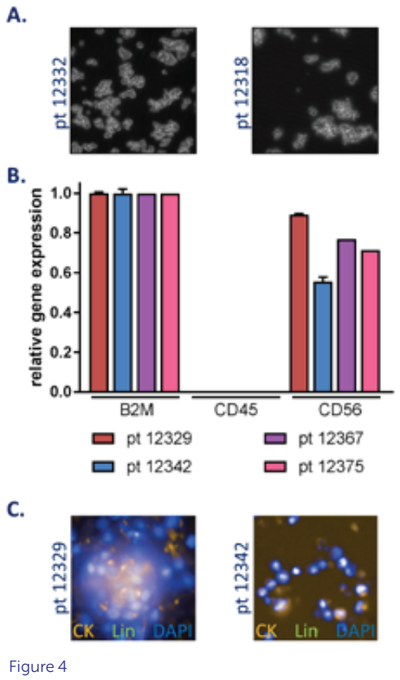
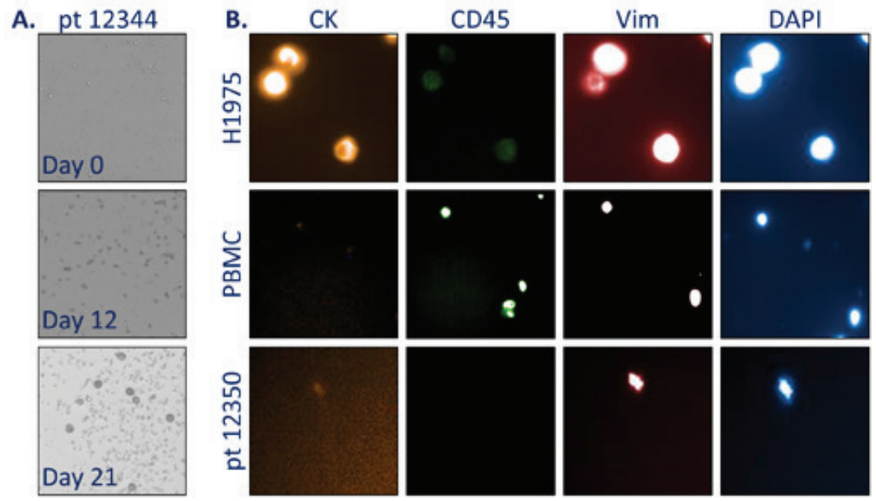


Figure 4

Figure 5. NSCLC ex vivo cultures. A) NSCLC CTCs enriched via RosetteSep were cultured in conditional reprogramming conditioned media under hypoxia for the indicated times and imaged at 20x. B) The H1975 NSCLC cell line, peripheral blood mononuclear cells (PBMC), and an NSCLC CTC culture were immunostained for pan-cytokeratin (CK), CD45, and vimentin to mark epithelial, lymphocyte, and mesenchymal cells, respectively, as well as DNA (DAPI).



treatment correlated with response to standard of care chemotherapy (Carter et al. Nature Medicine 2017). Ongoing studies are now focusing on extending both the number of patients examined and the number of individual CTCs profiled from each patient. To enable expansion of both patient and CTC numbers the team have established a simple means of banking enriched or isolated CTCs prior to analysis and have shown that samples can be stored for at least 2 years without any loss of integrity (Mesquita et al. Molecular Oncology 2017).

As part of the TRACERx (TRAcking non-small cell lung Cancer Evolution through therapy [Rx]) study led by Professor Charles Swanton (UCL), we have continued to examine the genetic status of single CTCs compared to tumour profiles from patients with resectable NSCLC. Building on our initial results, which identified CNA patterns that are shared between CTCs and the matched tumour, we have classified CTCs (identified via the CellSearch platform) on the basis of the expression of surface markers into three types (Figure 2). To better understand the nature of the three CTC types, we utilised single cell whole exome sequencing (WES) and are examining the WES data from single CTCs, primary tumour and relapse samples. The data indicate that the combined single cell CTC and tumour analysis identifies additional layers of heterogeneity that would not be seen by either approach alone furthering our understanding of how tumour clonal heterogeneity impacts upon therapeutic outcomes.

Development of the SCLC CDX panel and establishment of primary CTC cultures

Tumour biopsies are particularly challenging in SCLC, most notably so when patients have progressed during or after their chemotherapy whereas CDX models require only a 10ml blood sample from the patient. This year the PP team expanded our SCLC CDX panel to 45 models encompassing baseline chemosensitive and chemorefractory models and several 'matched pairs' generated from individual patients at baseline and again at progression after treatment with platinum/etoposide chemotherapy. Extensive molecular characterisation has highlighted considerable morphological and phenotypic heterogeneity between these, and the availability of the matched pairs has provided a unique opportunity to study SCLC heterogeneity following chemotherapy and disease progression. Until recently, SCLC was considered a relatively homogenous disease, thus patients received the same treatment with very poor outcomes that have remained unchanged in decades. Within our panel there are CDX models that represent the 'classic' and 'variant' molecular sub-types of SCLC with respect to morphology, expression of neuroendocrine lineage and EMT markers (Figure 3). Although development of CDX models and our ex vivo cultures from disaggregated CDX are a transformative step change for the study of lung cancers, the time it takes to generate and test therapeutic agents in CDX models is incompatible with a clinically actionable time frame. To overcome this limitation, we have successfully expanded CTCs from the blood of SCLC and NSCLC patients. Clusters of cells expressing the prototypical SCLC markers CD56 and cytokeratin were evident within one week of culturing (Figure 4). Similarly, both epithelial cytoketatin-positive and mesenchymal vimentin-positive were identified in NSCLC CTC cultures (Figure 5). Current efforts are focusing on further expanding CTCs as well as the development of small scale functional assays designed for future studies to prioritise clinical treatment selection.

Publications listed on page 58

www.cruk.manchester.ac.uk/Our-Research/Drug-Discovery

DRUG DISCOVERY

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The last year has been a time of change for the Drug Discovery Unit. In April the Head of the Unit, Dr Donald Ogilvie, retired. In October, we welcomed Professor Caroline Springer as our new Director. She joined with many of her existing team from the Institute of Cancer Research, bringing with them several new areas of research for the integrated team to progress. In parallel, many of our established projects are now successfully leaving the research laboratory as they start the path toward clinical testing; new projects, particularly focused on targets from our Manchester Institute colleagues, are now gaining momentum as they take their place in our portfolio of activities.

After disclosure of our PARG programme at the AACR Annual Meeting in 2016, we were invited to deliver two AACR talks this year, which led to a collaboration with a San Francisco biotech. Our joint effort has been very successful and we aim to select our pre-clinical candidate in 2018. We have an extensive network of collaborators, including Stephen Taylor at The University of Manchester, Keith Caldecott (CRUK, Sussex), Helen Bryant (Sheffield), Jos Jonkers (NKI, Amsterdam) and Jonathan Brody (Philadelphia, USA), investigating the role of PARG in various cancer types such as ovarian, breast, melanoma, pancreas and glioblastoma; the latter securing additional CRUK funds (with Anthony Chalmers in Glasgow) in this area of high unmet clinical need.

Our RET programme is advancing toward clinical trials. The Phase I clinical trial for our lead compound is in collaboration with the Experimental Medicines team at the Christie NHS Foundation Trust and our lead compound is progressing well toward Investigational New Drug approval.

In parallel, our on-going epigenetics target DNMT1 collaboration with GSK (Philadelphia, USA) has shown efficacy in several key models of leukaemia, with significant anti-tumour activity. We anticipate nomination of a pre-clinical candidate will follow shortly. Our related epigenetics project, which sought to develop novel, drug-like and reversible inhibitors of LSD1 (a key regulator of acute myeloid leukaemia) in collaboration with Professor Tim Somervaille at the CRUK MI, has also now successfully concluded. State-of-the-art computational chemistry and modelling techniques enabled the delivery of three new classes of inhibitors against this target (Mould et al. 2017a, b, c).

Whilst the licencing of our RET, DNMT1 and PARG projects is very impressive, it has left a significant gap in our late stage portfolio. The transfer of the Springer targets in panRAF, LOX and GDEPT, alongside our Cancer Stem Cell (CSC) targeting project, is very timely as it addresses this gap in our portfolio in the short term. Longer term, our portfolio will be repopulated from the impressive number of early targets which have been sourced from our CRUK MI collaborators and beyond, with leveraged additional expertise. We are particularly excited by the opportunity for discovery of inhibitors for lung cancer, given the academic and clinical expertise present in the Manchester Institute, the Christie Hospital and the CRUK Lung Cancer Centre of Excellence.

We have discovered and developed orally bioavailable, well-tolerated panRAF/SRC inhibitors that are effective in pre-clinical patient derived xenograft models at therapeutic doses, in collaboration with Professor Richard Marais (Girotti et al. 2015). These inhibitors are designed to treat mutant BRAF or mutant RAS melanoma and other tumour types driven by these mutant oncogenes. A Phase I clinical trial of our panRAF/SRC drug (BAL3833) continues at The Christie and The Royal Marsden NHS Foundation Trusts, with biomarker analysis in collaboration with Professor Caroline Dive at CRUK MI. The DDU remains involved and we are continuing our assessment of resistance and combinations in preclinical models in vivo. The panRAF programme was licenced to Basilea Pharmaceutica in 2015.

In lead optimisation, our lysyl oxidase (LOX) programme targets an enzyme that regulates cross-linking of structural proteins in the extracellular matrix and is important for the primary tumour growth and metastasis in several cancers, including breast, colorectal and pancreatic cancer. This is a challenging target for drug discovery as there is no protein crystal structure or homology model and it has proved impossible to express the recombinant active protein using existing systems. Despite these hurdles, with Wellcome Trust funding for a drug discovery programme against this target, and in collaboration with Professor Richard Marais, we have run an external high throughput screen and synthesised orally bioavailable small molecule LOX inhibitors. We have now identified a late stage preclinical candidate (10008) with good pharmacokinetics and significant therapeutic activity (Tang et al, 2017). This compound has efficacy against a number of primary tumour models. Very pleasingly, we are able to demonstrate reduction of established lung metastases using this compound following daily oral treatment in a genetically engineered breast cancer mouse model. Looking forward, we will be assessing the pancreatic stromal signalling networks changes on administration of our drugs in collaboration with Dr Claus Jørgensen at the CRUK MI. We will develop our inhibitors further and select drug candidates to progress to toxicology studies in 2019 before moving into early clinical trials in patients. Metastasis is the main cause of death in cancer, and we are excited to assess the potential of our LOX inhibitors to treat primary tumours and metastases in patients.

An earlier stage project aims to target cancer stem cells (CSCs), which are subpopulations of cells that evade therapy and perpetuate tumour growth. CSCs are described as initiating cancer, propagating cancer spread, driving drug resistance and leading to treatment relapse. Thus there is a need for drugs to target the CSC subpopulation within tumours, to use in combination with the standard of care. We have discovered potent and selective inhibitors of our CSC target and are progressing these compounds towards evaluation in efficacy studies. To support this project, crystallography studies are ongoing at Leicester University (funded by a CRUK Accelerator Award) and we are collaborating with Professor Richard Marais and University of Manchester based expert Dr Robert Clarke, in elucidating the biology of our target.

In addition, we are working on gene-directed enzyme prodrug therapy (GDEPT) treatments, in collaboration with Professor Richard Marais, using newly engineered vaccinia viral vectors designed to target tumours selectively. The tumours become 'mini-factories' expressing exogenous bacterial enzyme which is able to convert subsequently administered prodrugs to cytotoxic drugs. Using this approach, we have demonstrated selective tumour targeting with the viral vectors and long-term tumour xenograft regressions in mice. We will assess the potential to elicit beneficial immune responses to the virus and the expressed bacterial enzyme, in collaboration with Dr Santiago Zelenay at the CRUK MI.

To manage future opportunities, we will establish a scientific review committee for selection of new collaborative targets between the DDU and CRUK MI group leaders. We will ensure that all our DDU projects are integrated with Professor Caroline Dive's biomarker discovery programme so that all nominated targets have selection and predictive biomarkers and with the excellent committed clinicians in the Christie NHS Foundation Trust. In early 2018, we will move to our temporary laboratories in Alderley Park. After the disruption in 2017, we look forward with excitement to working in these new laboratories as a fully integrated DDU. We are extremely fortunate to have a portfolio of numerous projects with excellent CRUK MI collaborators, ranging from target validation to Phase I clinical trials, on many different targets involved in a range of cancer indications.

Publications listed on page 61

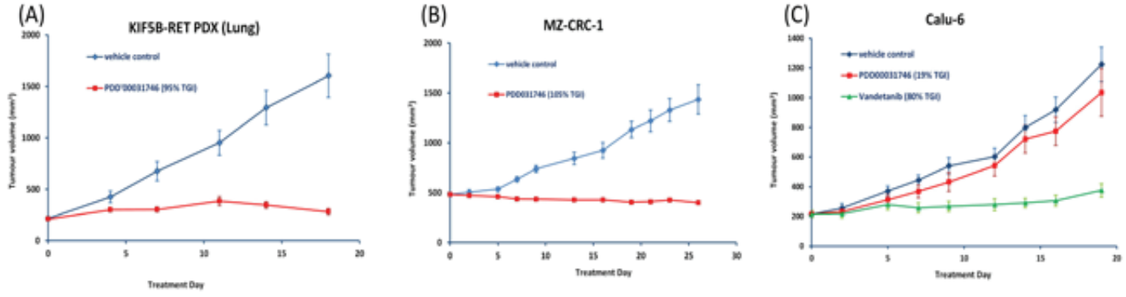

Figure 1. PDD00031746 significantly reduces the volume of three different RET-driven tumours.
PDD00031746 shows substantial tumour growth inhibition (TGI) in the (A) lung cancer patient derived model KIF5B-RET and the (B) thyroid cancer-derived MZ-CRC-1 at well-tolerated doses. Unlike the non-selective RET inhibitors such as vandetanib, (C) PDD00031746 does not inhibit the in vivo growth of non-RET driven cancer cells such as Calu6. This enhanced selectivity may translate to improved efficacy and fewer side effects in the clinic.

LEUKAEMIA BIOLOGY

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[1]Joined in 2017
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The cardinal pathologic feature of the acute leukaemias is a block to normal blood cell differentiation, which results in an accumulation in the bone marrow of incompletely differentiated blast cells, and failure of normal haematopoiesis. While the spectrum of mutations associated with these diseases is now well established, the biologic basis of how mutations interact with one another to establish the pathognomonic differentiation block is less well understood. We reported in 2015 that the Forkhead box transcription factor FOXC1 is misexpressed in approximately 20% of patients with acute myeloid leukaemia (AML), contributing to the development of the disease.

This year we completed our studies of another transcription factor gene called IRX3, which is misexpressed even more widely in acute leukaemia than FOXC1. While transcript levels are very low in normal haematopoietic cells, high IRX3 expression is found in ~30% of patients with AML, ~50% with T-acute lymphoblastic leukaemia (ALL) and ~20% with B-ALL and, like FOXC1, contributes to the differentiation block which is characteristic of the disease. Future studies will identify approaches to target these transcription factors for novel, pro-differentiation therapies aiming to improve patient outcome.

Transcription factors are critical regulators of cell fate decisions and it has long been known that some of the transcription factor genes which are essential for normal blood cell development are targeted by genetic mutation in acute leukaemia. Well known and frequently occurring examples include AML1-ETO (*RUNX1-RUNX1T1*), *CBFB-MYH11* and the range of *KMT2A* fusions. We recently noticed that some transcription factor genes which are highly expressed in AML, including in the AML stem cell compartment, are either minimally or completely unexpressed in normal haematopoiesis. The Forkhead box transcription factor gene *FOXC1* was the first example we studied. This gene is misexpressed in approximately 20% of patients with AML, in particular in those cases exhibiting high HOXA/B gene expression. *FOXC1* is neither required for nor expressed in normal hematopoietic cells, but is essential for normal development of mesenchymal tissues such as the skeleton, heart and eye, and for the normal function of bone marrow niche cells. Its misexpression in leukaemic hematopoiesis contributes to a block in differentiation along both monocytic and B-cell lineages, and is associated with inferior survival of patients. Given the lack of one-to-one correlation with any specific mutation, tissue inappropriate derepression of *FOXC1* is paradigmatic for a non-mutational mechanism contributing to cellular transformation in myeloid cancer.

Whether tissue inappropriate misexpression of other transcription factor genes contributes to the differentiation block of leukaemia is not known. One candidate is the Iroquois homeobox transcription factor gene *IRX3* which, like *FOXC1*, is expressed in a significant proportion of patients with AML (Figure 1) but minimally expressed in both normal human CD34$^+$ stem and progenitor cells and mature blood cells. IRX3 is a member of the three-amino-acid-loop-extension (TALE) superfamily of homeodomain transcription factors which also includes MEIS1 and PBX1. In embryogenesis it is strongly expressed in the developing nervous system, as well as in mesoderm-derived tissues such as the limb buds, kidney and heart. Of note, the developmental expression pattern of the *Irx3* paralog *Irx5*, which sits in the same 2MB topologically associating domain, is strikingly similar. These genes exhibit functional redundancy because although *Irx3* null and *Irx5* null mice are viable and fertile, mice lacking both genes die in utero due to severe cardiac and skeletal defects. Interestingly, non-coding variation in an enhancer region 500kB downstream of IRX3 provides the strongest genetic association with risk for human obesity. Pertinent to this, adult *Irx3* null mice display a 25-30% reduction in body weight due to loss of fat mass, increased basal metabolic rate and browning of white adipose tissue, attributable to loss of hypothalamic or preadipocyte *Irx3* expression. The rs1421085 single nucleotide variant present in the obesity risk region dictates the extent of local recruitment of ARID5B to the *IRX3* enhancer with consequent regulation of *IRX3* expression.

Whether IRX3 has a role in human malignancy has been unclear. One study reported that IRX3 is strongly expressed in colorectal adenomas by comparison with normal mucosa and negatively regulates TGFβ signalling in colorectal cancer cell lines. However, little else is known. Given this, and the observation that IRX3 is highly expressed in a subset of AML patients, we evaluated whether IRX3 has a functional role in acute leukaemia.

Based on quantitative PCR analysis of patient samples from The Christie, as well as analysis of published datasets, we established that while transcript levels are very low in normal hematopoietic cells, high level *IRX3* transcript and protein expression is found in ~30% of patients with AML. In contrast to our findings for FOXC1, high level IRX3 expression is also found in ~50% of patients with T- ALL and ~20% with B-ALL, almost invariably in association with high *HOXA* gene expression. From a functional point of view, IRX3 alone was sufficient to immortalise murine bone marrow stem and progenitor cells in myeloid culture and induce lymphoid leukaemias in vivo; knockdown of *IRX3* expression in human AML cells induced their terminal differentiation. Together these experiments indicate that IRX3 contributes to the differentiation block of human acute leukaemias of multiple lineages.

Given the frequent association of combined *IRX3* and *HOXA9* expression in human leukaemia cells, we performed co-expression experiments and observed that the addition of *IRX3* expression to *HOXA9* expression impeded normal T-progenitor cell differentiation and enhanced the differentiation block of the resulting AMLs in murine transplant experiments. Intriguingly, our murine experiments gave results that overlapped very significantly with data derived from human leukaemia cell transcriptomes: in human AML, high *IRX3* expression strongly associates with impaired myelomonocytic differentiation, likely through variable suppression of genes such as IRF5, IRF8, and GFI1 which are required for normal terminal myelomonocytic differentiation. Thus overall our data, which were published in Cell Reports in January 2018, reveal an entirely new and frequent oncogenic mechanism - the tissue-inappropriate derepression of the Iroquois homeodomain transcription factor gene *IRX3* which contributes to the pathognomonic differentiation block in acute leukaemia (Figure 2).

A key question for us moving forward is whether we can identify ways to target these misexpressed transcription factors to promote differentiation of leukaemia cells in patients. The aim would be to restore normal repression of genes such as *FOXC1* or *IRX3*, or to promote degradation of the protein products of these misexpressed genes in leukaemia cells. Critical next steps will be to understand the epigenetic regulation of these genes in normal and leukaemic cells, to map enhancer elements that regulate the misexpression and to understand which transcription factors are responsible for maintaining or reversing repression.

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Figure 1:
Representative images of IRX3 immune staining of human bone marrow trephine biopsies.

Figure 2:
Summary of consequences of IRX3 misexpression.

Figure 1

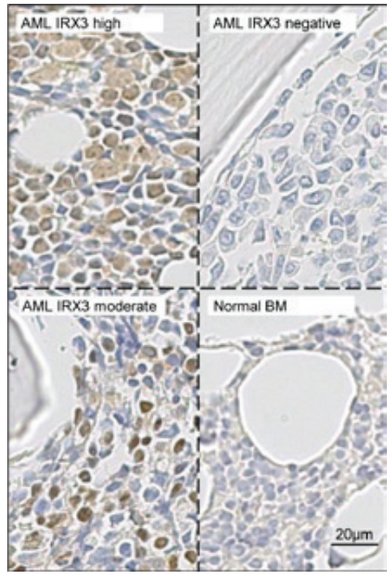
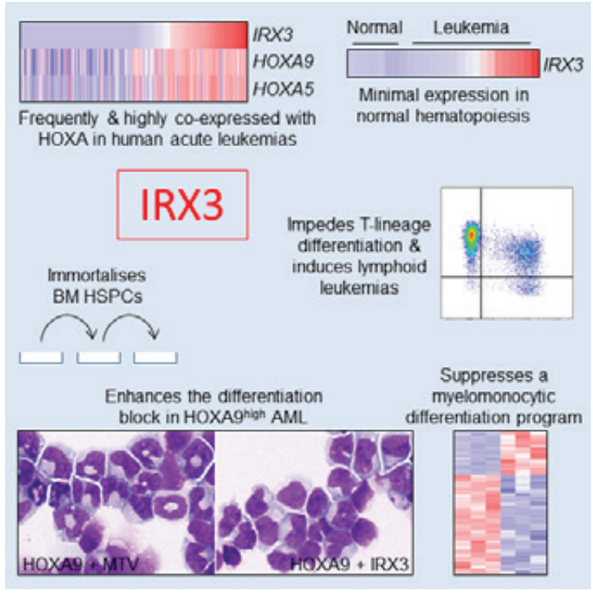


Figure 2



MOLECULAR ONCOLOGY

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Our group studies cancer biology and works to generate the new knowledge that we need to improve cancer patient care. Taking a multi disciplinary approach in which scientists and clinicians work closely together, we are making significant inroads into developing new diagnostic tools and optimising treatment modalities for cancer patients.

The past decade has seen enormous advances in treatment options for patients with metastatic melanoma, leading to significant improvements in patient survival. Treatments targeting the most common melanoma driver, a mutated form of the protein *BRAF*, and additional nodes in the signalling pathway in which *BRAF* is active, can stop tumour growth and extend patient survival for many months. Therapies designed to harness a patient's immune system to effectively attack the tumours have also been shown to prolong patient survival and in some cases even lead to cures. As more therapeutic options become available, and as they grow ever more effective, our ability to accurately assess how the disease course is affected by them, how they should be scheduled, who will benefit from them and how early disease can be detected and treatment initiated becomes crucial to optimise their use and provide the most patient benefit.

We have focused several studies on whether such questions can be answered by studying circulating cell free DNA (cfDNA) in patient blood samples. The benefits of being able to quickly and easily take these so-called 'liquid biopsies' at multiple stages of disease management are clear – particularly in the case of metastatic melanoma, where patients may have multiple sites of disease across a variety of often difficult to access organs. Moreover, CT scans are a costly and inconvenient way of assessing disease burden and tumour response to treatment, whereas taking blood is minimally invasive, relatively simple to do and inexpensive. cfDNA consists of DNA released into the circulation by dying cells and is thought to be derived from both healthy and cancer cells. Most cfDNA in healthy individuals is released from the bone marrow and white blood cells, whereas cfDNA in cancer patients (termed circulating tumour cell free DNA or ctDNA) is derived largely from cancer cells and carries the same genetic features present in the primary tumour and metastatic deposits.

CT scans to assess the extent of a patient's disease over the course of their treatment take significant time to analyse in depth, which is why clinical assessments and surrogate markers of disease burden, such as the levels of lactate dehydrogenase (LDH) in the blood, are currently more commonly used to calculate disease burden and progression. We wanted to examine if we could develop a more accurate way to estimate tumour burden and progression in melanoma patients, using a straightforward blood test. We therefore studied the levels of circulating cell free DNA (cfDNA) in the blood of melanoma patients and compared them to detailed summations of measurements of all tumour sites within a patient. We showed that total levels of cfDNA are a highly accurate reflection of disease burden and revealed that the comparative level of cfDNA in a patient with a given tumour burden is prognostic of their overall survival.

Liquid biopsies can also be used to tackle another challenge in melanoma patient care – assessing which patients are at highest risk of aggressive disease or relapse as early as possible. Eighty percent of melanoma patients are cured of their disease by surgery however, 20% go on to develop metastatic disease. We wanted to be able to identify these patients sooner to facilitate earlier treatment, which is known to give improved survival. In a recent study, we examined whether an increase in tumour ctDNA levels in patients' blood after surgery intended to cure their stage II/III disease was predictive of disease relapse. We showed that patients who had ctDNA 12 weeks after surgery had a decreased disease-free interval and distant metastatic disease-free interval, and also that their overall survival was significantly worse (Figure 1). Thus we were able to show that ctDNA levels after surgery can identify a subgroup of patients at higher risk of disease recurrence.

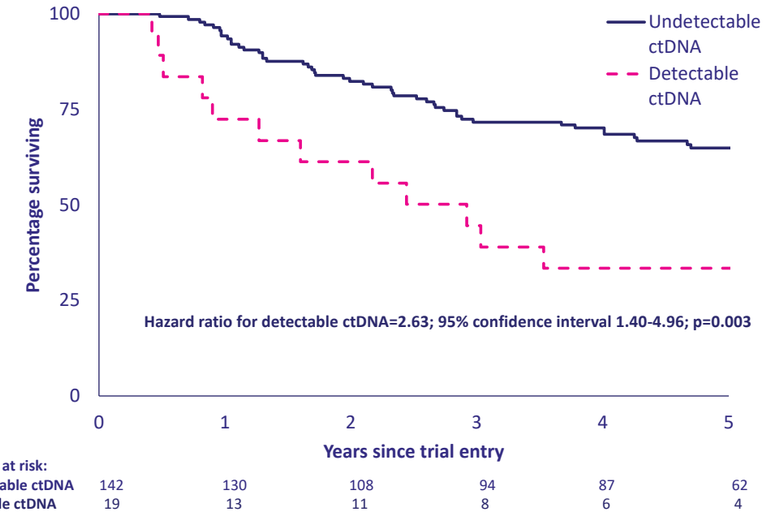

Figure 1:
Kaplan-Meier curve for overall survival (OS) of patients. Median OS was 2.9 years (95% CI 0.9–limit not reached) with detectable ctDNA within 12 weeks of surgery with curative intent compared with the median not reached for those with undetectable ctDNA (95% CI 6.0–limit not reached).

Our results suggest that ctDNA can be used to detect melanoma earlier and also to monitor how patients are responding to treatment. Our data suggests ctDNA can even be used to understand mechanisms of resistance. We are now testing our findings in clinical trials designed to test the potential of ctDNA. One such trial, which we will initiate in 2018, aims to maximise treatment response in melanoma by using changes in ctDNA levels to tell us when to bring in a second line of treatment. The development of this trial is a testament to our extraordinarily successful relationship between laboratory and clinic, allowing us to bring our scientific findings into clinical use.

At the heart of our vision to improve patient care we remain dedicated to understanding cancer biology, particularly the mechanisms of cancer initiation and progression and developing new therapeutic options for cancer patients. In a recent study, we demonstrate that lysyl oxidase (LOX), a protein whose presence in tumour tissue is linked to poor outcomes, regulates signalling through the epidermal growth factor receptor (EGFR). We showed that LOX traps EGFR at the surface of tumour cells and facilitates its activation by EGF. This, in turn, can drive tumour progression and metastatic spread. To measure LOX activity in live cells, we developed a biosensor that fluoresces in the presence of hydrogen peroxide, a by-product of LOX activity, and we showed that a pharmacological inhibitor of LOX, CCT365623, which has come out of our drug development programme with Caroline Springer (Director of the Drug Discovery Unit), was able to decrease LOX activity (Figure 2). The inhibitor disrupts EGFR retention and delays the growth of primary and metastatic tumour cells in an animal model of breast cancer. Thus, we have made some exciting discoveries about the role of LOX in cancer development, and in parallel are developing therapeutic agents to target this important protein.

By taking the approaches above and working closely with the clinical team, we are in a position to rapidly translate our scientific discoveries into the clinic. Our continued development of clinical trials will allow us to realise our aim of improving patient care.

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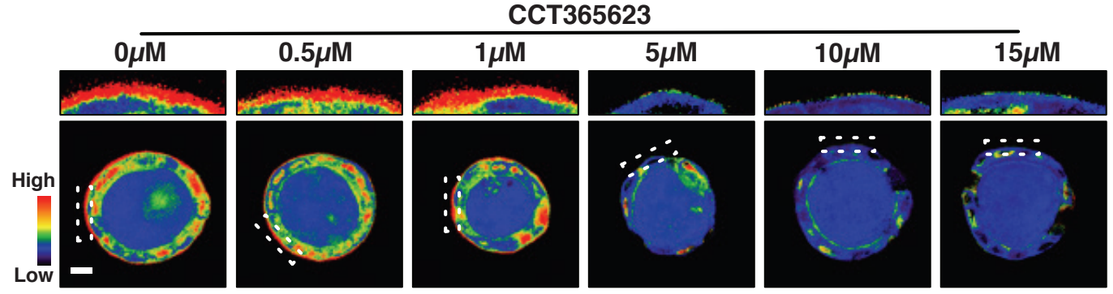

Figure 2:
CCT365623 inhibits LOX enzyme activity reported by a LOX biosensor. Confocal ratio images showing LOX activity (with red depicting highest activity) in MDCK cells grown as cysts in matrigel, treated with increasing concentrations of CCT365623. Scale bars, 10μm.

PROSTATE ONCOBIOLOGY

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A major challenge in cancer research is to understand the cellular and molecular basis of tumour heterogeneity and how this heterogeneity contributes to lethal cancer. Despite large-scale DNA sequencing, the mutational landscape of prostate cancer (PCa) cannot fully explain the clinical heterogeneity of the disease. Indeed, the occurrence of castration-resistant prostate cancer (CRPC) following androgen-deprivation therapy is a strong indicator that within the prostate tumour there may exist subpopulations of intrinsically castration-resistant progenitor cells capable of driving tumour progression toward more aggressive disease.

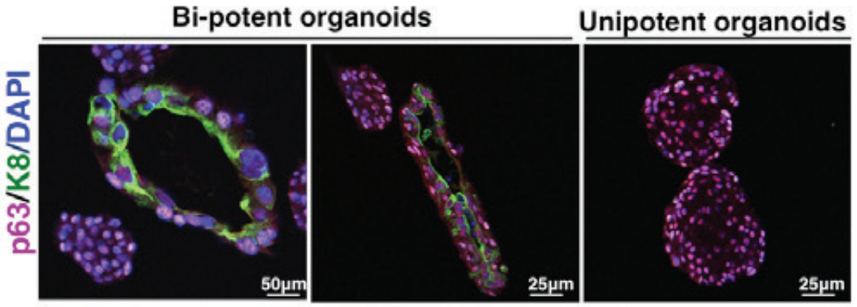
Upon castration, most basal cells and a small portion of luminal cells survive, suggesting these prostate cells may be intrinsically castration-resistant. However, the identity of such castration-resistant prostate cells and their contribution to CRPC remains elusive. Understanding the multistep process of prostate neoplastic transformation would undoubtedly facilitate the development of new diagnostic and prognostic markers as well as better therapies.

Intrinsic and acquired resistance place a substantial limit on the clinical effectiveness of current prostate cancer therapies. Advances in single-cell profiling, organoid-culture and in situ lineage-tracing analysis have enhanced the ability to identify stem/progenitor cell subpopulations in normal tissues, and interrogate their contribution to tumour subtypes thereby advancing patient stratification (Donati and Watt 2015; Visvader 2011; Clevers 2016). It is currently assumed that prostate basal cells and a subset of luminal cells are inherently resistant to androgen-targeted therapies (Isaacs 2008; Shibata and Shen 2015; Zong and Goldstein 2013). Despite these efforts, the identity of CR prostate cells in vivo, particularly CR luminal cells, and their contribution to CRPC remain largely unresolved. We have therefore been building a pipeline to define the identity of inherently castration-resistant (CR) prostate subpopulations and determine their role in the initiation of castration resistant prostate cancer (CRPC).

To address these, we have examined the heterogeneity of prostate epithelium by a novel Fluidigm multiplex quantitative PCR (qPCR)-based single cell expression analysis, focusing on cell surface markers. Importantly, in order to identify and characterise the cells resistant to androgen deprivation, we compared expression profiles of prostate cells sorted from hormone naïve (HN) versus surgically castrated mice at the single cell level, and coupled the analysis with organoid culture and in situ lineage-tracing. Our analysis shows that the prostate epithelium is highly heterogeneous, particularly in the luminal compartment. We identified a subset of HN luminal cells, characterised by castration-resistant growth (CR luminal cells), which exhibit a similar "lineage-intermediate" gene expression pattern, including expression of multiple prostate stem and progenitor marker genes and the androgen receptor gene. This finding led us to hypothesise that some of these cells may contribute directly to prostate cancer initiation and progression to the castration-resistant state. We have defined and validated a new marker for prostate luminal progenitors that has not been implicated in prostate biology. We found that this newly defined prostate subpopulation has a higher organoid-forming capacity, and they are multipotent in vitro (Figure 1). Of note, we found that they formed organoids in an androgen-independent manner.

Our immediate goal was then to determine whether CR prostate cells contribute to the initiation of treatment-resistant prostate cancer, and then determine whether targeting those cells would decrease tumour recurrence. In particular, we focused on the luminal lineage, as prostate tumours are characterised by a luminal phenotype and absence of detectable basal cells. Conditional genetic marking permits both lineage and temporal control of genetic alterations, thereby allowing initiation of PCa

Figure 1:
Co-IF analysis of organoids derived from CR luminal prostate cells for Keratins (K5 and K8) and p63 expression. Bipotent organoids contain $K5^+P63^+$ and K8+ cells, while unipotent organoids either $K5^+P63^+$ or $K8^+$ cells.



specifically in CR or HN luminal cells (Figure 2). By comparing the phenotypes of PCa arising from different luminal cells of origin, we obtained evidence to support that tumour lesions originating from CR luminal cells are more advanced. Indeed, PCa patients expressing high RNA levels of the inherently-CR markers at diagnosis correlates with aggressive disease and disease recurrence, and more importantly, with development of CRPC. To assess how mutations in the CR stem/progenitor subpopulations might promote tumour initiation/progression to CRPC, we are currently characterising mouse and human models with several genetic alterations such as Pten deficiency and ETS factors overexpression (i.e ETV1). We are currently evaluating in vitro and in vivo the requirements for selected ETV1/PTEN-associated targets for tumour growth and resistance to treatment using mouse tissue and human PCa primary cell organoid culture.

In parallel, these novel cell-surface markers are under evaluation in human prostate cells and patient tissue microarrays. We then wanted to assess the contribution, if any, of newly defined CR cells in the clonal localised tumours to metastatic sites, such as lymph node associated metastasis. For that purpose, we first focused on implementing a more comprehensive prostatectomies sampling method that sustains in depth characterisation of intra-patient heterogeneity. In close collaboration with the Manchester/Belfast Prostate Cancer of Excellence, we have established a new patient sample collection and its genetic characterisation, which demonstrates the need to incorporate new sampling procedures to improve patients' diagnosis and prognosis.

We envision that our current research program will facilitate the development of patient-specific therapies, and will serve as a valuable resource in understanding the roles of prostate luminal stem cells and their niche reprogramming in castration-resistant tumour development.

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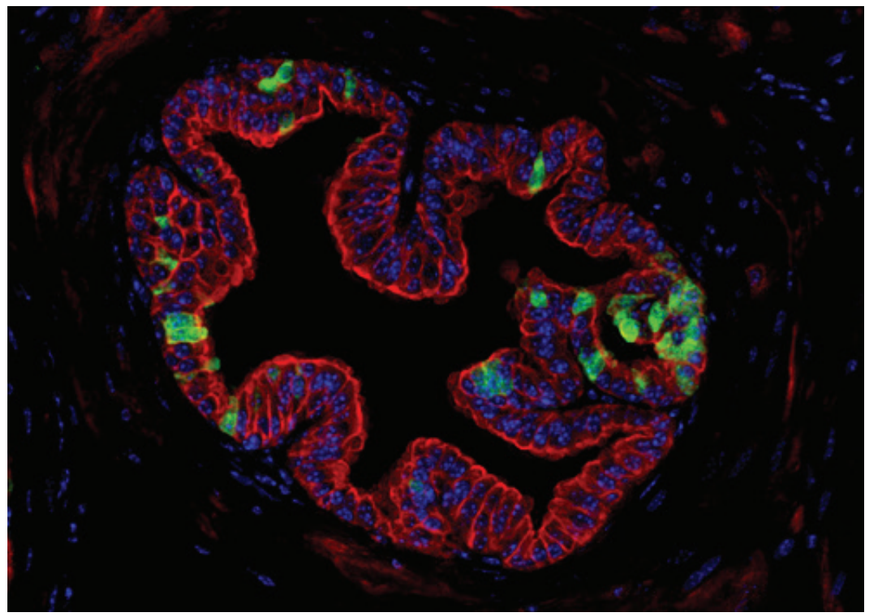
Figure 2:
In vivo luminal-cell-specific tracing. co-IF showing in the prostate of hormone-naive males carrying an inducible luminal-specific CRE traceable by YFP expression after tamoxifen administration. Red, keratin 8. Nuclei counterstained with DAPI.



RNA BIOLOGY

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The RNA Biology group is interested in how different sets of genes work together in a cell, and how their patterns of behaviour are altered in tumour cells. We are particularly interested in long noncoding RNAs (lncRNAs). These are encoded by genes that do not directly lead to the production of a protein, but are increasingly being shown to act as part of the regulatory systems within a cell.

Of the ~12 billion nucleotides that comprise the human genome, less than 2% directly encode amino acid sequences. Despite this, the majority of the genome is transcribed into RNA. We are focused on the extent to which these noncoding RNA sequences are functional, and how they impact on tumour growth and maintenance. Recent advances in genomic sequencing have led to the identification of tens of thousands of novel noncoding genes – to the extent that they are now known to outnumber protein-coding genes.

While the recent discovery of lncRNAs means that the vast majority have yet to be characterised, where they have been studied in detail they have been revealed to perform a wide variety of different functions in all parts of the cell. lncRNAs are therefore emerging as a critically important, but poorly understood aspect of the human genome that is increasingly implicated in the processes that underpin cancer.

In previous work we made substantial use of the model system fission yeast *(Schizosaccharomyces pombe)* to study the basic mechanisms by which noncoding RNAs function. These revealed an extensive network of regulatory noncoding RNAs that act upon the genome to control gene expression. Since the core mechanisms that underpin these processes are evolutionarily conserved within humans, this strongly suggests that there will be similar types of lncRNAs acting in human cells. Current work in the group is directed at seeking lncRNAs in human cells that act in a similar regulatory capacity. Our data are revealing many lncRNAs that interact directly with DNA sequences, and we are therefore particularly focused on those that directly associate with chromatin.

High performance computing and big data analysis

The RNA Biology group is highly interdisciplinary, and makes use of computer science and Artificial Intelligence techniques to analyse the increasingly large amounts of cancer genomics data that are becoming available. The CRUK MI has a large High Performance Computing (HPC) cluster (page 54) allowing us to employ big data techniques to cluster datasets, to seek patterns in the these data, and to ask how these patterns change in tumour cells. Part of the challenge of using these systems is to develop the underlying computational infrastructure needed to best exploit the computational power on offer (Smowton et al., 2017). Our initial work in fission yeast involved exploiting deep sequencing approaches to perform de novo re-annotation of genes within the genome, allowing us not only to identify new protein coding genes, but also to find novel noncoding RNAs. This historical interest in genome annotation has continued within the group and we have developed annotation pipelines that allow us to identify both known and novel noncoding RNAs and to ask how transcript structure changes according to context.

Long noncoding RNAs

We are then combining these global datasets derived from human tumours with cell line models in which we consider transcripts that change in levels in response to different stimuli. This allows us to find lncRNAs that are both clinically relevant and mechanistically important. We are then able to characterise these transcripts through conventional molecular biology approaches combined with with functional genomics techniques including ChIP-Seq, ChIRP-Seq, and RAP-MS.

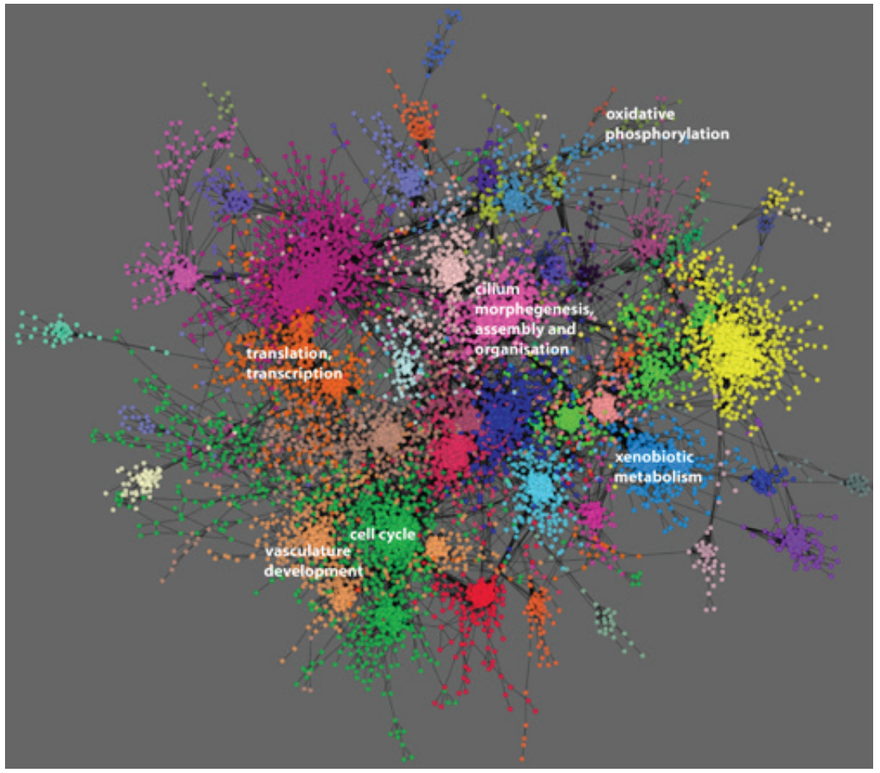

Figure 1: Mathematical techniques from Big Data analysis applied to gene expression data.
Co-expression networks were built from tumour data and partitioned using the Louvain optimisation algorithm to reveal modular architecture (modules indicted by colour). Different biological processes are performed by genes in different modules (representative annotation shown).

A major aspect of our work is to ask how noncoding RNAs influence the response of the cell to changes in oxygen levels. This is important because the majority of solid tumours are hypoxic, in part as a consequence of poorly formed vasculature within the tumours. Hypoxia is known to be associated with poor patient outcome and resistance to therapy. It impacts on multiple pathways throughout the cell. Understanding how noncoding RNAs affect these processes is therefore critical to understanding of the underlying mechanisms that govern tumour growth and maintenance.

We are also applying these integrated bench- and computational strategies to collaborative projects with other groups. A Prostate Cancer UK funded graduate student in the group is seeking noncoding RNAs that can help distinguish between indolent and progressive disease. He is doing this in collaboration with a bench student working in the Prostate Oncobiology group (page 32).

Sensitive and accurate processing of cancer genomics data sets

Our interest in genomics also leads to a focus on the computational techniques used to process and analyse these data. In the last year we have developed software to improve the quality of sequencing data arising from patient and circulating tumour cell derived xenograft (PDX/CDX) models. These tools improve the quality of mutation calling and differential expression analysis from high throughput DNA and RNA sequencing datasets (Khandelwal et al. 2017). In collaboration with the Clinical and Experimental Pharmacology group (page 22) we also developed a new Bioconductor package for analysing Digital Droplet PCR (ddPCR) data (Chiu et al. 2017). ddPCR is a new technique for quantifying specific nucleotide sequences. It is particularly sensitive and thus amenable to tasks that involve the detection of small amounts of DNA. One such application is the detection of small amounts of tumour DNA (ctDNA) from within a much larger background of normal DNA in circulating free DNA (cfDNA) extracted from patient blood.

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SKIN CANCER AND AGEING

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Cancer is more common in elderly patients and melanoma incidence continues to rise predominantly in this population. More than 80% of melanoma deaths occur in patients who are older than 50 years of age, and mortality is specifically increasing in the elderly. Older patients are more likely to suffer from multiple melanocytic and non-melanocytic skin cancers.

Melanomas in the elderly more frequently present as aggressive primary lesions, thick primary tumours that predominantly comprise the high-risk primary melanoma stages (Stage IIB-IIC). The overall survival for stage IIB-IIC patients of all ages at 5 years is 60% and 45%, respectively, despite being localised to the skin and non-metastatic at diagnosis. There is a gradual decline in 5-year survival with increasing decades of life, with an almost 20% decrease from ages 60 to 69, to ages greater than 80 years. Additional characteristics of poor prognosis, such as ulceration and elevated mitotic rate, are also more common in the elderly. However, even after taking the main prognostic factors into account there is a survival discrepancy between elderly and young patients, and age is the strongest independent adverse prognostic factor together with tumour thickness.

We focus our research on understanding the changes in aged skin that promote melanoma and the biology of aggressive disease affecting the elderly, in order to identify new strategies of adjuvant therapy.

This year, we have focused our study on the aged microenvironment. We have examined how the aged stroma contributes to melanoma invasion at the early stages of disease. We have been fortunate to obtain aged human normal skin specimens from skin cancer patients to model melanoma behaviour. Our preliminary data shows that aged stromal cells can influence melanoma cell migration, and this ultimately influences melanoma survival in the elderly population. Our preliminary work this year reveals possible adjuvant strategies for elderly patients with high-risk primary melanoma that has been surgically removed, as well as interventions that can decrease patient survival. Understanding the mechanisms that drive poor outcome in the elderly represents a critical current problem in melanoma care.

We have also performed initial studies to investigate how permanent damage to the epidermis in aged patients changes cutaneous homeostasis, premalignancy, and cancer initiation; and we will continue exploring this in depth in the coming months.

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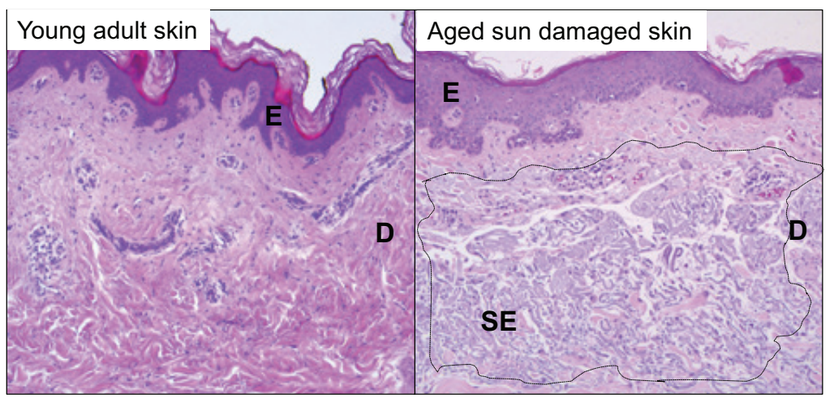
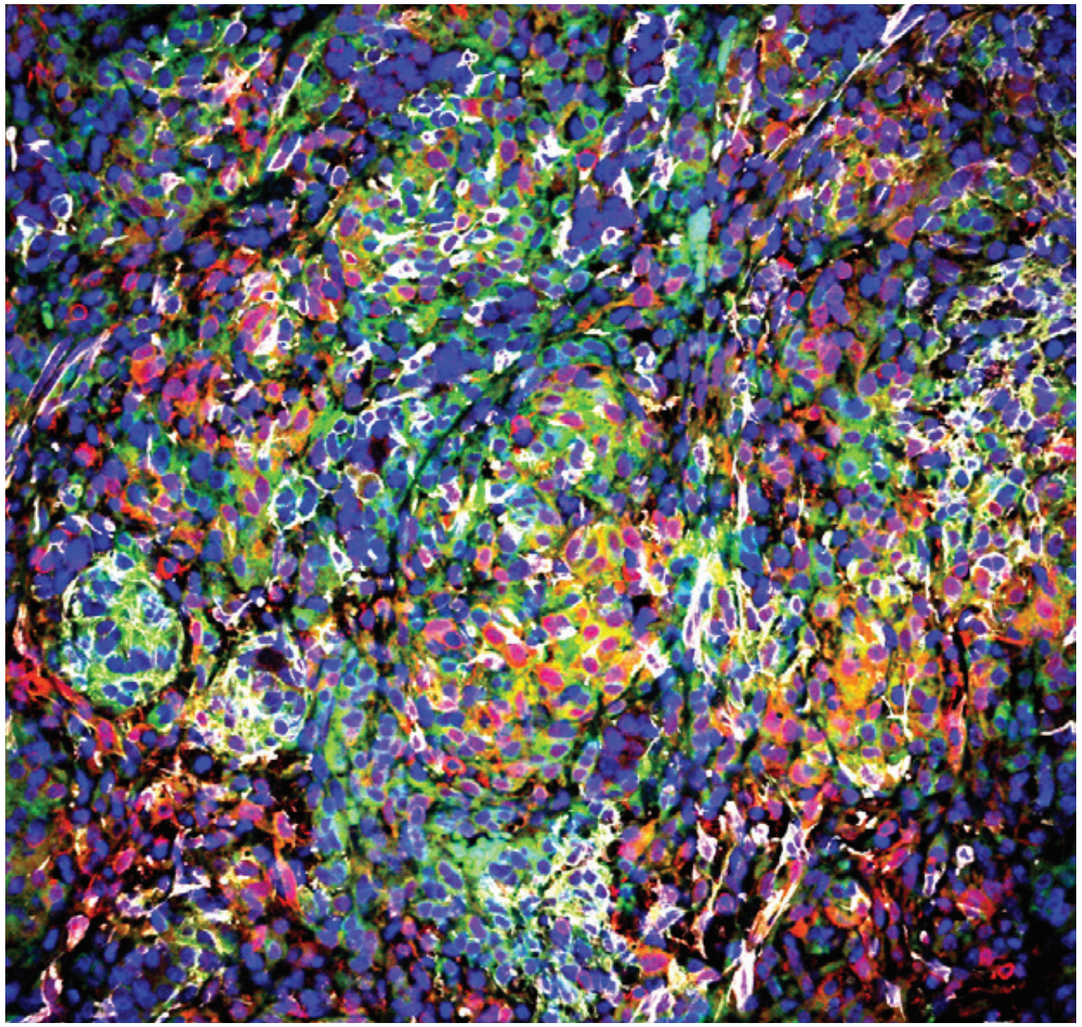


Figure 1:
Left panel: young skin depicting healthy epidermis (E) and dermis (D) of forearm skin. The epidermis presents natural ridges that penetrate into the epidermis. The collagen in the dermis is organised in bundles. Right panel: by contrast, the epidermis in the aged, sun damaged skin is thickened and has lost the ridges. In the dermis, there is collagen and elastic fiber degeneration forming solar elastosis (SE).



Confocal photomicrograph of PyMT mouse breast tumour. Cells were labelled with FGF1 (Red), pFGFR1 (Green), smooth muscle actin (Grey) and DAPI (Blue).
Image supplied by Haoran Tang (Molecular Oncology)

STEM CELL BIOLOGY

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Genes encoding the AML1/RUNX1 transcription factor and its cofactor CBFβ are frequently rearranged or mutated in human leukaemias, such as acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL). Consistent with its implication in leukaemia, RUNX1 has also been shown to be critical for haematopoietic development. Our group studies the function of RUNX1 in haematopoietic development and maintenance in order to better understand how alterations of these functions might lead to leukaemogenesis.

Unravelling the critical role of the RUNX1C isoform in mekagaryopoiesis

Haematopoiesis is a complex, multistep process involving increasingly restrictive cell fate decisions by self-renewing multipotent haematopoietic stem cells. Commitment to the different haematopoietic lineages - lymphoid, granulocytic/monocytic, megakaryocytic and erythroid - occurs through differentiation of immature progenitors. The Core Binding Factor transcription factors RUNX1 and CBFβ play key roles at different stages of this process, activating or repressing transcriptional targets. RUNX1 (also known as Acute Myeloid Leukaemia 1 or AML1) is a master regulator of definitive haematopoiesis critical for the embryonic establishment of normal adult haematopoiesis. Conditional deletion of *Runx1* in adult mice, meanwhile, results in haematological imbalances such as decrease of peripheral blood lymphocytes, expansion of monocytes and granulocytes, and impaired T cell maturation. RUNX1 is also critical in megakaryocytic maturation and platelet production. The importance of normal RUNX1 function extends to malignant haematopoiesis, with *RUNX1* or CBFβ mutations found in over 20% of acute myeloid and lymphoid leukaemia cases. More recently, RUNX1 has also shown to be recurrently mutated in solid tumours.

All vertebrate Runx loci possess two alternate promoters: a distal P1 promoter and a *proximal P2* promoter. In the case of RUNX1, the major protein products from the *P1* and *P2* promoters are the RUNX1C and RUNX1B isoforms respectively, which differ solely in their N-terminal amino acid sequences: RUNX1B begins with MRIPV, whereas RUNX1C is 14 amino acids longer and begins with MASDS. Another key difference between the *Runx1* promoters is the timing and localisation of their expression; although *P2* activity corresponds with the onset of embryonic definitive haematopoiesis, a switch to *P1*-dominated *Runx1* expression occurs from the fetal liver stage and into adult bone marrow haematopoiesis. We recently performed an extensive study of *Runx1* promoter activities in adult haematopoiesis, utilising a dual reporter mouse model to isolate highly purified haematopoietic subsets on the basis of *Runx1 P1* and/or *P2* activity. *P1* is the dominant promoter in adult haematopoiesis, active in all *Runx1*-expressing subsets, whereas *P2* activity is largely restricted to pro-lymphoid, granulocyte/macrophage and megakaryocytic progenitor subsets but absent from erythroid-restricted progenitors. Given the pre-eminence of *P1* activity, we also investigated the impact of its absence on adult haematopoiesis, utilising a *Runx1 P1-GFP* knock-in mouse line. Unlike the total RUNX1 knockout, deletion of *P1*-directed RUNX1C expression was not embryonic lethal but resulted in numerous multilineage haematopoietic defects in adults, including mild thrombocytopaenia, therefore suggesting a partial recapitulation of the megakaryocytic lineage defects associated with total RUNX1-deficiency in this lineage.

We therefore decided to pursue a detailed investigation of the impact of altered RUNX1 isoform expression, in particular RUNX1C-deficiency, on Mk/Ery lineage specification. For this, we developed and utilised a novel mouse line *(P1-MRIPV)* in which the RUNX1C-specific N-terminal amino acid sequence (MASDSIFESFPSYPQCFMR) was replaced by MRIPV, the *P2*-driven RUNX1B-specific amino acids. The *P1*-MRIPV mouse line therefore lacks RUNX1C expression but has normal total RUNX1 levels, due entirely to RUNX1B. Utilising this mouse line we established a specific requirement for the P1-RUNX1C isoform in megakaryopoiesis, which cannot be entirely compensated for by RUNX1B expression. *P1* knock-in Megakaryocyte Progenitors have reduced proliferative capacity and undergo increased cell death, resulting in thrombocytopaenia. *P1* knock-in Pre-Megakaryocyte/Erythroid Progenitors demonstrate an erythroid-specification bias, evident from increased erythroid colony-forming ability and decreased megakaryocyte output. At a transcriptional level, multiple erythroid-specific genes are upregulated and megakaryocyte-specific transcripts are downregulated. In addition, pro-apoptotic pathways are activated in *P1* knock-in Pre-Megakaryocyte/Erythroid Progenitors, presumably accounting for the increased cell death in the MkP compartment. Unlike in the conditional adult *Runx1* null models, megakaryocytic maturation is not affected in the *P1* knock-in mice, suggesting that RUNX1B can regulate endomitosis and thrombopoiesis. Therefore, our data demonstrate that, despite the high degree of structural similarity, RUNX1B and RUNX1C isoforms have distinct roles at different stages of adult megakaryopoiesis.

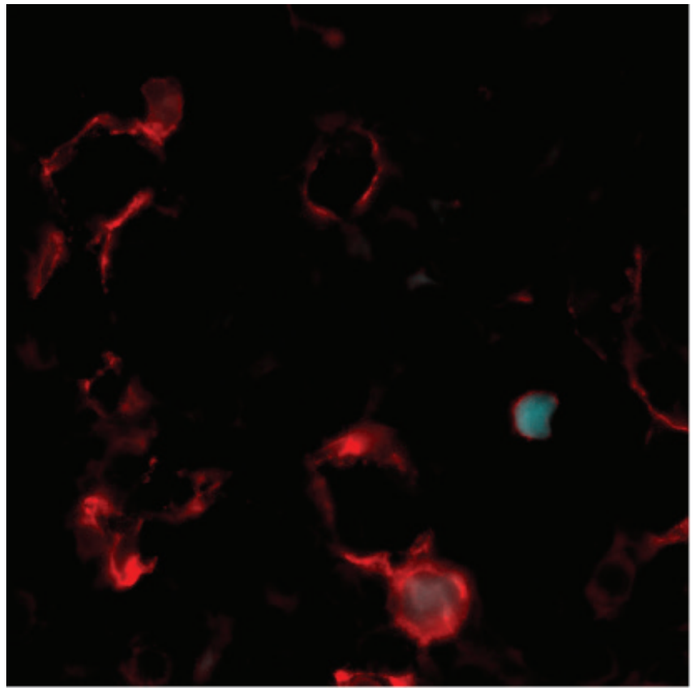

Figure 1:
Staining of fetal liver sections for P2-RFP (blue) and CD31 (red). Note the presence of double positive round cells.

A novel prospective identification of murine fetal liver progenitors to study in utero haematopoietic defects and malignancies

In recent years, highly detailed characterisation of adult bone marrow myeloid progenitors has been achieved and, as a result, the impact of somatic defects on different haematopoietic lineage fate decisions can be precisely determined. In comparison, fetal liver haematopoietic progenitor cells are poorly characterised, hindering the study of genetic alterations on mid-gestation haematopoiesis. Numerous disorders, for example infant acute leukaemias, have in utero origins and their study would therefore benefit from the ability to isolate highly purified progenitor subsets. High-resolution purification of myeloid progenitor cells would facilitate the identification of the specific gene regulatory networks and the evaluation of haematopoietic output disrupted in infant leukaemia. We therefore endeavored to improve our understanding of the specification of fetal liver progenitor cells.

To identify progenitor subsets with different line preferences, we characterised expression of Runx1-P1-GFP and P2-hCD4 in fetal liver using a *Runx1 distal promoter (P1)-GFP::proximal promoter (P2)-hCD4* dual-reporter mouse *(Mus musculus)* model. In this study, we undertook the characterisation of the expression of *Runx1-P1-GFP* and *P2-hCD4* in fetal liver. Within the Megakaryocyte-Erythroid Progenitor the *P2-hCD4*$^+$ fraction possessed the entirety of its bipotential megakaryocytic/erythroid output. The *P2-hCD4*$^+$ Common Myeloid progenitor population, meanwhile, had more balanced myeloid output, in particular decreased erythroid specification, than its *P2-hCD4*$^-$ counterpart. We subsequently identified CD31, CD45 and CD48 as candidate markers whose expression correlated with *P2-hCD4* providing a new strategy for prospective identification of highly purified fetal myeloid progenitors without requiring our transgenic mouse model. We utilised this methodology to compare the impact of the deletion of either total RUNX1 or the RUNX1C isoform alone and to determine the progenitor lineages most substantially affected. Using this approach we revealed fundamental differences between the two RUNX1 deletions. Whereas total RUNX1 mutant CD31 common myeloid progenitors have impaired erythroid specification and maturation, RUNX1C mutant CD31 common myeloid progenitors have impaired megakaryocyte specification but normal megakaryocytic/erythroid maturation. Our findings indicate that this new prospective identification of fetal liver progenitors raises the prospect of identifying the underlying gene networks affected in leukaemia with greater precision than previously possible.

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SYSTEMS ONCOLOGY

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Pancreatic cancer, and specifically Pancreatic Ductal Adenocarcinoma (PDA), is a dismal disease with a median survival below six months and an average five-year survival rate below 5%. This is due to the aggressive nature of the cancer, a lack of effective therapy as well as late diagnosis. Consequently, while PDA is only the 11th most common occurring cancer in the UK, it is currently the 4th largest contributor to cancer related deaths. The most frequent occurring genetic mutations have been identified with activating mutations in the oncogene KRAS and inactivation of the tumour suppressor CDKN2A in more than 90% of all cases and loss of SMAD4 and TP53 function occurring in 55% and 85% of all cases respectively.

Treatments targeting tumour cell dependencies on these mutations are not currently available in the clinic. Less frequent mutations can be grouped according to the deregulated pathways, where DNA repair mechanisms are inactive in ~20% of all PDA. This offers novel ways to treat PDA in the clinic, some of which will be tested through the PRECISION-Panc framework (see below). A hallmark of PDA is an extensive stromal infiltrate that makes up 80% of the tumour volume. This desmoplastic reaction consists of a pathological remodelled extracellular matrix and influx of fibroblasts and immune cells (Figure 1). The microenvironment has been shown to contribute to therapeutic resistance, immune tolerance and tumour progression. Importantly, very little is still known about the mechanisms whereby the tumour cells co-opt hosts cells to establish this hostile environment. Delineating these mechanisms is therefore important and may lead to the identification of novel therapeutic targets in both the tumour and stromal cells.

Tumour-stroma signalling in Pancreatic Ductal Adenocarcinoma

The central aim of the Systems Oncology laboratory is to determine how tumour cells exchange information with host cells to support tumour growth and resistance to therapies. Specifically, we aim to describe the key mechanisms whereby tumour cells co-opt stromal cells and conversely how the contextual impact of the microenvironment steer specific cancer cell phenotypes. Understanding these rules will enable development of synergistic combination therapies co-targeting tumour cell intrinsic dependencies together with tumour cell extrinsic dependencies on stromal reciprocal signals.

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

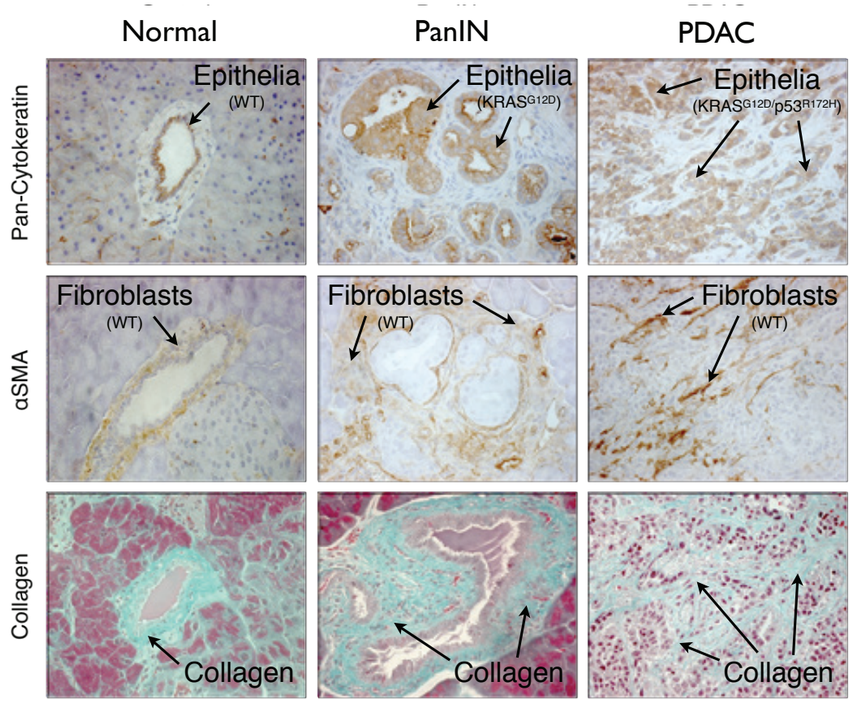

Figure 1:
Pancreatic Ductal Adenocarcinoma (PDAC) is characterised by extensive stromal reaction and desmoplasia. Immunohistochemistry for epithelia (pan-cytokeratine), activated fibroblasts (alpha Smooth muscle actin, αSMA) and collagen (Massons Trichrome) shown on pancreatic tissue isolated from Genetic 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. Of note, the epithelia loses its structure progressively as disease develops alongside an extensive fibroblast activation and Collagen deposition.

Defining and targeting the tumour microenvironment in PDA

In order to define interdependencies between tumour and stromal cells it is critical to map the cellular and extracellular component in the microenvironment. We have therefore started to catalogue, isolate and characterise individual stromal elements (including both cellular and extracellular components). The critical aim of these analyses is to determine whether individual stromal cell populations (or extracellular matrix components) differentially alter the tumour cell phenotype and whether this results in a differential sensitivity to therapy. Using a combination of proteomics and transcriptomics analyses we are defining the key pathways regulating tumour cell resistance. In parallel we are identifying targetable pathways in the tumour stroma and optimising their use for combination therapy. We have recently completed a screen to identify candidate targets in the pancreatic stroma and have in initial experiments described how targeting these stromal cells results in their 'normalisation' to ablate their pro-tumorigenic effect on the tumour cells. We are currently working closely with the Drug Discovery Unit at CRUK Manchester Institute, directed by Professor Caroline Springer, to develop this project further.

Delivering personalised medicine in PDA – PRECISION-Panc

Personalised therapy, the selection of a therapy that is matched to specific characteristics of individual tumours, has benefitted cancer patients enormously, but is still not available to patients with PDA. In an effort to improve treatment options and patient selection in PDA, we are involved in establishing a national infrastructure where individual tumours are subjected to molecular profiling such that patients can be matched with selected treatments. These clinical trials are underpinned by the development of biomarkers and pre-clinical research to further refine treatment strategies targeting specific dependencies. Understanding the role of the microenvironment in shaping the therapeutic response across selected patient populations is critical to define whether approaches targeting the tumour stroma should be delivered in a personalised manner, or whether a broader non-selected approach can be taken.

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TRANSCRIPTIONAL NETWORKS IN LUNG CANCER

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[1]Joined in 2017
[2]Left in 2017

Lung cancer causes the most cancer-related deaths in the world and the main obstacle to a cure is chemoresistance. The main interest of our group is to identify the causes behind lung cancer development and resistance to chemotherapy. Over the last decade, a growing number of non-coding transcripts (ncRNAs) have been found to have a pivotal role in gene regulation and cell biology.

The most well- known ncRNAs are microRNAs (miRNAs), single stranded RNAs of 19–25 nucleotides in length, which negatively regulate gene expression by translational inhibition or degradation of the mRNA targets. MiRNAs are differentially expressed in almost all types of human cancers versus the normal tissue counterpart and are key players in cancer onset and progression, functioning as tumour promoters (TP) or tumour suppressors (TS). The application of miRNAs to cancer therapeutics and diagnostics is emerging as an important field of gene therapy. Thus far both miRNA replacement and miRNA inhibition strategies have been successfully used to restore normal gene networks *in vitro* and *in vivo*, evidencing the huge potential of microRNAs in the fight against cancer. MiRNAs are released in the bloodstream, where they are highly stable, embedded in exosomes and microvesicles and could be used as prognostic and diagnostic biomarkers to predict disease onset or progression.

PDGFRs axis

Lung cancer ranks first in cancer morbidity and mortality rates globally. The most frequently diagnosed histological sub-type, non-small cell lung cancer (NSCLC), accounts for 80–85 % of cases, with a disappointing 5 year survival rate of 15.9% (Chen Z et al., 2014). During the last decade several effective targeted therapies have been developed; Erlotinib, Gefitinib and most recently Osimertinib and crizotinib/ceritinib for patients with EGFR activating mutations and ALK/EML4 translocations, respectively. However, *EGFR*-mutant and ALK-rearranged cancers constitute less than one-fifth of all NSCLC cases and patients that initially respond well to therapy inevitably relapse a few months later. Thus, identification of other potential molecular targets and novel therapeutic approaches is of primary importance. It is now accepted that NSCLC is not a singular entity but a heterogeneous disease and therefore requires targeted therapies. In this regard, a pathway that holds significant promise involves platelet-derived growth factors (PDGFs) which bind to PDGF receptors resulting in the activation of a downstream signalling cascade that promotes cellular proliferation, migration and survival. There are five different isoforms of PDGF that activate cellular response through receptors alpha (PDGFR-α) and beta (PDGFR-β). Both PDGF ligands and the receptors have been detected in lung cancer cells but not in normal cells and are markers of worse prognosis.

Several tyrosine kinase inhibitors, including imatinib, have been developed to block PDGFRs, however they are not selective and also inhibit other kinases (Camorani et al., 2014). Furthermore, these inhibitors did not show significant effects in vivo (von Mehren et al., 2005). We have identified miR-23b-miR-27b-miR-24-1 (referred as miR-23b cluster) and miR-125a-5p as PDGFR-modulated microRNAs (Naidu S. et al., 2017). Enforced expression of miR-23b cluster and miR-125a-5p silenced directly or indirectly multiple genes involved in the KRAS and NF-kB signaling reducing cell proliferation and enhanced drug-induced apoptosis. We demonstrated that miR-23b cluster and miR-125a-5p are transcriptionally activated by p53 and negatively regulated by NF-kB p65 transcription factors (Figure 1). In vivo delivery of these microRNAs suppressed the growth of a highly aggressive tumour derived from a patient with metastatic NSCLC and who was unresponsive to standard-of-care chemotherapy. These results shed new light on the mechanisms involved in lung tumorigenesis and lay the foundations to potentially develop more effective therapeutic strategies for both PDGFR- and KRAS-driven NSCLC.

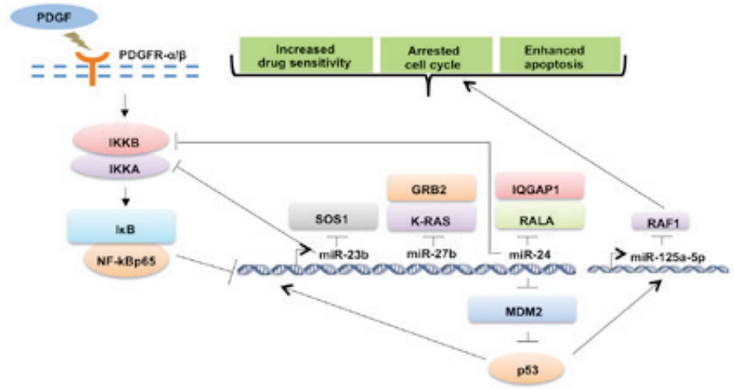

Figure 1. Model depicting the regulatory network controlling miR-23b cluster and miR-125a-5p.
PDGFR-α and PDGFR-β downregulate miR-23b cluster and miR-125a-5p via NF-kB p65. MiR-23b cluster and miR-125a-5p are transcriptionally activated by p53 and silence several important oncogenes involved in the KRAS and NF-kB pathways.

Figure 2. MiR-30c and miR-21 silence RASSF8 and RASA1.
Immunofluorescence (IF) showing downregulation of miR-30c and miR-21 target genes in H1299 cells. Scale bar 20 µm.

Figure 3A.
LNA-anti-miR-21 halts tumorigenesis in vivo. A) Representative pictures of the effect of LNA-anti-miR-21 (A-C) and LNA-anti-miRNA control (D-F) treatment 12 weeks post adenoCRE inhalation in KRAS^{G12D} transgenic mice. Lungs from mice treated with anti-miR-21 presented a normal alveolar texture with normal bronchiolar structures and no or minimal signs of fibrosis/flogosis (A). Normal representation of bronchiolar epithelial cells (B) and of alveolar framework (C) is shown. KRAS^{G12D} mice treated with anti-miR control developed the usual preneoplastic lesions observed in this model. A significant disarrangement of the pulmonary parenchyma (D), with the accumulation of preneoplastic lesions such as atypical adenomatous hyperplasias (E) or adenomas (F; the largest adenoma is showed). Scale bars 100 µm.

Figure 3B.
KI67 in lungs from mice treated with anti-miR-ctr and anti-miR-21.

KRAS and non-coding RNAs

The proto-oncogene *RAS* encodes three different RAS proteins: *HRAS, NRAS* and *KRAS,* regulated by guanine nucleotide exchange factors (GEFs), which stimulate RAS activation through GDP for GTP exchange, and by GTPase-activating proteins (GAPs), which catalyse the hydrolysis of GTP to GDP to switch off the KRAS signalling. Mutations in KRAS are very frequent in NSCLC (~30) (Timar J, 2014) and in lung adenocarcinoma harbouring K-Ras mutations, so far no specific drug has demonstrated efficacy. One of our current interests is to identify K-RAS-regulated microRNAs that, by targeting molecules involved in the RAS pathway, can be used as therapeutic tools in lung cancer. By overexpressing wild type or mutant KRAS (KRAS^{G12D}) and using inducible human and mouse cell lines, we analysed KRAS-regulated microRNAs in non-small cell lung cancer. We showed that miR-30c and miR-21 are significantly upregulated by both KRAS isoforms and induce drug resistance and enhance cell migration/invasion via inhibiting crucial tumour suppressor genes, such as RASA1 and RASSF8 (Figure 2). MiR-30c and miR-21 levels were elevated in tumours from patients that underwent surgical resection of early stage NSCLC compared to normal lung and plasma from the same patients. Systemic delivery of LNA-anti-miR-21 in combination with cisplatin in vivo completely suppressed the development of lung tumours in a KRAS^{G12D}-driven genetic mouse model of lung cancer (Figure 3A). Mechanistically, we demonstrated that ELK1 is responsible for miR-30c and miR-21 transcriptional activation by direct binding to the miRNA proximal promoter regions. In summary, our study defines that miR-30c and miR-21 may be valid biomarkers for early NSCLC detection and their silencing could be beneficial for therapeutic applications (Shi L. et al., in press).

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Figure 2

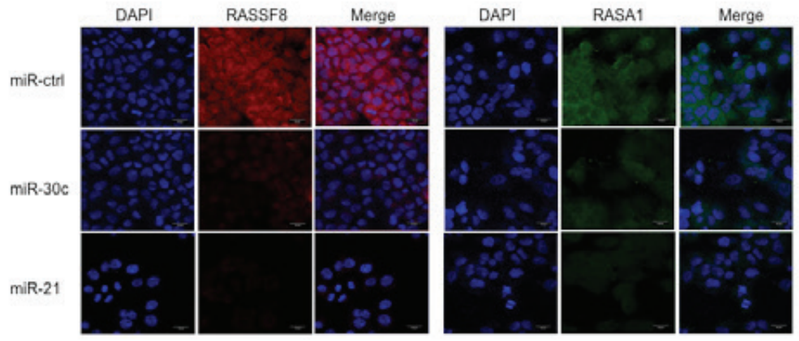


Figure 3A

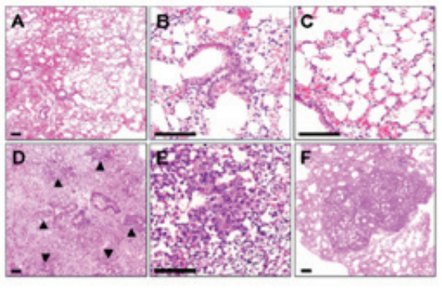
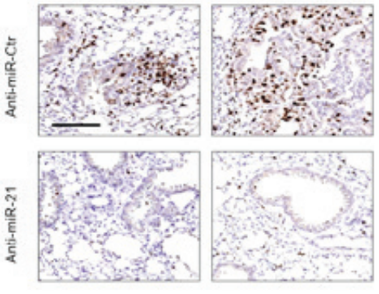


Figure 3B



TUMOUR SUPPRESSORS

Group Leader
Patricia Muller[1]

[1]Joined in 2017

Small cell lung cancer (SCLC) is dominated by mutations in the tumour suppressor gene TP53. These mutations result in the loss of p53 expression or expression of a mutated or truncated p53 protein. A mutated protein has lost most of its wild type functions, but can also exert new functions in promoting tumourigenesis, conferring chemoresistance and inducing invasion. Despite the important role of p53 as a tumour suppressor, p53 status is currently not used in the clinic for therapeutical decisions. Insufficient knowledge of mutant p53 gain-of-function as well as insufficient understanding of the differences between mutant p53 proteins could be a reason for this lack of clinical use. Our group aims to characterise the molecular pathways underlying mutant p53 gain-of-function and aims to classify different mutant p53 proteins occurring in SCLC based on molecular function and patient characteristics.

Mutant p53 proteins use several strategies to exert gain-of-function (GOF). They hijack transcription factors, alter the function of binding partners, or bind to DNA both specifically and non-specifically. Our previous work has characterised a role for several mutant p53 proteins in promoting the recycling of growth factor receptors and integrins via p63, Dicer and Rab Coupling Protein (RCP), which in turn promoted cell migration, invasion and chemoresistance. More recently, we determined that mutant p53 dependent regulation of EGFR and integrins caused engulfment of neighbouring lung cancer cells and subsequent genomic instability. Our future work will build on these findings to further understand how EGFR and integrins are regulated by RCP and mutant p53, which molecular mechanisms underlie engulfment, how engulfment leads to tumour progression and what role it plays in SCLC.

In cancers almost every amino acid of p53 has been found mutated making mutant p53 not simply one protein but a large variety of proteins that are likely to have similarities, but also differences in function. Some mutant p53 proteins will have a GOF, whereas others will have a partial or full loss of WT function or dominant negative activity. Most likely all of these, alone or in combination, can contribute to the oncogenic effects of mutant p53 seen in cancers, dependent on the circumstances and selective advantages of the tumour.

From a therapeutic perspective mutant p53 is an interesting target as it is often expressed to very high levels in many cancers. Current strategies aim to deactivate or degrade the mutant protein, to reactivate wild type p53 using stabilising drugs, to target downstream pathways activated by mutant p53 or to use synthetic lethality approaches exploiting mutant p53's specific requirements to target cancer cells. Although progress has been made in all of these areas, a better understanding of how each of the different mutant p53s function is needed to fully exploit therapeutic possibilities in mutant p53 positive tumours. Using public databases and through collaboration with Professor Caroline Dive's group, we have determined the most frequently occurring mutations in *TP53* in SCLC.

We are currently collecting these mutations in a cDNA library to express in a variety of cell lines and test for differences in mutant p53 gain-of-function and any remaining wild type function. We will then classify these mutations based on functional differences and determine whether there is a correlation to SCLC patient clinical or pathological characteristics. This is illustrated in Figure 1.

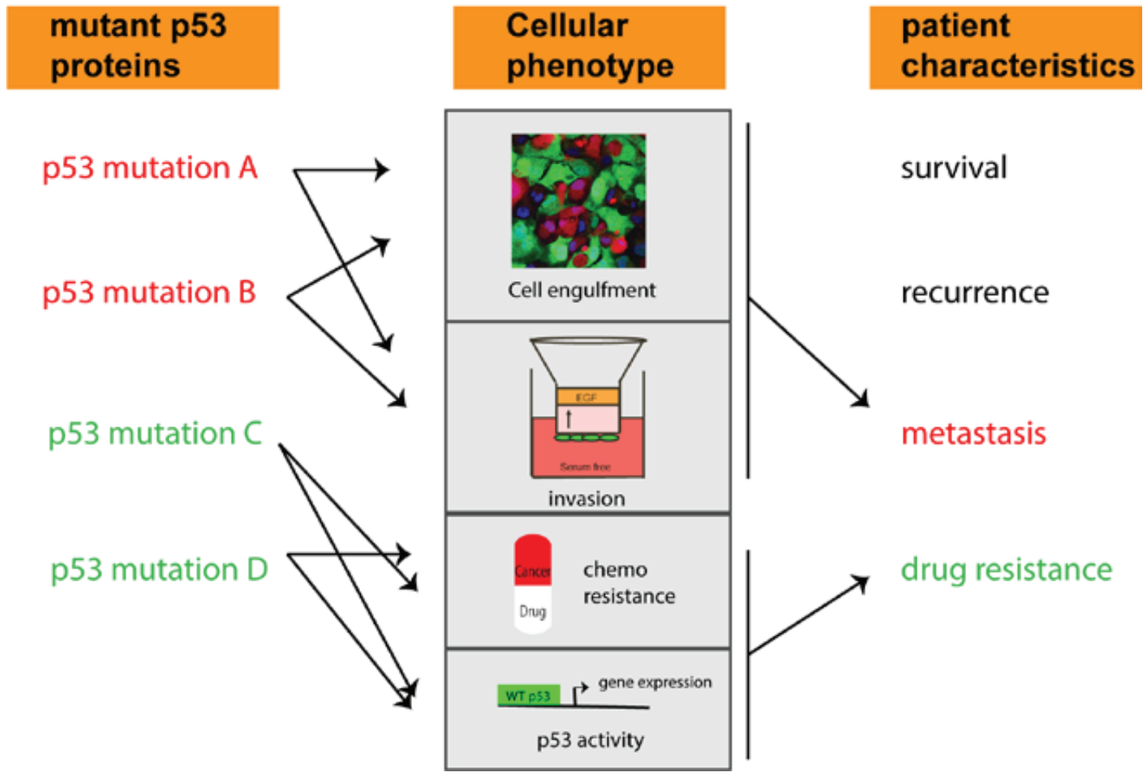


Figure 1.
The most frequent mutations in SCLC will be studied for cellular phenotypes in a variety of cell lines using a variety of tests to determine GOF and p53 activity. It is expected that different mutations will classify into groups regulating certain phenotypes that will then be correlated to SCLC patient characteristics.

CANCER RESEARCH UK MANCHESTER INSTITUTE

RESEARCH SERVICES

RESEARCH SERVICES

Chief Laboratory Officer
Stuart Pepper

The last twelve months have created some extreme challenges for the core facilities; the initial impact of the fire was that we lost access to almost all our main technology platforms. For the first six months after the fire the core facilities operated by a combination of outsourcing work, taking up many kind offers from other University of Manchester and CRUK core facilities to share space or equipment and, in some cases, using equipment loaned by companies. As the year has progressed we have started to move into our new labs at Alderley Park and by the end of the year the majority of equipment is coming back on line.

Chief Laboratory Officer
Stuart Pepper

Despite the complexities of dealing with the aftermath of the fire, this year has also seen the introduction of some new equipment. The Molecular Biology Core Facility has worked with the Oxford Nanopore platform to develop long read sequencing, and in Advanced Imaging and FACS we have installed a CyTOF platform to allow mass spectrometry based cytometry. Alongside this we have seen continual development in workflows in Histology and the introduction of new procedures in the in vivo team.

Whatever the situation, the most important aspect of the core facilities is that the focus remains on supporting the cutting edge research programs run by the Institute. Over the last year the core facilities have kept this single aim in mind whilst adapting to difficult situations, and will continue to do so as we look ahead to the next year.

Advanced Imaging and Flow Cytometry
Steve Bagley, Jeff Barry, Antonia Banyard, Helen Carlin, Abi Johnson, Isabel Peset Martin, Bogdan Potereas[1], Heather Woodhouse, Kang Zeng

[1]left in 2017

In response to the fire, we have spent most of this year running the service on multiple sites around Manchester. However, one positive outcome of the disruption has been that we have had time to review the facility, the services and support being offered and consider how we can develop the facility in response to several recent developments in flow cytometry, imaging and screening.

By assessing the up and coming research requirements, several systems have been purchased and techniques developed. A Zeiss Airyscan Fast has been purchased with several modifications to allow both super resolution imaging whilst using low levels of laser light, and a UV laser to induce DNA damage. This system will be operational from February 2018.

In the field of flow cytometry analysis, it is becoming more evident that there is a requirement for high complexity, multiple labels. With light based flow cytometry analysis, it is routine within the laboratory to be assessing up to 14 cellular markers but for our ongoing work into the heterogeneity of tumours, phenotype, biomarker discovery and characterisation of the cellular environment, the level of marker complexity requires at least a doubling of present technologies. A pilot study with the Systems Oncology laboratory (Claus Jørgensen) has proven the suitability of mass cytometry (CyTOF), which uses rare metal labels rather than light emitting fluorophores at a far higher level of complexity. As a consequence of this case study a CyTOF will be installed in the Institute in April 2018.

Complexity of data capture, whether this be single cells in a flow stream, a multi-well plate using high content screening, or tissues requires complex software and informatics. Our dependence upon IT and Scientific Computing to host software means that these departments are essential to the service we offer. For example, high content screening software permits anyone to process data through a web page interface to enable distribution. In 2018 we will be supporting scientists across multiple sites as well as assisting research collaborators to access data; consequently there is an essential requirement for distributed software. Work is also ongoing alongside the Biological Resources Unit, Histology, and the Molecular Biology Core Facility where our technologies overlap and we can assist other facilities. For example, single cell sorting for molecular biology processes has become an essential development and is now a routine process.

2017 saw two members of the team being promoted to the role of Senior Scientific Officer. Antonia Banyard was recognised for her work in developing high dimensional experimental design and analysis in flow cytometry to ask questions about molecular relationships. Isabel Peset Martin has been working closely with our research groups, clinicians and the Histology facility in developing routines for the analysis of tissue. Over the last few years the complexity of Antonia and Isabel's work has increased and many research groups depend upon the developments made in these fields.

Early next year will see several new hardware developments. The team are presently putting in place several new applications and software is being installed to enable the visualisation and analysis of complex data. The new facility laboratories at Alderley Park will be spread across the whole site so that researchers are close to essential equipment rather than having them located centrally, thus responding to the fragility of biological samples and the needs of the research groups.

Biological Mass Spectrometry Facility
Duncan Smith, Yvonne Connolly

The role of the Biological Mass Spec facility is to support the protein analysis requirements of all onsite research groups. Liquid Chromatography Mass Spectrometry (LCMS) has become a very powerful tool in the analysis of proteins in cancer research. The ability to probe the physiochemical properties of one or thousands of protein molecules with LCMS has enabled the emergence of a vast array of proteomic approaches to answer previously intractable biological questions. These questions often involve how the abundance of a molecule might change, how a protein interaction network is modulated or how a particular post translational modification dynamic is associated with a biological phenotype.

In addition to providing service to approximately 18 research groups, the facility has invested significant development time in the area of high performance sample preparation and clean up approaches. These approaches maximise sample quality by eliminating chemical contaminants whilst minimising protein losses. The net result is a significant improvement in overall sensitivity of protein detection meaning we can now analyse very low abundance samples that were previously beyond our capability.

Biological Resources Unit
Transgenic Breeding
Team Leader: Kim Acton

The BRU transgenic mouse breeding facility is at the University Incubator Building. Live mice are not brought into the Incubator Building directly to ensure the high health status of the facility is maintained and to avoid possible contamination. Newly imported lines are now brought into the University Stopford Building Quarantine area, until rederivation (via embryos) to the Incubator Building is complete.

Approximately 24 new lines were set up at the Incubator Building during 2017, mostly by crossing existing lines, but included two newly imported lines and three lines created by embryo microinjections.

Cryopreservation of embryos and sperm has resulted in the archiving (closing of live colonies) for 20 lines during 2017. A further 17 live colonies were closed in 2017 where cryopreservation was not required.

Currently, the Transgenic Facility has around 130 breeding lines of genetically altered mice, cared for by 10 staff, for the Institute's scientists. Although the numbers of lines have decreased, the number of cages has remained at around 2,300. This is due to smaller, maintenance lines having been closed, with increased breeding for the newer lines, to establish stock of appropriate genotypes for experimental use. Additional services include timed mating set up and the import and export of strains; exports in 2017 were to Japan and within the UK.

The BRU Transgenic Team manages the genotyping service, where all samples are sent to external company Transnetyx in USA, and then genotype results downloaded into the stock records.

Twice weekly shipments of stock mice are now transferred to the BRU Experimental facility at the Alderley Park site. Mice are transferred, on request, allowing a minimum of one week acclimatisation for the mice from arrival at the Alderley Park facility prior to starting in an experiment. New carriers were sourced and adapted to each hold six cages and improve handling and transport of the mice from the Incubator Building to Alderley Park.

Welfare improvement has also been made for male mice needing to be singly housed, e.g. for stud use, with increased cage enrichment and when reducing numbers in cages - unsuitable

genotypes, or removed for breeder set up - leaving a 'companion' in the cage where possible.

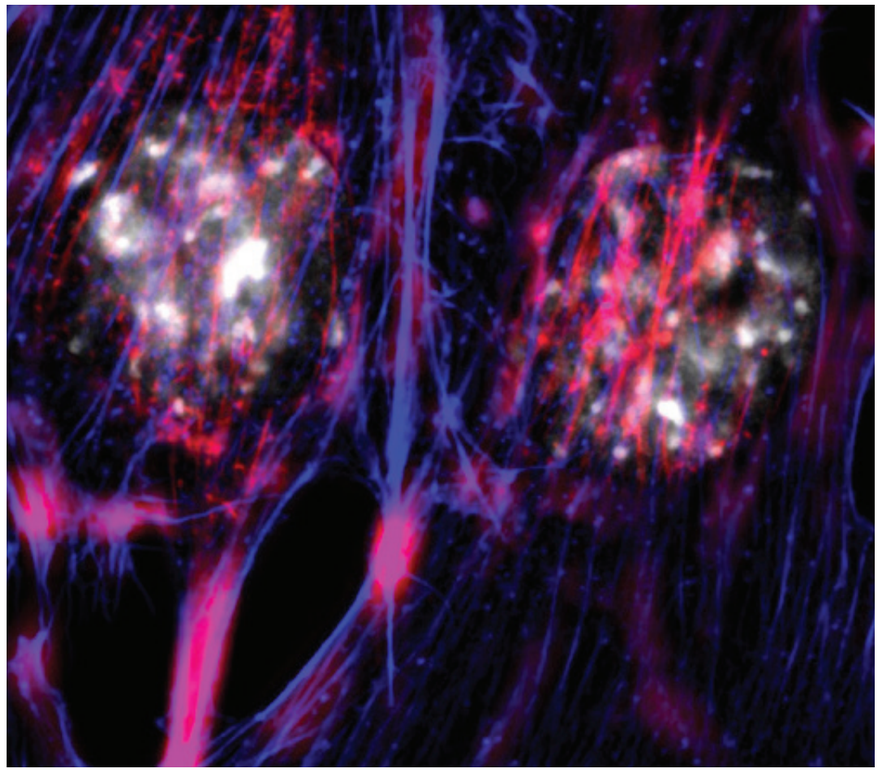
Biological Resources Unit

Experimental Services
Team Leader: Lisa Doar

In the first quarter of 2017 the experimental unit continued to expand steadily, taking us to around 1200 cages by the end of Q1. One of our main aims for 2017 was to increase the expertise of the BRU technicians in the areas of imaging and surgery as these are two key areas where we see progression of a great deal of new and exciting projects. Working alongside our vet we have also looked at refinements such as improving our analgesia regimes and encouraging anaesthesia and imaging contrast agents to be given via the sub-cutaneous injection route rather than by intra-peritoneal route as has been traditionally used, which is a less invasive and lower risk route.

At the end of last year, I entered a competition called the 'Janet Wood Innovation Award' with our idea for cable tie swings as a form of mouse enrichment and we were absolutely delighted that it won. The product has been further developed and is now being produced for sale by a company called Datesand. It is renamed the 'mouse swing' and has had a lot of interest from different institutes and is so far selling really well.

The Paterson Building fire in April 2017 caused a huge disruption to our research. The animal facility was initially moved up to The University of Manchester's Biological Services Facility (BSF) and from early October we started relocating to AstraZeneca's Alderley Park facility. During these difficult months the BRU staff worked really hard to keep the studies running smoothly and we are one of the only core facilities to have been in a position to maintain a steady level of work going throughout this year. Whilst working at the BSF we managed to facilitate a good number of imaging, X-ray and surgical studies and by Q3 we were providing similar levels of training to those before the fire. Our first animals arrived at Alderley Park in the second week of October and by Christmas we were already up to approximately 400 cages, ready to begin at full speed in 2018.



Actin fibres at the apical (top) surface of the cell (shown in red) are distinct from basal fibres (shown in blue.) Apical actin fibres form a 'cap' over the cell nucleus (shown in white) which help orient and shape the nucleus. This control of the cell nucleus is important for cell migration, and for invasion of cancer cells, which have to navigate complex and confined environments within the body.

Image supplied by Andrew Porter (Cell Signalling)

There will still be many adjustments to be made in early 2018 as we adapt to our new work environment and the workload increases, but our aims are to continue where we left off earlier in 2017 - continuing to develop our surgical and imaging models. Another project is to produce training guidance sheets and DOPS (Directly Observed Procedural Skills) documents to further enhance our training processes. The staff involved in training will also attend an assessor's course to enhance their own skillset.

Transgenic Production Facility

Natalia Moncaut, Mark Willington, Athina Papaemmanouil

The application of the Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) technology, with its remarkable ability to edit the genome in a precise manner, has revolutionised the field of transgenic mouse production. With this new technology, mouse models carrying gene disruptions or small insertions can be generated in a routine basis with high efficiency. Though more complex genome modifications can take longer or even require the use of mouse embryonic stem cells, the time frame using CRISPR compared with traditional genome targeting approaches is much reduced.

The Transgenic Production Facility (TPF) works closely with the researchers providing a comprehensive service in the generation of new genetically modified mouse lines using the new CRISPR technology. We are responsible for the design of the best targeting strategy and for the production of all the reagents required for the process. We have a fully equipped laboratory for molecular biology and a dedicated area for mouse embryonic stem cell culture. Additionally we have a microinjection suite with inverted microscopes equipped with micromanipulators, where we carry out the embryo manipulations and mouse surgeries.

Since establishing the TPF, we have successfully run several projects including the generation of double gene disruptions and insertion of small point mutations. We are also working on more complex projects like conditional point mutant and knockout lines or bigger genome modifications. The TPF also provides regular assistance in the design of targeting strategies using the CRISPR technology for in vitro applications, such as cell lines.

Genome editing is constantly improving and new tools are being tested in genetically engineered mouse lines in a more efficient and precise way to make the technology more versatile for any application. At TPF we constantly participate in annual meetings and courses in order to keep up with the latest advances of this technology. One aspect of genome editing that the TPF would be interested in developing in the near future is in vivo genetic targeting to produce mouse models of cancer. By using this approach with the appropriate delivery method, specific tissues can be targeted with the CRISPR system and precise genome modifications are generated. This strategy will circumvent the generation of new lines with their associated time and breeding costs.

Histology

Garry Ashton, Caron Abbey, Emma Watson, Marta Madureira da Graca, Janice Kerrigan[1], Usman Mahmood, Deepti Wilks (Haematological Malignancy Biobank)

[1]Joined in 2017

The Histology facility allows for the adoption of tissue-based experimental approaches to a large number of both basic and translational research groups within the CRUK MI. Despite the challenges this year, the range and complexity of the services offered has continued to grow allowing us to adopt and incorporate sophisticated labelling techniques into routine practice. The training and continued professional development of staff has ensured the unit continues to offer a comprehensive and flexible service at all times. A new scientific officer was recruited in 2017 with further posts to be filled in 2018. A lot of effort has also gone into preparing for the relocation of the lab and its services, which will take place in early 2018.

The unit's remit is to offer a full range of both routine and advanced histological services for oncology research. The facility routinely processes, embeds and sections both human and mouse tissues in addition to organotypic assays, spheroids, agar plugs and cell pellets. Vibratome sections from fresh tissue (50–250µm) have also been prepared for the set up of ex vivo cultures of tumours and to evaluate and develop three dimensional studies. Several special stains have been used by various groups including Masson Trichrome, PAS and reticulin stains. In addition, the facility continues to process FFPE and frozen samples for the MCRC Biobank. To date, samples from over 10,000 patients have been collected. In addition blood, bone marrow and plasma from haematological malignancy patients has also been collected and processed. The samples are of the highest quality ensuring maximum value to any research program.

The range and availability of automation within the unit is now excellent. Both the Leica Bond RX and the Ventana Discovery platforms allow us to offer both in situ hybridisation and multiplex immunohistochemistry as a routine service. In situ hybridisation allows for the analysis of gene expression data in the context of tissue/cell morphology and has been employed in several research groups' projects using either chromogenic or fluorescent markers in both

single and multiplex formats. Multiplex immunohistochemistry allows the study of 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. Numerous groups are using the data this technique produces. Evaluation and development of the technique has shown experimental design and full validation is vital to ensure good quality data is produced.

The high throughput routine immunohistochemistry service, troubleshooting and antibody validation services continue to see exceptional demand. These services are run on the Leica BondMax and Ventana Benchmark platforms and are available for both mouse and human tissues.

The Prostate Oncobiology group are specifically interested in understanding the cellular heterogeneity of the prostate tissue and identification of the clones responsible for disease progression to lethal castration resistant prostate cancer. For that purpose it is key for the team to assess the spatial location of prostate subpopulations in situ and their association with prostate cancer progression. In collaboration with the group we have optimised the protocols for IHC and multiplexed IF on mouse and human prostate tissue. Validation of the expression of the markers of interest, by co-evaluating the protein (IF) and RNA (RNAscope), has been performed. Evaluation of a novel marker in prostate cancer patients' outcome, using multiplex IF on a prostate cancer patients' tissue-microarray to stratify patients and predict their clinical outcome has now been completed.

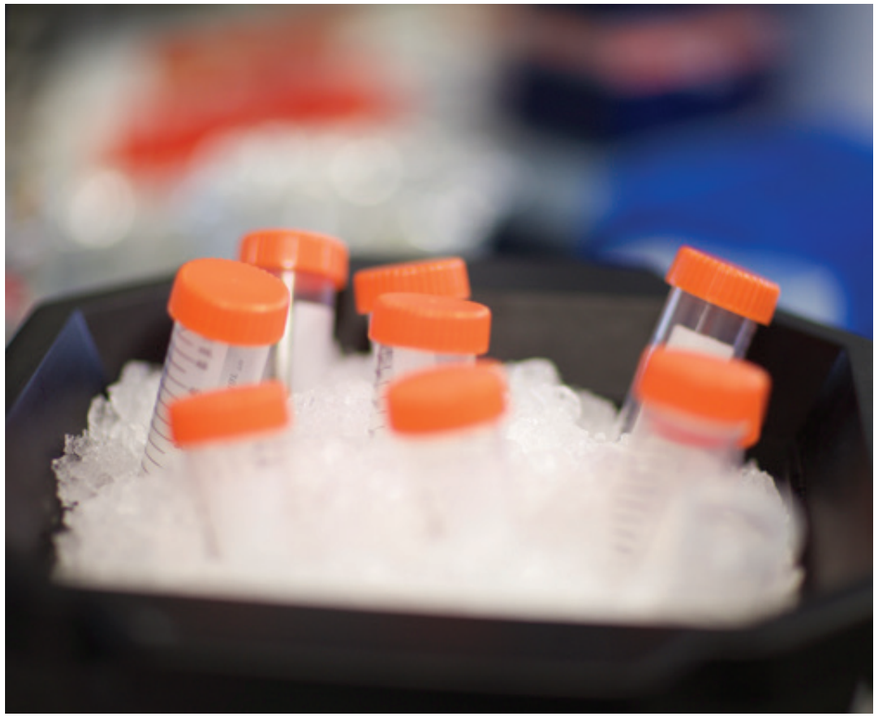
The Cell Signalling group studies the role of RAC signalling in tumourigenesis. They concentrate on the RAC activator, TIAM1, as previous work revealed an important role for TIAM1 in the formation and progression of skin and colorectal cancer (CRC). To further address the impact of TIAM1 on CRC and increase the understanding of its clinical significance, IHC was used to evaluate a tissue microarray comprising 650 samples, including Dukes stages A-C, from a well-characterised patient cohort. Results indicated TIAM1 expression is negatively associated with colon cancer progression, consistent with the Cell Signalling group's previous finding that TIAM1 antagonised progression of intestinal tumours in *Apc*$^{MIN/+}$ mice, an animal model for CRC. Further analysis of these data also showed that patients with high nuclear TIAM1 had significantly better survival than those with low nuclear TIAM1 (Diamantopoulou et al. Cancer Cell 2017, 31:621).

One of the Stem Cell Biology group's scientific interests is to study the role of the transcription factor RUNX1 in the biology and pathology of mouse and human prostates. The unit has been instrumental in optimising multiplexed panels of markers to define the localisation of RUNX1 expression within the mouse prostate and in the study of how RUNX1 contributes to prostate organoid development. Following extensive validation, the unit has also stained human prostate cancer arrays using RUNX1 in combination with other markers using multiplexed immunofluorescence to study how RUNX1 expression correlates with clinical outcomes. The unit has also used in situ hybridisation (chromogenic and fluorescent, single and multiplex) in order to validate these findings at the protein level. These sets of experiments have been essential to determine the contribution of RUNX1 to prostate homeostasis and prostate cancer.

The Systems Oncology group are focused on understanding the role of the tumour microenvironment in pancreatic cancer. Having used mass cytometry to identify several potential surface markers of distinct lineages of fibroblasts in murine tumours, IHC work has allowed confirmation of these observations in FFPE mouse tumours and normal tissue, in addition to FFPE tumours from other genetically engineered mouse models provided by a collaborator. Further, a panel of anti-human antibodies have allowed an extension of these observations from murine tumours to human tumours.

One of the most advanced projects within the Drug Discovery Unit's portfolio is on inhibitors of poly(ADP) ribose glycohydrolase (PARG). Together with collaborators they had identified a synthetic lethal interaction between depletion of XRCC1 and PARG inhibition. Previous IHC using Biobank samples confirmed a subset of breast tumours with low levels of XRCC1 that therefore suggested a clinical population. Together with the Division of Cancer Science's Breast Biology group headed by Rob Clarke we have been using IHC to identify possible patient derived xenografts with low levels of XRCC1. If found these will enable the Drug Discovery Unit to test PARG inhibitors in 2D and 3D models.

The unit continues to be used heavily by the CEP preclinical team and IHC biomarkers teams. CDX



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

Laboratory Services

Mark Craven, Tony Dawson, Corinne Hand, Petra Kubinova, Abigail Pienaar[1], Adriana Tudelo, Christine Whitehurst

[1]Left in 2017

During 2017 Lab Services has continued to support the various research buildings across the site; initially having the Paterson Building as our main base, and then transferring to the MCRC Building. We provide our primary function of supplying clean and sterilised glass, plastics and fluids by using the glass washing and sterilising equipment in the MCRC Building and can deliver to our customer base using the Porters/Logistics team and their shuttle service across the site.

The department also supports the research by organising the allocation of clean lab coats used for general and tissue culture work and for visitors. We arrange monthly pipette calibration clinics and operate a film-based dark room based in the MCRC Building. We supply and monitor the first aid supplies across the site. We also supply a range of microbiological media and agar plates using a standardised recipe.

We have extended the sites we visit to include the research groups temporarily located in other University of Manchester buildings and to those already at or preparing to move to the Alderley Park site. We are developing the service and plan to site members of the team at Alderley Park to support the growing research presence there whilst also remaining at the MCRC Building.

The department and I have also been involved with the relocation process away from our Withington site and have assisted with the planned emptying of the laboratory areas. I have also been involved in supporting the Chief Laboratory Officer, Stuart Pepper, with the plans to transfer the Institute's research groups and Core Facilities to Alderley Park.

Molecular Biology Core Facility

Wolfgang Breitwieser, Andzhela Abu Rashed[1], Chris Clark, Gillian Newton, Bogdan Potereas[2], Amy Priestman[1], Leanne Wardleworth and John Weightman

[1]Joined in 2017 [2]Left in 2017

Over the last year the Molecular Biology Core Facility has strived to maintain, and improve on, high quality and reliable services for a wide range of molecular biology as well as high throughput automation applications.

A significant part of the services that the facility supports is dedicated to Next Generation Sequencing (NGS). Using our range of Illumina NGS platforms we continue to provide a host of applications, including whole genome, whole exome, RNA and ChIP sequencing. In addition we undertook the testing and validation of protocols for more bespoke applications including RIP-Seq, ATAC-Seq, or Immuno-Seq.

CRISPR has become a predominant method of choice for phenotypic cell screening and is heavily dependent on NGS. In support of these applications we developed a workflow for CRISPR screen validations, which we rolled out as a service at the beginning of 2017. In further developments we also explored novel NGS methods such as Nanopore sequencing. This technology exploits the physical properties of nucleic acid molecules transferring across nanopore protein complexes and allows the sequencing of nucleic acid fragments of thousands to tens of thousands of nucleotides in length, as opposed to the short-read technology that Illumina instruments provide. Using the Oxford Nanopore Technologies MinION platform we supported RNA-Seq projects in which long-read sequencing complements short-read, high-throughput sequencing.

In a separate line of technical developments, we established a comprehensive workflow for large-scale RNA-Seq of single cells. For a range of biological questions, studying single cells has clear advantages over bulk tissue, as it allows for the detection of tissue heterogeneity, or the identification of distinct cell populations within a tissue. This can be particularly relevant in cancer, where it is recognised that tumours consist of heterogeneous pools of distinct clonal populations rather than a homogeneous mass. Adapting the MBCF's automation and high-throughput platforms we developed workflows for cDNA and sequence library preparation. This workflow now allows us to process many hundreds of single cells for RNA sequencing in a single experiment, in a time and cost effective manner.

High-throughput automation was also adapted to further enhance the MBCF's compound library service. By adding a high specification microplate reader (BMG Labtech PHERAstar FSX) to the existing Labcyte Access robotic platform we are now capable of undertaking automated plate assays. Thus we can provide comprehensive support for high-throughput compound plating as well as assaying. Workflows on the Access platform have also been developed for molecular biology applications including the single cell processing.

Scientific Computing and Computational Biology Support

These two services provide high performance computing and data analysis solutions for the Institute's scientists.

Scientific Computing

Marek Dynowski, Jack Heal[1], Neil Venables[1]
ZhiCheng Wang, Christopher Wirth[2]

[1]Joined in 2017 [2]Left in 2017

Two new programmers joined the Scientific Computing team (SciCom) at the beginning of the year; Neil Venables is focused on software development and pipeline implementation, while Jack Heal concentrates on the management of scientific data, as well as pipeline implementation. New bioinformatics pipelines have been developed in cooperation with the Computational Biology team. These are based on the workflow management software Snakemake, introduced in 2016, and exploits improvements to the High-Performance Computing (HPC) infrastructure. We have contributed additional HPC-related features to the official Snakemake codebase, and a new continuous integration pipeline for performing automatic unit tests has been designed by SciCom. It is based on the open source version control system, Gitlab, and is used for automatic testing and quality checking of bioinformatics pipelines on the CRUK MI HPC cluster. This guarantees the generation of consistent results, and is especially important for running and developing Good Clinical Practice (GCP) compliant pipelines for personalised medicine.

Before Christopher Wirth left the SciCom team in September 2017 in order to pursue a PhD, he implemented a mutation-calling pipeline using the Snakemake workflow management system. This forms part of the TARGET trial and we are now collaborating further with the CRUK MI digitalECMT and eTarget (see page 23) teams to extend this work.

Fortunately, the majority of computing and storage hardware in the water-cooled server room in the Paterson Building was not damaged by the fire or water used to fight the fire, and was in sufficiently good condition to be quickly repaired. Most importantly, no data were lost, validating our robust backup strategy. We were therefore able to rapidly establish an interim HPC cluster to support mission critical workloads, including analysis pipelines to support CRUK MI clinical data. We were also able to draw on the support of our friends and colleagues at St Mary's Hospital, Manchester, in order to resume bioinformatics analysis for a limited set of clinical trial data before the interim system was available. In the meantime, we have designed and refurbished a server suite at Alderley Park where we will relocate the complete CRUK MI IT infrastructure.

Reconstructing the HPC cluster also gave us the opportunity to improve and update the system. It will be put into production in the beginning of January 2018 with newly introduced data and software management tools.

Computational Biology Support

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

[1]Left in 2017 to join the Molecular Oncology group

With the advent in high-throughput technologies, biological data is being generated at an unprecedented pace in recent years. Much of these data are derived from studies of patients with cancers. For example, under The Cancer Genome Atlas (TCGA) initiative, comprehensive molecular profiling of 11,000 patients has been performed to generate multidimensional maps of key genomic changes for 33 types of cancers. The availability of these data and the insights they may provide into the biology of cancer open exciting possibilities for precision medicine. However, mining the sheer volume of these "big data" to answer complex biological questions remains a challenge. The Computational Biology Support (CBS) team is committed to assist researchers in CRUK Manchester Institute (CRUK MI) to overcome this bottleneck.

The main role of CBS is to provide data analysis and a statistics consultation service to CRUK MI researchers, and develop data analysis pipelines to support the processing and interpretation of data generated by large-scale high-throughput technologies.

Next generation sequencing (NGS) applications continued to be the major theme for CBS over the past year, with ongoing development of our data analysis pipelines for RNA-seq, ChIP-seq, small RNA-seq, whole-exome and whole-genome sequencing. During the course of 2017, we collaborated with experimental researchers within the Institute on a range of NGS projects and provided rigorous bioinformatics support tailored to each project, examples include:

- The development of dedicated data analysis workflows for detecting copy number aberration events in circulating tumour DNA from small cell lung cancer patients. This was done in collaboration with the Clinical and Experimental Pharmacology group.
- Integrative analysis of RNA-seq and small RNA-seq datasets to gain a better understanding of the modulation of microRNAs in lung cancers. This was done in collaboration with the Transcriptional Networks in Lung Cancer group.
- The development of customised scripts and approaches for assessing the prognostic power of COX2 gene signatures across different cancer datasets in TCGA. This work was done in collaboration with the Cancer Inflammation and Immunity group.

In addition to well-established applications, we have also been working closely with the Molecular Biology Core Facility (MBCF) to help support scientific advances in other research technologies such as CRISPR/Cas9 knockout screens and NGS of formalin-fixed paraffin embedded (FFPE) samples.

NGS pipelines are computationally intensive. We have been working in synergy with the Scientific Computing team to establish bioinformatics infrastructure to support automated data processing, which enables efficient distribution of data analysis tasks across the high performance computing cluster.

Besides NGS, we also collaborated with the Biological Mass Spectrometry Facility and the Systems Oncology group to develop infrastructure and pipelines for mass spectrometry data analysis, including protein identification, label-free quantitative proteomics, SILAC/iTRAQ and SWATH.

CBS has contributed to three publications this year (Naidu et al. Sci Rep 2017; Garofalo et al. Cell Death and Disease 2017; Khandelwal et al. Mol Cancer Res 2017).

CANCER RESEARCH UK MANCHESTER INSTITUTE

PUBLICATIONS AND ADMINISTRATION

Confocal photomicrograph of PyMT mouse breast cancer showing stromal cells (Red), IL33 (Green), basal epithelial cells (Grey) and cell nuclei (Blue).
Image supplied by Haoran Tang (Molecular Oncology)

RESEARCH PUBLICATIONS

Cancer Inflammation and Immunity (page 12)
Santiago Zelenay

Refereed research publications
Molgora M, Bonavita E, Ponzetta A, Riva F, Barbagallo M, Jaillon S, Popović B, Bernardini G, Magrini E, Gianni F, Zelenay S, Jonjić S, Santoni A, Garlanda C, Mantovani A. (2017)
IL-1R8 is a checkpoint in NK cells regulating anti-tumour and anti-viral activity. ***Nature***, 551(7678):110-114.

Cell Division (page 14)
Iain Hagan

Refereed research publications
Chan KY, Alonso-Nuñez M, Grallert A, Tanaka K, Connolly Y, Smith DL, Hagan IM. (2017)
Dialogue between centrosomal entrance and exit scaffold pathways regulates mitotic commitment. ***Journal of Cell Biology***, 216(9):2795-2812.

Other publications
Grallert A, Hagan IM. (2017)
Elementary Protein Analysis in Schizosaccharomyces pombe. ***Cold Spring Harb Protoc***. doi: 10.1101/pdb.top079806.

Grallert A, Hagan IM. (2017)
Large-Scale Immunoprecipitation from Fission Yeast Cell Extracts. ***Cold Spring Harb Protoc***. doi: 10.1101/pdb.prot091595.

Grallert A, Hagan IM. (2017)
Small-Scale Immunoprecipitation from Fission Yeast Cell Extracts. ***Cold Spring Harb Protoc***. doi: 10.1101/pdb.prot091587.

Grallert A, Hagan IM. (2017)
Preparation of Protein Extracts from Schizosaccharomyces pombe Using Trichloroacetic Acid Precipitation. ***Cold Spring Harb Protoc***. doi: 10.1101/pdb.prot091579.

Cell Signalling (page 20)
Angeliki Malliri

Refereed research publications
Diamantopoulou Z, White G, Fadlullah MZH, Dreger M, Pickering K, Maltas J, Ashton G, MacLeod R, Baillie GS, Kouskoff V, Lacaud G, Murray GI, Sansom OJ, Hurlstone AFL, Malliri A. (2017)
TIAM1 antagonizes TAZ/YAP both in the destruction complex in the cytoplasm and in the nucleus to inhibit invasion of intestinal epithelial cells. ***Cancer Cell***, 31(5):621-634.e6.

Myant KB, Cammareri P, Hodder MC, Wills J, Von Kriegsheim A, Győrffy B, Rashid M, Polo S, Maspero E, Vaughan L, Gurung B, Barry E, Malliri A, Camargo F, Adams DJ, Iavarone A, Lasorella A, Sansom OJ. (2017)
HUWE1 is a critical colonic tumour suppressor gene that prevents MYC signalling, DNA damage accumulation and tumour initiation. ***EMBO Mol Med***., 9(2):181-197.

Clinical and Experimental Pharmacology (page 22)
Caroline Dive

Refereed research publications
Salem A, Mistry H, Backen A, Hodgson C, Koh P, Dean E, Priest L, Haslett K, Trigonis I, Jackson A, Asselin MC, Dive C, Renehan A, Faivre-Finn C, Blackhall F.
Cell Death, Inflammation, Tumor Burden, and Proliferation Blood Biomarkers Predict Lung Cancer Radiotherapy Response and Correlate With Tumor Volume and Proliferation Imaging. ***Clin Lung Cancer***. [Epub 11 Dec 2017]

Naidu S, Shi L, Magee P, Middleton JD, Laganá A, Sahoo S, Leong HS, Galvin M, Frese K, Dive C, Guzzardo V, Fassan M, Garofalo M. (2017)
PDGFR-modulated miR-23b cluster and miR-125a-5p suppress lung tumorigenesis by targeting multiple components of KRAS and NF-kB pathways. ***Sci Rep***., 7(1):15441.

Germano G, Mauri G, Siravegna G, Dive C, Pierce J, Di Nicolantonio F, D'Incalci M, Bardelli A, Siena S, Sartore-Bianchi A.
Parallel Evaluation of Circulating Tumor DNA and Circulating Tumor Cells in Metastatic Colorectal Cancer. ***Clin Colorectal Cancer***. [Epub 1 Nov 2017]

McGranahan N, Rosenthal R, Hiley CT, Rowan AJ, Watkins TBK, Wilson GA, Birkbak NJ, Veeriah S, Van Loo P, Herrero J, Swanton C; TRACERx Consortium. (2017)
Allele-Specific HLA Loss and Immune Escape in Lung Cancer Evolution. ***Cell***. 171(6):1259-1271.

Mesquita B, Rothwell DG, Burt DJ, Chemi F, Fernandez-Gutierrez F, Slane-Tan D, Antonello J, Carter M, Carter L, Parry M, Franklin L, Marais R, Blackhall F, Dive C, Brady G. (2017)
Molecular analysis of single circulating tumour cells following long-term storage of clinical samples. ***Mol Oncol***. 11(12):1687-1697.

Chiu A, Ayub M, Dive C, Brady G, Miller CJ. (2017)
twoddpcr: an R/Bioconductor package and Shiny app for Droplet Digital PCR analysis. ***Bioinformatics***, 33(17):2743-2745.

Abbosh C, Birkbak NJ, Wilson GA, Jamal-Hanjani M, Constantin T, Salari R, Le Quesne J, Moore DA, Veeriah S, Rosenthal R, Marafioti T, Kirkizlar E, Watkins TBK, McGranahan N, Ward S, Martinson L, Riley J, Fraioli F, Al Bakir M, Grönroos E, Zambrana F, Endozo R, Bi WL, Fennessy FM, Sponer N, Johnson D, Laycock J, Shafi S, Czyzewska-Khan J, Rowan A, Chambers T, Matthews N, Turajlic S, Hiley C, Lee SM, Forster MD, Ahmad T, Falzon M, Borg E, Lawrence D, Hayward M, Kolvekar S, Panagiotopoulos N, Janes SM, Thakrar R, Ahmed A, Blackhall F, Summers Y, Hafez D, Naik A, Ganguly A, Kareht S, Shah R, Joseph L, Marie Quinn A, Crosbie PA, Naidu B, Middleton G, Langman G, Trotter S, Nicolson M, Remmen H, Kerr K, Chetty M, Gomersall L, Fennell DA, Nakas A, Rathinam S, Anand G, Khan S, Russell P, Ezhil V, Ismail B, Irvin-Sellers M, Prakash V, Lester JF, Kornaszewska M, Attanoos R, Adams H, Davies H, Oukrif D, Akarca AU, Hartley JA, Lowe HL, Lock S, Iles N, Bell H, Ngai Y, Elgar G, Szallasi Z, Schwarz RF, Herrero J, Stewart A, Quezada SA, Peggs KS, Van Loo P, Dive C, Lin CJ, Rabinowitz M, Aerts HJWL, Hackshaw A, Shaw JA, Zimmermann BG; TRACERx consortium; PEACE consortium, Swanton C. (2017)
Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. ***Nature***, 545(7655):446-451.

Jamal-Hanjani M, Wilson GA, McGranahan N, Birkbak NJ, Watkins TBK, Veeriah S, Shafi S, Johnson DH, Mitter R, Rosenthal R, Salm M, Horswell S, Escudero M, Matthews N, Rowan A, Chambers T, Moore DA, Turajlic S, Xu H, Lee SM, Forster MD, Ahmad T, Hiley CT, Abbosh C, Falzon M, Borg E, Marafioti T, Lawrence D, Hayward M, Kolvekar S, Panagiotopoulos N, Janes SM, Thakrar R, Ahmed A, Blackhall F, Summers Y, Shah R, Joseph L, Quinn AM, Crosbie PA, Naidu B, Middleton G, Langman G, Trotter S, Nicolson M, Remmen H, Kerr K, Chetty M, Gomersall L, Fennell DA, Nakas A, Rathinam S, Anand G, Khan S, Russell P, Ezhil V, Ismail B, Irvin-Sellers M, Prakash V, Lester JF, Kornaszewska M, Attanoos R, Adams H, Davies H, Dentro S, Taniere P, O'Sullivan B, Lowe HL, Hartley JA, Iles N, Bell H, Ngai Y, Shaw JA, Herrero J, Szallasi Z, Schwarz RF, Stewart A, Quezada SA, Le Quesne J, Van Loo P, Dive C, Hackshaw A, Swanton C; TRACERx Consortium. (2017)
Tracking the Evolution of Non-Small-Cell Lung Cancer. ***N Engl J Med***., 376(22):2109-2121.

Khandelwal G, Girotti MR, Smowton C, Taylor S, Wirth C, Dynowski M, Frese KK, Brady G, Dive C, Marais R, Miller C. (2017)
Next-Generation Sequencing Analysis and Algorithms for PDX and CDX Models. ***Mol Cancer Res***., 15(8):1012-1016.

Arce Vargas F, Furness AJS, Solomon I, Joshi K, Mekkaoui L, Lesko MH, Miranda Rota E, Dahan R, Georgiou A, Sledzinska A, Ben Aissa A, Franz D, Werner Sunderland M, Wong YNS, Henry JY, O'Brien T, Nicol D, Challacombe B,

Beers SA; Melanoma TRACERx Consortium; Renal TRACERx Consortium; Lung TRACERx Consortium, Turajlic S, Gore M, Larkin J, Swanton C, Chester KA, Pule M, Ravetch JV, Marafioti T, Peggs KS, Quezada SA. (2017)
Fc-Optimized Anti-CD25 Depletes Tumor-Infiltrating Regulatory T Cells and Synergizes with PD-1 Blockade to Eradicate Established Tumors. ***Immunity***, 46(4):577-586.

Russell MR, D'Amato A, Graham C, Crosbie EJ, Gentry-Maharaj A, Ryan A, Kalsi JK, Fourkala EO, Dive C, Walker M, Whetton AD, Menon U, Jacobs I, Graham RL. (2017)
Novel risk models for early detection and screening of ovarian cancer. ***Oncotarget***, 8(1):785-797.

Thistlethwaite FC, Gilham DE, Guest RD, Rothwell DG, Pillai M, Burt DJ, Byatte AJ, Kirillova N, Valle JW, Sharma SK, Chester KA, Westwood NB, Halford SER, Nabarro S, Wan S, Austin E, Hawkins RE. (2017)
The clinical efficacy of first-generation carcinoembryonic antigen (CEACAM5)-specific CAR T cells is limited by poor persistence and transient pre-conditioning-dependent respiratory toxicity. ***Cancer Immunol Immunother.***, 66(11):1425-1436

Other publications

Mohan S, Chemi F, Brady G. (2017)
Challenges and unanswered questions for the next decade of circulating tumour cell research in lung cancer. ***Transl Lung Cancer Res.***, 6(4):454-472.

Foy V, Fernandez-Gutierrez F, Faivre-Finn C, Dive C, Blackhall F. (2017)
The clinical utility of circulating tumour cells in patients with small cell lung cancer. ***Transl Lung Cancer Res.***, 6(4):409-417.

Lallo A, Schenk MW, Frese KK, Blackhall F, Dive C. (2017)
Circulating tumor cells and CDX models as a tool for preclinical drug development. ***Transl Lung Cancer Res.***, 6(4):397-408.

Blackhall F, Dive C. (2017)
Spotlight on circulating tumour cells. ***Transl Lung Cancer Res.***, 6(4):396.

Blandin Knight S, Crosbie PA, Balata H, Chudziak J, Hussell T, Dive C. (2017)
Progress and prospects of early detection in lung cancer. ***Open Biol.***,7(9). pii: 170070.

Dive C, Brady G. (2017)
SnapShot: Circulating Tumor Cells. ***Cell***, 169(1):176.

Dive C, Shishido SN, Kuhn P. (2017)
Cancer Moonshot Connecting International Liquid Biopsy Efforts Through Academic Partnership. ***Clin Pharmacol Ther.***, 101(5): 622-624.

Drug Discovery (page 26)

Donald Ogilvie / Caroline Springer

Refereed research publications

Mould DP, Bremberg U, Jordan AM, Geitmann M, McGonagle AE, Somervaille TCP, Spencer GJ, Ogilvie DJ. (2017)
Development and evaluation of 4-(pyrrolidin-3-yl)benzonitrile derivatives as inhibitors of lysine specific demethylase 1. ***Bioorg Med Chem Lett.***, 27(20):4755-4759.

Mould DP, Alli C, Bremberg U, Cartic S, Jordan AM, Geitmann M, Maiques-Diaz A, McGonagle AE, Somervaille TCP, Spencer GJ, Turlais F, Ogilvie D. (2017)
Development of (4-Cyanophenyl)glycine Derivatives as Reversible Inhibitors of Lysine Specific Demethylase 1. ***J Med Chem***. 60(19):7984-7999.

Mould DP, Bremberg U, Jordan AM, Geitmann M, Maiques-Diaz A, McGonagle AE, Small HF, Somervaille TCP, Ogilvie D. (2017)
Development of 5-hydroxypyrazole derivatives as reversible inhibitors of lysine specific demethylase 1. ***Bioorg Med Chem Lett.***, 27(14):3190-3195.

Gravells P, Grant E, Smith KM, James DI, Bryant HE. (2017)
DNA Specific killing of DNA damage-response deficient cells with inhibitors of poly(ADP-ribose) glycohydrolase. ***DNA Repair***, 52:81-91.

Fitzpatrick PA, Akrap N, Söderberg EMV, Harrison H, Thomson GJ, Landberg G. (2017)
Robotic mammosphere assay for high-throughput screening in triple-negative breast cancer. ***SLAS Discovery***, 22(7):827-836.

Other publications

Allan Michael Jordan and Kate Mary Smith
"Beyond PARP: inhibitors of DNA repair processes as potential therapeutic agents for cancer", Medicinal Chemistry Reviews, Volume 52, 2017, in press.

Palazzo L, James DI, Waddell ID, Ahel I. (2017)
Studying Catabolism of Protein ADP-Ribosylation. ***Methods Mol Biol.***, 1608:415-430.

Active Patents/Filings

Allan Jordan, Rebecca Newton
"Heterocyclic Compounds as RET Kinase Inhibitors", PCT Int. Appl. WO2017178844, 19th October, 2017

Allan Jordan, Rebecca Newton
"Heterocyclic Compounds as RET Kinase Inhibitors", , PCT Int. Appl. WO2017178845, 19th October, 2017

"Substituted Pyridines as Inhibitors of DNMT1", Nicholas David Adams, Andrew B. Benowitz, Maria Lourdes Roueda Benede, Karen Anderson Evans, David T. Fosbenner, Bryan Wayne King, Mei Li, Juan Ignacio Luengo, William Henr Miller, Alexander Joseph Reif, Stanley J. Schmidt, Roger J. Butlin, Kristen M. Goldberg, Allan M. Jordan, Christopher S. Kershaw, Ali Raoof, Bohdan Waszkowycz, PCT Int. Appl. WO2017216727, 21st December 2017.

Leukaemia Biology (page 28)

Tim Somervaille

Refereed research publications

Mould DP, Bremberg U, Jordan AM, Geitmann M, McGonagle AE, Somervaille TCP, Spencer GJ, Ogilvie DJ. (2017)
Development and evaluation of 4-(pyrrolidin-3-yl)benzonitrile derivatives as inhibitors of lysine specific demethylase 1. ***Bioorg Med Chem Lett.***, 27(20):4755-4759.

Mould DP, Alli C, Bremberg U, Cartic S, Jordan AM, Geitmann M, Maiques-Diaz A, McGonagle AE, Somervaille TCP, Spencer GJ, Turlais F, Ogilvie D. (2017)
Development of (4-Cyanophenyl)glycine Derivatives as Reversible Inhibitors of Lysine Specific Demethylase 1. ***J Med Chem***. 60(19):7984-7999.

Mould DP, Bremberg U, Jordan AM, Geitmann M, Maiques-Diaz A, McGonagle AE, Small HF, Somervaille TCP, Ogilvie D. (2017)
Development of 5-hydroxypyrazole derivatives as reversible inhibitors of lysine specific demethylase 1. ***Bioorg Med Chem Lett.***, 27(14):3190-3195.

Pearson S, Williamson AJK, Blance R, Somervaille TCP, Taylor S, Azadbakht N, Whetton AD, Pierce A.

Proteomic analysis of JAK2V617F-induced changes identifies potential new combinatorial

therapeutic approaches. ***Leukemia***. [Epub 19 December 2017]

Igoe N, Bayle ED, Fedorov O, Tallant C, Savitsky P, Rogers C, Owen DR, Deb G, Somervaille TC, Andrews DM, Jones N, Cheasty A, Ryder H, Brennan PE, Müller S, Knapp S, Fish PV. (2017)
Design of a Biased Potent Small Molecule Inhibitor of the Bromodomain and PHD Finger-Containing (BRPF) Proteins Suitable for Cellular and in Vivo Studies. ***J Med Chem***., 60(2):668-680.

Other publications

Wiseman DH, Somervaille TCP. (2017)
Nanofluidic Allele-Specific Digital PCR Method for Quantifying IDH1 and IDH2 Mutation Burden in Acute Myeloid Leukemia. ***Methods Mol Biol***. 1633:235-255.

Molecular Oncology (page 30)

Richard Marais

Refereed research publications

Yates LR, Seoane J, Le Tourneau C, Siu LL, Marais R, Michiels S, Soria JC, Campbell P, Normanno N, Scarpa A, Reis-Filho JS, Rodon J, Swanton C, Andre F.
The European Society for Medical Oncology (ESMO) Precision Medicine Glossary. ***Ann Oncol***. [Epub 13 November 2017]

Lee RJ, Gremel G, Marshall A, Myers KA, Fisher N, Dunn J, Dhomen N, Corrie PG, Middleton MR, Lorigan P, Marais R.
Circulating tumor DNA predicts survival in patients with resected high risk stage II/III melanoma. ***Ann Oncol***. [Epub 13 November 2017]

Green AC, Viros A, Hughes MC, Gaudy-Marqueste C, Akhras V, Cook MG, Marais R.
Nodular Melanoma: A Histopathologic Entity? ***Acta Derm Venereol***. [Epub 28 Nov 2017]

Negrouk A, Lacombe D, Cardoso F, Morin F, Carrasco E, Maurel J, Maibach R, Aranda E, Marais R, Stahel RA. (2017)
Safeguarding the future of independent, academic clinical cancer research in Europe for the benefit of patients. ***ESMO Open***, 2(3):e000187.

Nieto P, Ambrogio C, Esteban-Burgos L, Gómez-López G, Blasco MT, Yao Z, Marais R, Rosen N, Chiarle R, Pisano DG, Barbacid M, Santamaría D. (2017)
A Braf kinase-inactive mutant induces lung adenocarcinoma. ***Nature***, 548(7666):239-243.

Mesquita B, Rothwell DG, Burt DJ, Chemi F, Fernandez-Gutierrez F, Slane-Tan D, Antonello J, Carter M, Carter L, Parry M, Franklin L, Marais R, Blackhall F, Dive C, Brady G. (2017)
Molecular analysis of single circulating tumour cells following long-term storage of clinical samples. ***Mol Oncol***., 11(12):1687-1697.

Cook MG, Massi D, Blokx WAM, Van den Oord J, Koljenović S, De Giorgi V, Kissin E, Grant M, Mandal A, Gremel G, Gaudy C, Viros A, Dhomen N, Khosrotehrani K, Marais R, Green AC, Mihm MC Jr. (2017)
New insights into naevoid melanomas: a clinicopathological reassessment. ***Histopathology***, 71(6):943-950.

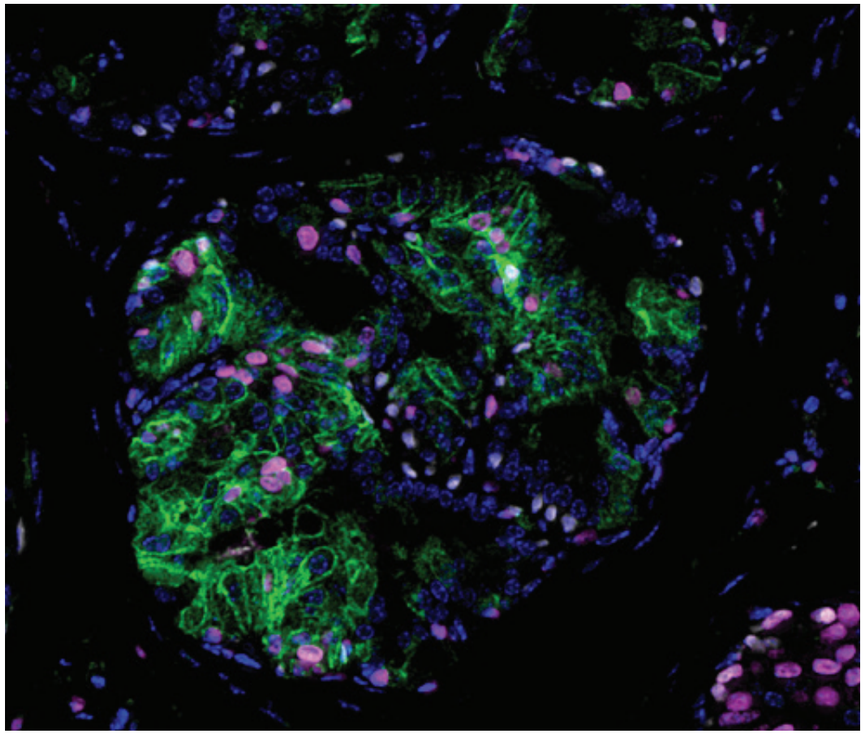
Haston S, Pozzi S, Carreno G, Manshaei S, Panousopoulos L, Gonzalez-Meljem JM, Apps JR, Virasami A, Thavaraj S, Gutteridge A, Forshew T, Marais R, Brandner S, Jacques TS, Andoniadou CL, Martinez-Barbera JP. (2017)
MAPK pathway control of stem cell proliferation and differentiation in the embryonic pituitary provides insights into the pathogenesis of papillary craniopharyngioma. ***Development***, 144(12):2141-2152.

Khandelwal G, Girotti MR, Smowton C, Taylor S, Wirth C, Dynowski M, Frese KK, Brady G, Dive C, Marais R, Miller C. (2017)
Next-Generation Sequencing Analysis and Algorithms for PDX and CDX Models. ***Mol Cancer Res***. 15(8):1012-1016.

Tang H, Leung L, Saturno G, Viros A, Smith D, Di Leva G, Morrison E, Niculescu-Duvaz D, Lopes F, Johnson L, Dhomen N, Springer C, Marais R. (2017)
Lysyl oxidase drives tumour progression by trapping EGF receptors at the cell surface. ***Nat Commun***. 8:14909.

Other publications

Lee RJ, Marais R. (2017)
Cancer: Tumours addicted to drugs are vulnerable. ***Nature***, 550(7675):192-193



Pten loss in the luminal compartment of mouse prostate tissue. The tissue is stained for pAKT (green), P63 (white), Ki67 (pink) and DAPI (blue).
Image supplied by Ivana Steiner (Prostate Oncobiology)

Prostate Oncobiology (page 32)

Esther Baena

Refereed research publications

Lessard S, Gatof ES, Beaudoin M, Schupp PG, Sher F, Ali A, Prehar S, Kurita R, Nakamura Y, Baena E, Ledoux J, Oceandy D, Bauer DE, Lettre G. (2017)
An erythroid-specific ATP2B4 enhancer mediates red blood cell hydration and malaria susceptibility. ***J Clin Invest***., 127(8):3065-3074.

Ali A, Hoyle A, Baena E, Clarke NW. (2017)
Identification and evaluation of clinically significant prostate cancer: a step towards personalized diagnosis. ***Curr Opin Urol***., 27(3):217-224.

RNA Biology (page34)

Crispin Miller

Refereed research publications

Khandelwal G, Girotti MR, Smowton C, Taylor S, Wirth C, Dynowski M, Frese KK, Brady G, Dive C, Marais R, Miller C. (2017)
Next-Generation Sequencing Analysis and Algorithms for PDX and CDX Models. ***Mol Cancer Res***., 15(8):1012-1016.

Chiu A, Ayub M, Dive C, Brady G, Miller CJ. (2017)
twoddpcr: an R/Bioconductor package and Shiny app for Droplet Digital PCR analysis. ***Bioinformatics***, 33(17):2743-2745.

Smowton, C, Balla, A, Antoniades, D, Miller, C, Pallis, G, Dikaiakos, MD, Xing, W (2017)
A cost-effective approach to improving performance of big genomic data analyses in clouds. ***Future Generation Computer Systems***, 67: 368-381.

Draper JE, Sroczynska P, Leong HS, Fadlullah MZH, Miller C, Kouskoff V, Lacaud G. (2017)
Mouse RUNX1C regulates premegakaryocytic/erythroid output and maintains survival of megakaryocyte progenitors. ***Blood***, 130(3):271-284.

Skin Cancer and Ageing (page 36)

Amaya Virós

Refereed research publications

Martin-Gorgojo A, Llinares M, Virós A, Requena C, Garcia-Casado Z, Traves V, Kumar R, Nagore E. (2017)
Cutaneous melanoma primary site is linked to nevus density. ***Oncotarget*** 8(58):98876-98886.

Green AC, Viros A, Hughes MC, Gaudy-Marqueste C, Akhras V, Cook MG, Marais R. Nodular Melanoma: A Histopathologic Entity? ***Acta Derm Venereol***. [Epub 28 Nov 2017]

Cook MG, Massi D, Blokx WAM, Van den Oord J, Koljenović S, De Giorgi V, Kissin E, Grant M, Mandal A, Gremel G, Gaudy C, Viros A, Dhomen N, Khosrotehrani K, Marais R, Green AC, Mihm MC Jr. (2017) New insights into naevoid melanomas: a clinicopathological reassessment. ***Histopathology***, 71(6):943-950.

Tang H, Leung L, Saturno G, Viros A, Smith D, Di Leva G, Morrison E, Niculescu-Duvaz D, Lopes F, Johnson L, Dhomen N, Springer C, Marais R. (2017) Lysyl oxidase drives tumour progression by trapping EGF receptors at the cell surface. ***Nat Commun***. 8:14909.

Other publications

Winder M, Virós A. Mechanisms of Drug Resistance in Melanoma. ***Handb Exp Pharmacol***., [Epub 9 March 2017]

Stem Cell Biology (page 38)

Georges Lacaud

Refereed research publications

Stefanska M, Batta K, Patel R, Florkowska M, Kouskoff V, Lacaud G. (2017) Primitive erythrocytes are generated from hemogenic endothelial cells. ***Sci Rep***., 7(1):6401.

Lilly AJ, Mazan A, Scott DA, Lacaud G, Kouskoff V. (2017) SOX7 expression is critically required in FLK1-expressing cells for vasculogenesis and angiogenesis during mouse embryonic development. ***Mech Dev***., 146:31-41.

Draper JE, Sroczynska P, Leong HS, Fadlullah MZH, Miller C, Kouskoff V, Lacaud G. (2017) Mouse RUNX1C regulates premegakaryocytic/erythroid output and maintains survival of megakaryocyte progenitors. ***Blood***, 130(3):271-284.

Diamantopoulou Z, White G, Fadlullah MZH, Dreger M, Pickering K, Maltas J, Ashton G, MacLeod R, Baillie GS, Kouskoff V, Lacaud G, Murray GI, Sansom OJ, Hurlstone AFL, Malliri A. (2017) TIAM1 antagonizes TAZ/YAP both in the destruction complex in the cytoplasm and in the nucleus to inhibit invasion of intestinal epithelial cells. ***Cancer Cell***, 31(5):621-634.e6.

Other publications

Bonifer C, Levantini E, Kouskoff V, Lacaud G. (2017) Runx1 Structure and Function in Blood Cell Development. ***Adv Exp Med Biol***., 962:65-81.

Lacaud G, Kouskoff V. (2017) Hemangioblast, hemogenic endothelium, and primitive versus definitive hematopoiesis. ***Exp Hematol***., 49:19-24.

Systems Oncology (page 40)

Claus Jorgensen

Other publications

Tape CJ, Jørgensen C. (2017) Cell-specific labeling for analyzing bidirectional signaling by mass spectrometry. ***Methods Mol Biol***., 1636:219-234.

Transcriptional Networks in Lung Cancer (page 42)

Michela Garofalo

Refereed research publications

Naidu S, Shi L, Magee P, Middleton JD, Laganá A, Sahoo S, Leong HS, Galvin M, Frese K, Dive C, Guzzardo V, Fassan M, Garofalo M. (2017) PDGFR-modulated miR-23b cluster and miR-125a-5p suppress lung tumorigenesis by targeting multiple components of KRAS and NF-kB pathways. ***Sci Rep***., 7(1):15441.

Additional Publications

Meehan TF, Conte N, Goldstein T, Inghirami G, Murakami MA, Brabetz S, Gu Z, Wiser JA, Dunn P, Begley DA, Krupke DM, Bertotti A, Bruna A, Brush MH, Byrne AT, Caldas C, Christie AL, Clark DA, Dowst H, Dry JR, Doroshow JH, Duchamp O, Evrard YA, Ferretti S, Frese KK, Goodwin NC, Greenawalt D, Haendel MA, Hermans E, Houghton PJ, Jonkers J, Kemper K, Khor TO, Lewis MT, Lloyd KCK, Mason J, Medico E, Neuhauser SB, Olson JM, Peeper DS, Rueda OM, Seong JK, Trusolino L, Vinolo E, Wechsler-Reya RJ, Weinstock DM, Welm A, Weroha SJ, Amant F, Pfister SM, Kool M, Parkinson H, Butte AJ, Bult CJ. (2017) PDX-MI: Minimal Information for Patient-Derived Tumor Xenograft Models. ***Cancer Res***., 77(21):e62-e66.

Ramos M, Schiffer L, Re A, Azhar R, Basunia A, Rodriguez C, Chan T, Chapman P, Davis SR, Gomez-Cabrero D, Culhane AC, Haibe-Kains B, Hansen KD, Kodali H, Louis MS, Mer AS, Riester M, Morgan M, Carey V, Waldron L. (2017) Software for the Integration of Multiomics Experiments in Bioconductor. ***Cancer Res***. 77(21):e39-e42.

Edwards ZC, Trotter EW, Torres-Ayuso P, Chapman P, Wood HM, Nyswaner K, Brognard J. (2017) Survival of Head and Neck Cancer Cells Relies upon LZK Kinase-Mediated Stabilization of Mutant p53. ***Cancer Res***., 77(18):4961-4972.

Castle J, Morris K, Pritchard S, Kirwan CC. (2017) Challenges in enumeration of CTCs in breast cancer using techniques independent of cytokeratin expression. ***PLoS One***, 12(4):e0175647.

Hessmann E, Patzak MS, Klein L, Chen N, Kari V, Ramu I, Bapiro TE, Frese KK, Gopinathan A, Richards FM, Jodrell DI, Verbeke C, Li X, Heuchel R, Löhr JM, Johnsen SA, Gress TM, Ellenrieder V, Neesse A. (2017) Fibroblast drug scavenging increases intratumoural gemcitabine accumulation in murine pancreas cancer. ***Gut***, 67(3):497-507.

Newton AC, Brognard J. (2017) Reversing the Paradigm: Protein Kinase C as a Tumor Suppressor. ***Trends Pharmacol Sci***., 38(5):438-447.

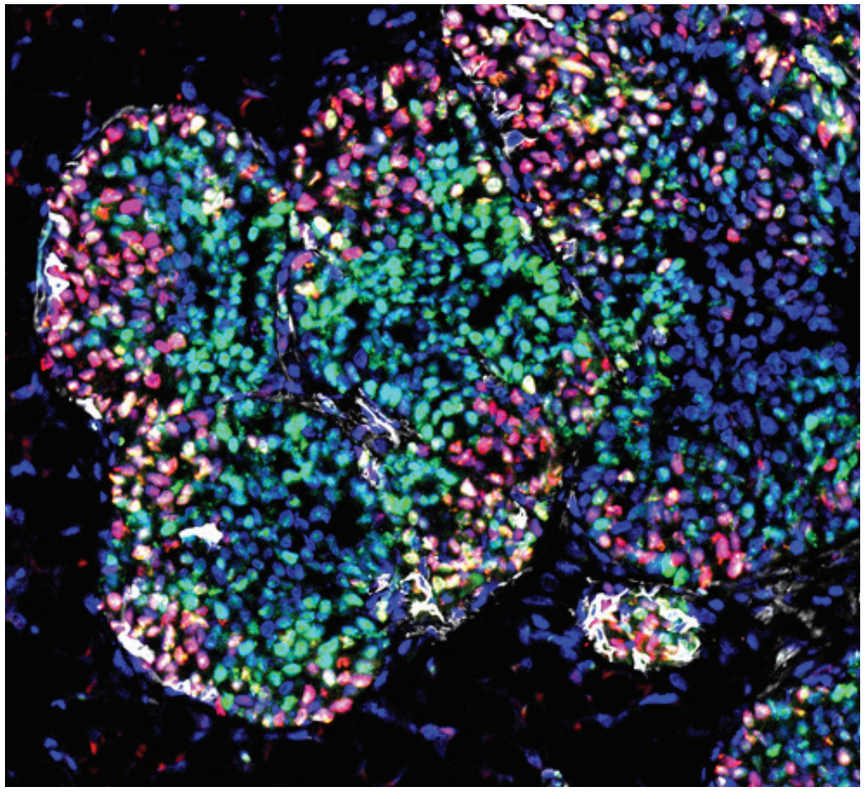
Lorigan P, Green AC. (2017) Immunotherapy: Does adjuvant ipilimumab have little adverse effect on quality of life? ***Nat Rev Clin Oncol***., 14(7):395-396.

Plasmeijer EI, Nguyen TM, Olsen CM, Janda M, Soyer HP, Green AC. (2017) The Natural History of Common Melanocytic Nevi: A Systematic Review of Longitudinal Studies in the General Population. ***J Invest Dermatol***., 137(9):2017-2018.

Olsen CM, Green AC. Risk of invasive melanoma in patients with rheumatoid arthritis treated with biologics: an updated meta-analysis. ***Ann Rheum Dis***., [Epub 22 August 2017]

Confocal photomicrograph of PyMT mouse breast tumour. Cells were labelled with Ki67 (Red), Cre (Green) and DAPI (Blue).

Image supplied by Haoran Tang (Molecular Oncology)



Dusingize JC, Olsen CM, Pandeya NP, Subramaniam P, Thompson BS, Neale RE, Green AC, Whiteman DC; QSkin Study. (2017) Cigarette Smoking and the Risks of Basal Cell Carcinoma and Squamous Cell Carcinoma. J ***Invest Dermatol***., 137(8):1700-1708.

Webb PM, Green AC, Jordan SJ. (2017) Trends in hormone use and ovarian cancer incidence in US white and Australian women: implications for the future. ***Cancer Causes Control***, 28(5):365-370.

Olsen CM, Wilson LF, Green AC, Biswas N, Loyalka J, Whiteman DC. (2017) Prevention of DNA damage in human skin by topical sunscreens. ***Photodermatol Photoimmunol Photomed***., 33(3):135-142.

Blomberg M, He SY, Harwood C, Arron ST, Demehri S, Green A, Asgari MM; NCI Keratinocyte Carcinoma Consortium. Research gaps in the management and prevention of cutaneous squamous cell carcinoma in organ transplant recipients. ***Br J Dermatol***. [Epub 30 October 2017]

Olsen CM, Pandeya N, Thompson BS, Dusingize JC, Subramaniam P, Nagle CM, Green AC, Neale RE, Webb PM, Whiteman DC; QSkin Study. (2017) Hormonal and reproductive factors and incidence of basal cell carcinoma and squamous cell carcinoma in a large, prospective cohort. ***J Am Acad Dermatol.*** pii: S0190-9622(17)32438-6.

Green AC, Hughes MCB, von Schuckmann LA, Khosrotehrani K, Smithers BM. Clustering of prevention behaviours in patients with high-risk primary melanoma ***Psychooncology***. 2017 Oct 16. doi: 10.1002/pon.4565. [Epub 16 October 2017]

Madeleine MM, Patel NS, Plasmeijer EI, Engels EA, Bouwes Bavinck JN, Toland AE, Green AC; the Keratinocyte Carcinoma Consortium (KeraCon) Immunosuppression Working Group. (2017) Epidemiology of keratinocyte carcinomas after organ transplantation. ***Br J Dermatol***., 177(5):1208-1216.

Jiyad Z, O'Rourke P, Soyer HP, Green AC. (2017) Clinical comparison of actinic changes preceding squamous cell carcinoma vs. intraepidermal carcinoma in renal transplant recipients. ***Clin Exp Dermatol***., 42(8):895-897.

von Schuckmann LA, Smith D, Hughes MCB, Malt M, van der Pols JC, Khosrotehrani K, Smithers BM, Green AC. (2017) Associations of Statins and Diabetes with Diagnosis of Ulcerated Cutaneous Melanoma. ***J Invest Dermatol***., 137(12):2599-2605.

Youlden DR, Baade PD, Aitken JF, Green AC, Khosrotehrani K. (2017) Prognostic importance of a second invasive primary melanoma according to tumour stage. ***Br J Dermatol***., 177(6):e336-e337.

Law MH, Medland SE, Zhu G, Yazar S, Viñuela A, Wallace L, Shekar SN, Duffy DL, Bataille V, Glass D, Spector TD, Wood D; MuTHER Consortium, Gordon SD, Barbour JM, Henders AK, Hewitt AW, Montgomery GW, Sturm RA, Mackey DA, Green AC, Martin NG, MacGregor S. (2017) Genome-Wide Association Shows that Pigmentation Genes Play a Role in Skin Aging. ***J Invest Dermatol***., 137(9):1887-1894.

Karimkhani C, Green AC, Nijsten T, Weinstock MA, Dellavalle RP, Naghavi M, Fitzmaurice C. (2017) The global burden of melanoma: results from the Global Burden of Disease Study 2015. ***Br J Dermatol***., 177(1):134-140.

Green AC, Olsen CM. (2017) Cutaneous squamous cell carcinoma: an epidemiological review. ***Br J Dermatol***., 177(2):373-381.

Ghiasvand R, Rueegg CS, Weiderpass E, Green AC, Lund E, Veierød MB. (2017) Indoor Tanning and Melanoma Risk: Long-Term Evidence From a Prospective Population-Based Cohort Study. ***Am J Epidemiol***. 185(3):147-156.

Jiyad Z, O'Rourke P, Soyer HP, Green AC. (2017) Actinic keratosis-related signs predictive of squamous cell carcinoma in renal transplant recipients: a nested case-control study. ***Br J Dermatol***., 176(4):965-970.

THESES

Daniel Mould
Drug Discovery

Design and synthesis of small molecule inhibitors of lysine specific demethylase 1

Emma Williams
Leukaemia Biology

Inhibition of LSD1 as a candidate therapeutic strategy in myeloproliferative neoplasms

EXTERNAL SEMINAR SPEAKERS 2017

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

Nicola Aceto
University of Basel

Constance Alabert
University of Dundee

Sam Au
Imperial College London

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

Jonathan Blackburn
Sengenics

Christian Blank
Netherlands Cancer Institute

Dr Paul Boutros
Ontario Institute for Cancer Research

Robert Brown
Imperial College London

Siggeir F. Brynjolfsson
University of Gothenburg

Francesca Demichelis
Centre for Integrative Biology (CIBIO) - University of Trento

Jessica Anne Downs
The Institute of Cancer Research

Jorrit M. Enserink
University of Oslo - Oslo University Hospital

Christian Frezza
MRC Cancer Unit - University of Cambridge

Silke Gillessen
Kantonsspital St.Gallen

Susana Godinho
Barts Cancer Institute - Queen Mary University of London

Romina Goldszmid
National Cancer Institute (USA) - Center For Cancer Research

John Haanen
The Netherlands Cancer Institute

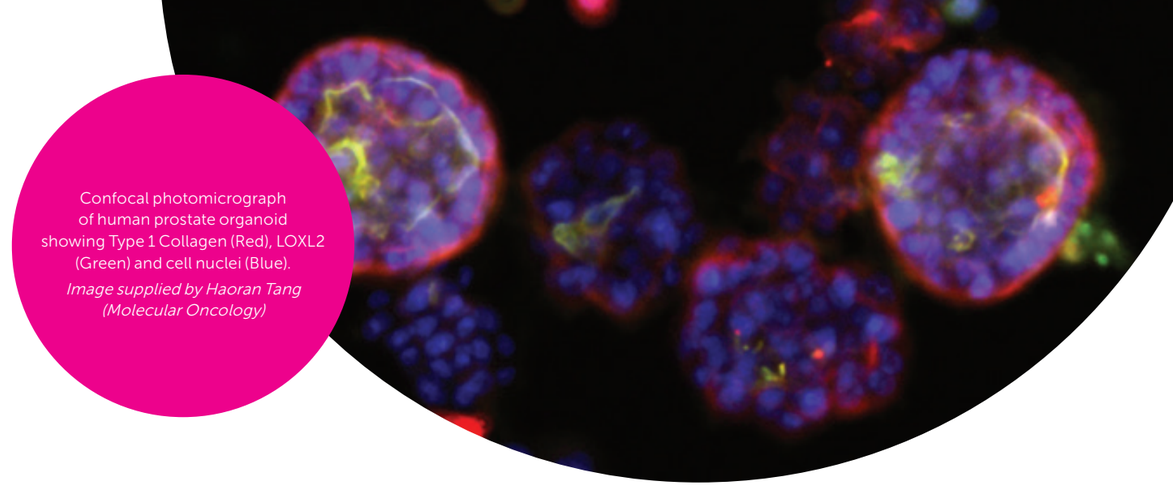
Benjamin Haibe-Kains
University of Toronto

Kevin Janes
University of Virginia

Tobias Janowitz
Cambridge University Hospitals NHS Foundation Trust

Ricky Johnstone
Peter MacCallum Cancer Centre

Claire Lewis
University of Sheffield



Confocal photomicrograph of human prostate organoid showing Type 1 Collagen (Red), LOXL2 (Green) and cell nuclei (Blue).
Image supplied by Haoran Tang (Molecular Oncology)

Helder Maiato
University of Porto

Marc Mansour
UCL

Leticia De Mattos-Arruda
Vall d'Hebron Institute of Oncology

Florian Muller
The University of Texas MD Anderson Cancer Center

Maddy Parsons
Nikon Imaging Centre - King's College London

Simona Polo
The FIRC Institute for Molecular Oncology

Hans-Reimer Rodewald
German Cancer Research Center (DKFZ)

Jan Schellens
Netherlands Cancer Institute - NKI

Sheila Singh
McMaster Children's Hospital

Stephen Tait
Cancer Research UK Beatson Institute

Peter Van Loo
The Francis Crick Institute

Fiona Watt
King's College London

Breast Cancer Now Seminars

Matthew Ellis
Baylor Breast Centre

Richard Iggo
Bergonié Cancer Institute - University of Bordeaux

Louise Jones
Barts Cancer Institute - Queen Mary University of London

Jos Jonkers
The Netherlands Cancer Institute

Lone Rønnov-Jessen
University of Copenhagen

Pepper Schedin
Oregon Health & Science University

Nicholas Turner
The Institute of Cancer Research

POSTGRADUATE EDUCATION

Postgraduate Education Manager

Julie Edwards

Postgraduate Tutor

Angeliki Malliri

Postgraduate Director and Chair of the Education Committee

Tim Somervaille

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

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

In 2017, we welcomed ten Graduate Students and three Clinical Research Fellows to our PhD programme, working in a variety of fields from leukaemia biology, molecular oncology, skin cancer and ageing, cancer inflammation and immunity through to cell biology.

The Cancer Research UK Manchester Graduate Programme

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

The CRUK MI runs an external seminar series featuring talks from many of the key players in cancer research, and students are expected to attend. The speakers are internationally renowned scientists and we consider it essential that our students are exposed to outstanding research from leaders in different disciplines, which will give them a broad understanding of many aspects of cancer research and basic biology. In addition, we hold a series of weekly postdoctoral research seminars and attendance from PhD students is an integral part of the seminar programme. While students themselves are asked to give talks at key points during their PhD, they also have opportunities to present their work at lab meetings and during student forums within the Institute. STAy (short for Science TakeAway) is a group run by junior scientists in the CRUK Manchester Institute and PhD students sit on the committee. Meetings are open to all early career scientists - PhD students, Postdoctoral Fellows and Scientific Officers. The aim is to provide a forum for discussions and training related to research, communication of scientific engagement and development of social and networking opportunities. Topics in 2017 have included a careers panel, discussions on the funding of science, and science communications.

The CRUK Manchester Institute Colloquium takes place annually in September, and is an excellent opportunity for our new intake of students to meet other established PhD students, members of the Institute, including Group Leaders, Postdoctoral Fellows, and Scientific Officers. This forum communicates up to date science in the form of oral presentations given by Group Leaders and second year PhD students, as well as poster presentations from a range of scientists across the Institute covering all aspects of cancer research. Poster prizes are awarded, including the Lizzy Hitchman Prize for the best poster presented by a PhD student or clinical fellow. In 2017 PhD student Alice Lallo from the Clinical Experimental and Pharmacology group was the recipient of this prize for her work on circulating tumour cells and drug resistance in small cell lung cancer.

Cancer Research UK contributes towards an annual International PhD Student Cancer Conference (IPSCC) allowing students (typically in 2nd or 3rd year) from top cancer research institutes across Europe to organise and present at their own scientific conference. Core participating Institutes include The Francis Crick Institute, CRUK Cambridge Institute, CRUK Beatson Institute, Netherlands Cancer Institute, European School of Molecular Medicine, Milan and the German Cancer Research Centre.

In 2017, the 11th IPSCC was organised by PhD students from the Max Delbruck Centre for Molecular Medicine, Berlin and 11 students from the CRUK MI attended the conference in June. Student delegates submit an abstract and showcase their research either through a poster or oral presentation.

PhD studentships

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

POSTGRADUATE EDUCATION (CONTINUED)

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.

Education Committee 2017

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

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

Education Committee Members

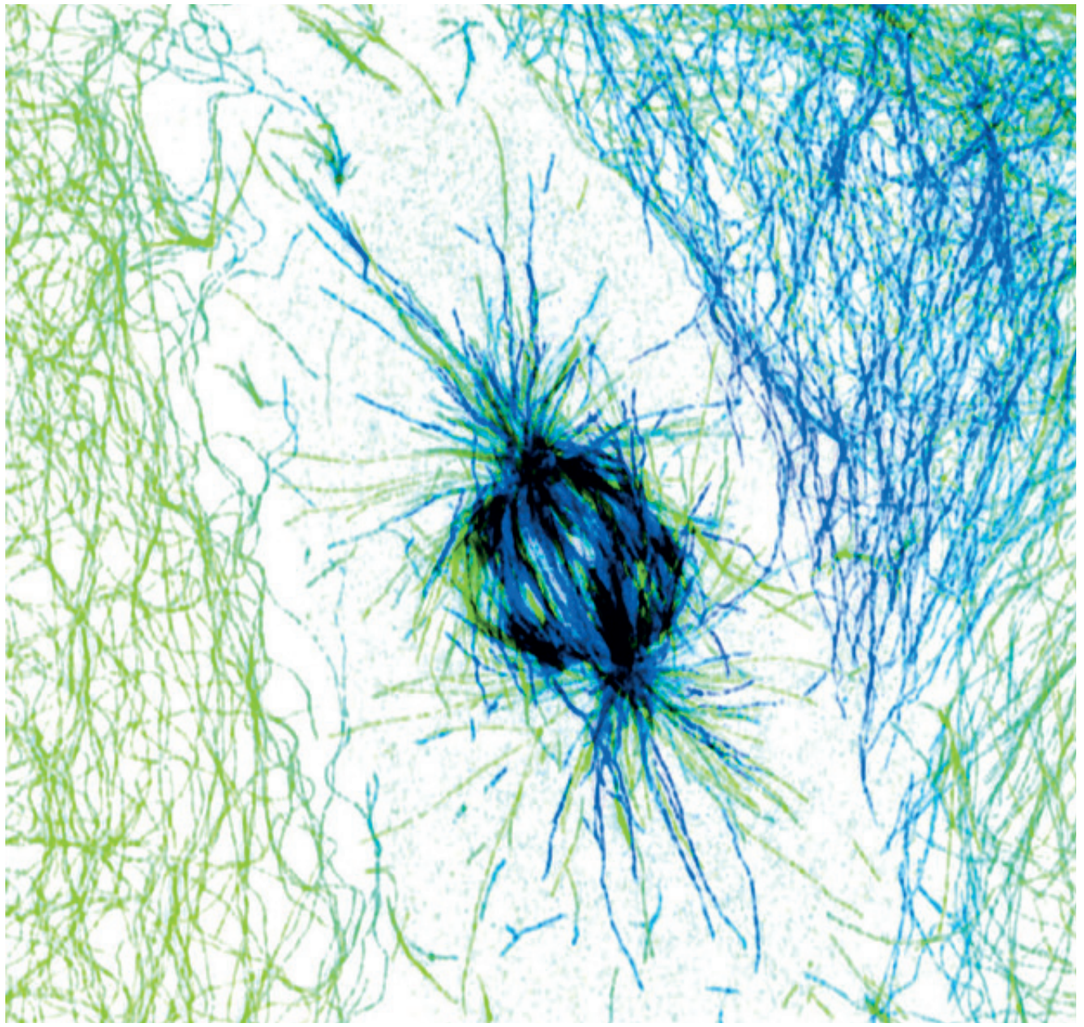
Tim Somervaille
Postgraduate Director and Chair

Angeliki Malliri
Postgraduate Tutor
Richard Marais
Ex-Officio Member
Wolfgang Breitwieser
Julie Edwards
Postgraduate Manager
Claus Jørgensen
Georges Lacaud
Donald Ogilvie[2]
Jonathan Tugwood
Ian Waddell[2]
Caroline Wilkinson

Student Representatives

Amy McCarthy[2]
Denys Holovanchuk
Jakub Chudziak[1]

[1]Joined in 2017
[2]Left in 2017



Super-resolution microscopy allows us to uncover otherwise undetectable details within individual cells. This Zeiss AiryScan image shows the dramatic rearrangement of the microtubule cytoskeleton of a mitotic epithelial cell, with ultra-fine astral microtubules radiating from the mitotic spindle to the cell cortex, compared with the dense, overlapping microtubules in the surrounding cells. These astral microtubules help ensure cells divide in the plane of tissue; misorientation of cell division has been linked to the formation of tumours. The colouring from blue to green represents the height of the microtubules as captured in the original three-dimensional image.

Image supplied by Andrew Porter (Cell Signalling)

OPERATIONS

Chief Operating Officer
Caroline Wilkinson

Chief Laboratory Officer
Stuart Pepper

Head of Finance
Margaret Lowe

Head of Human Resources
Rachel Powell

The Operations' team provides the necessary infrastructure and services to facilitate the running of the Institute. Finance and purchasing fall under the leadership of Margaret Lowe while Stuart Pepper oversees IT, Estates, Logistics and Health and Safety; Rachel Powell is Head of HR and Caroline Wilkinson is responsible for all aspects of scientific administration and communications and acts as the primary point of operational contact within the Institute for both The University of Manchester and Cancer Research UK. This year, staff from across the team have played a key role in the recovery and relocation efforts following the Paterson Building fire. This has involved a vast project together with the Institute's scientists to empty the building and catalogue its contents. In tandem with this, there was a huge piece of work to move to our interim facilities at Alderley Park. We said goodbye to Steve Alcock and Graham Hooley and welcomed Neil Carne while Klara Berne and Lee Anne van Winkel provided invaluable temporary cover in the finance team and Director's office respectively.

Director's Office and Administration Services

Ruth Perkins, Maria Belen Conti, Lee Anne van Winkel[1]

[1]Temporary cover

The office provides administrative support to the Director and to the Institute Group Leaders. In addition, the department has assisted with the organisation of several events over the course of the year, including the successful Quinquennial Review of the Institute and the Institute Colloquium.

Administrative support is provided for the external seminar series, which has continued to be a great success in 2017, hosting around two seminars per week. The seminars serve to foster collaboration and encourage positive interaction within the wider scientific community. We aim to provide a varied programme of national and international speakers. Details can be found at www.cruk.manchester.ac.uk/seminars.

During the year, we had support from Lee Anne van Winkel, who provided invaluable temporary cover to the office. She has now returned to New Zealand and we wish her all the best.

Estates

Neil Carne[2], Steve Alcock[1], Graham Hooley[1], Steven Powell, Tony Woollam

[1]Left in 2017 [2]Joined in 2017

Last year Steve Alcock and Graham Hooley both retired after many years of service to the Institute. Neil Carne took over the Estates Manager position from Steve, continuing the existing projects including the refurbishment of the Biobank office in the Kay Kendall Laboratories, along with several other projects.

The Estates team has also been proactive in energy saving initiatives and essential heating and ventilation plant upgrades; with Air Handling Units 2, 3 and 4 being completed the previous year, only AHU 6 was outstanding. AHU6 was upgraded over the winter months and was completed along with the commissioning of the new energy efficient Blue Cube cooling units in April.

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

In the aftermath of the fire in April, the team worked hard to reinstate some of the electrical supplies to restore power, lighting and ventilation where possible for the many -80°C and -20°C freezers that were salvaged and which needed to be maintained and relocated to more suitable areas within the Paterson Building. They also assisted with the daily monitoring of -80°C freezers temperature checks, ensuring the various room temperatures were managed accordingly.

We also provided assistance in facilitating the various mechanical and electrical engineers who came in to test and verify the lab equipment was working correctly, installing temporary power supplies where they were required, prior to the equipment being packed away for transportation.

Moving forward, Neil is now on secondment to the Faculty of Biology, Medicine and Health (FBMH) Estates team at The University of Manchester Oxford Road campus, gaining additional experience and cementing ties with the University Estates team until such time that the Institute returns to its Withington site. Steve and Tony have also taken up new roles, assisting our electronics engineer Yunis Al-Hassan with the electrical testing and installation of the large volumes of laboratory equipment arriving at Alderley Park, along with delivering the maintenance program to ensure the lab equipment is maintained and usable for the researchers.

Finance and Purchasing

Margaret Lowe, Klara Berne[1,2], David Jenkins, Denise Owen, Muhammad Raja, Vikki Rosheski, Debbie Trunkfield

[1]Left in 2017 [2]Joined in 2017

The Institute Finance team supports the Director with the management of the Institute's £27m 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 team supports the research groups by providing effective and efficient professional advice when costing new research proposals and contracts. Group Leaders are encouraged to continue to apply for external funding to allow us to carry out the breadth of research that we need to undertake.

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

Health and Safety

Colin Gleeson

The clearing of the Paterson Building and subsequent relocation to Alderley Park (AP) has been the main focus of work since the fire. The complete emptying of the contents of the Paterson Building of all equipment, consumables, chemicals and biological agents was an enormous challenge. It involved working closely with numerous contractors and many of our own staff. Programmes were developed for the emptying of laboratories and offices utilising standard operating procedures that were drawn up for safe working in an unusual environment. This work took months due to the need to log everything for insurance purposes.

Once much of the equipment, chemicals and biological agents had been removed and either sent for storage or disposal, the relocation of our research groups was next on the agenda. Working with University colleagues we ensured that those temporarily relocating to University buildings received inductions and could apply the same risk control measures as before to enable safe working in their new environment.

The preparations for the relocation of staff to Alderley Park were also undertaken. This necessitated the speedy development of working relationships with safety and

operational staff from our new landlords (AstraZeneca and Alderley Park Limited). From a legal standpoint, amendments to our GM centre status, animal by-products and duty free spirits licences were required to remain compliant with the law. On a more practical level, operational procedure at AP was sometimes different to that of CRUK MI and so we have had to adapt at all levels to this new working environment. Service provisions, such as the delivery of equipment and chemicals, and waste removal, are provided by sub-contractors on the AP site and this makes the operational framework more complex. The development of working relationships with key AP staff was useful in flagging up and troubleshooting issues that have arisen.

As of December 2107 about half of CRUK MI have relocated to AP. Work continues, including laboratory space redesign projects, to enable all those that are relocating to AP to continue their research programmes. This year health and safety has very much operated within a business continuity framework, enabling our staff to continue their research in new but safe working environments.

Human Resources

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

[1]Joint with administration

Over the past year, the HR Department has continued to deliver a high quality proactive service to the Institute and its staff under difficult circumstances. The team provides advice and guidance to managers and staff on all employment-related matters such as recruitment, policy guidance, employment legislation and best practice. Due to the success and the expansion of the Institute, the HR Department increased its headcount in October when Rachel Craven, Recruitment Coordinator and Natalie Taylor, HR Administrative Assistant became substantive members of the team.

During 2017, 76 individuals were successfully appointed to enhance the work of the Institute. We are delighted to have appointed a new Director of the Drug Discovery Unit and we have welcomed two new Institute Fellows. The team administered the successful promotion of 10 individuals.

The Institute is committed to working towards obtaining Athena Swan accreditation. A self-assessment team has been established and is jointly chaired by Professor Caroline Dive and Dr Caroline Wilkinson. This will be a priority over the next 12 months.

During the Institute's relocation to Alderley Park, the HR Department has continued to provide support to staff. We have managed to continue the employment of all staff without the requirement for redundancies and we have successfully secured a new place of work for the domestics team at the MCRC Building. The HR department has established a transport network between the MCRC Building and Alderley Park by arranging several shuttle buses at various times throughout the day. The department has worked hard to ensure that 400 individuals attended the Alderley Park Induction and carried out security screenings as part of the relocation.

Next year, the focus will be on the Athena Swan accreditation, recruitment of new research groups and the implementation of a new candidate management system to support the application process, whilst relocating the remainder of the Institute to Alderley Park.

Information Technology

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

The CRUK Manchester Institute IT team provides a full catalogue of IT services upon which our researchers and support staff alike now depend for nearly every aspect of their work. In 2017 we were planning to replace our SAN based storage solution, then end of life, along with several other infrastructure upgrades. Unfortunately, the fire at the Paterson Building in April resulted in much of our IT infrastructure being damaged, both in the datacentre and network 'hubs' around the Paterson Building.

As a result, 2017 became a year of unprecedented change. Effectively divided into thirds, January to April was largely business as usual. Late April to mid-August was spent in the primary stages of disaster recovery. The focus during this phase was to firstly reestablish our lines of communication. To this end, we reestablished network connectivity into our temporary base at the Manchester Cancer Research Centre Building, which was up and running the day after the fire. We then restored our corporate email service, which was operational from two days after the fire. Then started the lengthy process of rebuilding services and recovering all the Institute's research datasets from hundreds of backup tapes.

The final 'third' part of 2017, from mid-August to December, was spent planning and executing our return to 'business as usual'. To this end the CRUK MI chose to relocate the majority of staff (over 400) to Alderley Park, a bioscience hub which is an expansive site of laboratory and office space.. Once leases were signed, the IT team rebuilt the CRUK MI computer network out across several buildings within AP to provide the first incoming wave of researchers with a fit for purpose computer network. This is connected back to The University of Manchester via two (diversely routed) 10G links, giving our researchers access to storage and a server farm, currently being hosted by The University of Manchester IT Services, VoIP telephony and the Internet. Our next steps will be to push our network out to all our research groups as they arrive at AP and re establish an enterprise class file storage facility for our research datasets. This will be a replicated design, hosted in two datacentres, to provide a high availability, redundant, fit for purpose storage facility.

Logistics

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

The past year the team has had to deal with new challenges and lots of change including the relocation of the team to the MCRC Building. In the early months of the year we were able to establish small stores in the MCRC Building so we did not have to rely on the Paterson Stores to support the research. After the fire, this store became crucial in continuing to provide essential stores items for the research groups not only in the MCRC, but for staff in the Wolfson Molecular Imaging Centre and the Incubator Building at The University of Manchester.

The team has continued to deliver an efficient and effective service providing support for the research carried out. This includes the receipting, checking, booking in and distribution of goods ordered by staff as well as the collection and removal of waste from the MCRC Building.

The Logistics team continues to provide liquid nitrogen collection and refill service three days a week and a dry ice service with deliveries taking place twice a week. The team is also monitoring the levels of all nitrogen in the cell banks that are still located in the Paterson Building and monitoring the temperatures of the -80°C freezers. Gas cylinders are also monitored and replaced by the team.

Researchers can still order central stores stock items either by email or in person. The intranet ordering system became operational again in early January 2018. We currently stock over 100 stores items from tissue culture essentials to cleaning products. Included in this system are the enzymes and media stored in the Institute freezers (Sigma, Life Tech, Roche, Promega, New England Bio labs, Fisher kits and Qiagen). Media became available again in the New Year. We continue to make savings by buying in bulk from suppliers. We have set up numerous "call off orders" for stores items, again making significant savings but also guaranteeing a stable stock.

The team also provides assistance with moving heavy equipment or furniture therefore helping facilitate internal rearrangements and the arrival of new groups. The last few months have been especially busy as the team has helped with the relocation of groups to the Michael Smith Building, WMIC and have helped with moving IT equipment from storage to the Alderley Park site and the MCRC Building. We also helped relocate staff and move lab equipment around the MCRC Building to accommodate the influx of staff from the Paterson Building.

This year the Logistics team has introduced a new service. We now offer a goods and sample transfer service. We have a second van so are able to offer a delivery/collection service from the University main campus to Alderley Park at least once a day. This enables time critical samples to be processed more efficiently. We have also been working closely with the BRU team based at Alderley Park and the Stopford Building, transferring twice weekly between the two sites. The MCRC goods-in bay has become the base camp for the team and a main sample collection point. Since October we have had a team of two working at Alderley Park assisting the receipting, checking, booking in and distribution of goods and coordinating any sample collections and deliveries onsite. As the Institute starts to relocate to Alderley Park in the New Year, we are aiming to run a small stores service and increase staff numbers working at the new site.

Scientific Operations and General Administration

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

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

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

Prior to the Paterson Building fire, the team were busy preparing the necessary documentation for the Institute Quinquennial Review that was planned for the end of June. Despite the fire, the QQR still went ahead with the remaining preparation conducted alongside efforts towards the recovery and relocation efforts. This included work towards the emptying of the Paterson Building and the resulting insurance claim process.

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

A new development for 2017 was the introduction of a Research Integrity Committee, chaired by Caroline Wilkinson, which oversees and review all manuscripts before final submission for publication to help ensure that our research is conducted in accordance with the highest standards of integrity.

The team also worked on a new external website for the Institute which was developed by Tom Bolton. This will be launched in spring 2018. Tom also provided invaluable help to the IT team and the wider Institute in the months after the fire. Tom has continued to work with our Postgraduate Education Manager, Julie Edwards, to refine the online PhD application portal that they developed and introduced in 2016.

The team have also been preparing for the introduction in 2018 of the EU General Data Protection Regulation which is due to be adopted into UK law. David Stanier has been appointed as an Information Governance Co-ordinator, in addition to his general administration role, in order to support Caroline Wilkinson's role as the Institute's Information Governance Guardian and they have worked with The University of Manchester's Information Governance Office over preparations for the GDPR. This has included an audit of information assets and rolling out a new data protection training module for all staff and students.

In the aftermath of the fire, Steve Morgan helped with maintaining security for the Paterson Building and has now been reassigned into a new role on reception at the MCRC Building working closely with staff from The University of Manchester's Faculty of Biology, Medicine and Health to ensure the smooth operations of the building.

Animal Welfare

Simon Poucher, Regulatory Liaison and Training Officer, **Janet Watson**, Animal Welfare and Ethical Review Body (AWERB) Chair, **Caroline Wilkinson**, Establishement Licence Holder, **Stuart Pepper**, (Deputy AWERB Chair)

The Institute upholds the highest standards of welfare for the laboratory mice used in our research. All animal research activities are conducted in full compliance with the Animals (Scientific Procedures) Act 1986 and are scrutinised by the Institute's Animal Welfare and Ethics Review Body (AWERB). This consists of experienced animal husbandry staff, a veterinary surgeon, Institute scientists, a statistician and lay members. The AWERB supports all staff involved with animal research, ensuring the provision of appropriate management structures and processes, staff training and facilities for the care and use of mice, encouraging implementation of the 3Rs principles (replacement, reduction and refinement of animals). It also reviews the ethics of proposed collaborations and all grant applications involving animal research.
In 2017, our AWERB met formally on six occasions with one meeting open to all Institute staff. Five new applications for research project licences and seven amendments to existing licences were reviewed during the year; all were subsequently granted by the Home Office. A total of 27,234 mice were used in regulated procedures under the Act in 2017. AWERB sponsored two annual meetings for licensees to share information on animal research achievements and improvements, along with the latest news on animal welfare practices and legislation; these meetings are regularly attended by our Home Office Inspector.

The fire at the Paterson Building, which housed our experimental animal facility, necessitated the transfer of the mice to temporary facilities at The University of Manchester. This unfortunate event demonstrated the dedication of our staff to the welfare of their animals: four staff were allowed into the building under supervision of Greater Manchester Fire and Rescue Service to check on the well-being of the mice on the day of the fire and, within 36 hours, all animals were safely removed. Their actions were commended by the Home Office. Caroline Wilkinson gave a presentation on the fire and resulting rescue efforts to the Laboratory Animal Science Association winter meeting.

Our scientists are proud to communicate with the public on our animal research and strive to continuously improve animal welfare. In 2017, animal technologists at the Institute won an industry award, the Janet Wood Innovation Award for the development of a swing for mouse cages to enhance their environment and sociability.

In May, we were delighted to be invited to the Home Office Inspectorate Annual meeting and contribute presentations and debate to a session on oncology research. Caroline Wilkinson and Stuart Pepper from the Operations team attened together with researchers Kris Frese and Jamie Honeychurch (The University of Manchester, Division of Cancer Sciences) for a very constructive discussion.

Cancer Research UK Commercial Partnerships Team

Martyn Bottomley

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

Our exclusive focus on oncology provides an unrivalled depth of knowledge and experience in cancer-specific translational development and commercialisation. We also have access to the CRT Pioneer Fund; this £70m fund has been established with 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, CRUK owns and is responsible for the development and commercialisation of intellectual property arising from CRUK funded research at The University of Manchester. To effectively facilitate this, Martyn Bottomley, a CRUK CP Business Manager is based within Manchester and is currently working out of the MCRC Building, UMIP and Alderley Park to work closely with the staff funded by CRUK at 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.

Martyn continues to work very closely with the Drug Discovery Unit (DDU) to facilitate the development of drug therapies to satisfy the unmet clinical needs of cancer patients. The DDU has recently appointed a new Director of the Unit, Professor Caroline Springer formerly of the Institute of Cancer Research; Martyn has been working closely with Caroline to transfer her existing projects to Manchester and facilitate a smooth integration into the DDU. Martyn continues to be involved with the management of collaborations with Pharmaceutical partners such as GSK, Basilea and AstraZeneca and also the filing and management of a number of patent applications for the Drug Discovery Unit to protect novel compounds resulting from their research.

CP is also actively managing a broad portfolio of development programmes and exciting licensing opportunities originating from the Cancer Research UK Manchester Institute that continue to attract commercial partners. This includes negotiation and completion of a significant number of agreements between the Clinical Experimental Pharmacology group and commercial collaborators. We look forward to building on our successes and continuing to work closely with the Cancer Research UK funded researchers in Manchester to advance discoveries to beat cancer in the years ahead.

Guests go on a VR tour of the Paterson Building labs at The Late Lab

CANCER RESEARCH UK'S RESEARCH ENGAGEMENT

Cancer Research UK's Research Engagement Manager

Tim Hudson

A vast and varied programme of bringing research to life for Cancer Research UK's supporters, partners and the wider public continued in 2017, with many researchers from across our community volunteering their time to inspire and educate.

Almost 8,500 people interacted with our work, at events in our own labs or externally at fundraising events and science festivals. Activities within our own labs and event spaces (27 this year) gave donors, fundraisers, volunteers, ambassadors and corporate partners the opportunity to see the work that their support helps to fund. Highlights include our large public Open Day in the Spring, in partnership with the Manchester Cancer Research Centre, a visit by the Greater Manchester mayoral candidates and a tour with the team from Co-Op Legal Services, who raised £42,000 for CRUK in 2017.

Our new CRUK Study Day, organised with Senior Research Nurse Clare Dickinson was a huge success, bringing over 100 guests to the Manchester Cancer Research Centre Building to find out more about cancer prevention, early diagnosis and the development of better treatments. It was rounded off by a keynote lecture and Q&A from Cancer Research UK's CEO, Sir Harpal Kumar. The first of its kind nationally, we hope it will be rolled out across all CRUK's research locations.

Research engagement is also about giving visitors an excellent all-round experience. Thanks go to Café Vivo for their special opening arrangements during our weekend Open Days and for their extra support as activity increased in the MCRC Building through the year.

Our scientists took their work out into the community at over 30 external events, raising awareness of the life-saving research taking place on our supporters' doorstep.

CANCER RESEARCH UK'S RESEARCH ENGAGEMENT

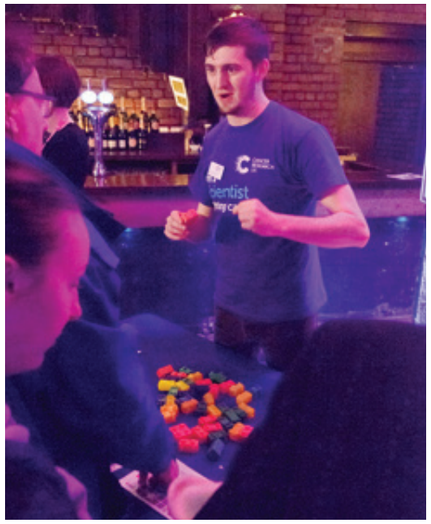
In the summer, the 12th Relay for Life Stockport took place, featuring a team of researchers from the Manchester Institute headed-up by Senior Scientific Officer, Steve Lyons. The team contributed over £1,500 to the event's fundraising total and hosted a research stall where participants could extract DNA from strawberries and take part in VR lab tours whilst being informed about our research.

Individuals have continued to play an important role in thanking those groups and partners who have reached significant fundraising milestones, through visits to fundraising committees and annual dinners, and helping to create 'Thank You' videos. One such video, made with the DDU's Head of Chemistry Allan Jordan, was played out during a live-streamed online gaming event resulting in over 3,700 views, reaching an online audience of 1.7million and raising over £1,000 for CRUK's Bobby Moore Fund. European Researchers' Night took place in in September, when four members of the BRU, together with Postdoctoral Fellow Julia Draper, engaged with The University of Manchester's research community at the Manchester Museum, talking about our animal research and how this is beneficial to basic and translational research. The Scientific Computing team's Jack Heal was in the spotlight at the event, where he delighted audiences with a fast and furious Lightening Talk.

The biggest external event this year saw our researchers engage with over 600 people during the Manchester Science Festival. Four hundred budding scientists took part in our Molecular Model Makers workshop as part of Science Spectacular at the Manchester Museum, and we were honoured to showcase the Etch-a-Cell citizen science project, developed by the Microscopy team from the Francis Crick Institute, to over 200 visitors at the University of Salford at MediaCityUK.

This year saw a new introduction to the CRUK events calendar with The Late Lab. Trialled in London and rolled out to Manchester in the autumn, The Late Lab was an opportunity for researchers to engage with the army of CRUK volunteers from across the North West. Forty shop volunteers, events volunteers and media ambassadors enjoyed the social setting of Victoria Warehouse to meet researchers from various groups, engage in science activities and talk about the important work we do.

Huge thanks go to all the volunteer group leaders, researchers, scientists and staff who donate their time, energy and enthusiasm to support our engagement activities.



Sam Humphrey challenges CRUK volunteers to decode cancer at The Late Lab

Clockwise from top left: Allan Jordan appears as part of the Pro Evolution Soccer 2017 live-stream fundraiser; Members of the BRU with Julia Draper at Science Uncovered for European Researchers' Night; Victoria Pelly demonstrates tumour heterogeneity with guests at The Late Lab; Visitors took part in the Etch-a-Cell citizen science project during the Manchester Science Festival; Julia Draper and Toni Banyard bring some science to Relay for Life Stockport; Steve Bagley meets guests at our September Open Day in the MCRC Building.

Clockwise from top: The CRUK MI Relay for Life Stockport team; Families gather at our Molecular Model Making workshop as part of Science Spectacular at the Manchester Science Festival; Jack Heal entertained an audience with a Lightening Talk at Science Uncovered.

ACKNOWLEDGEMENT FOR FUNDING FOR THE CANCER RESEARCH UK MANCHESTER INSTITUTE

The total funding of the CRUK Manchester Institute for 2017 was £27m. The major source of this funding was awarded by Cancer Research UK via a core grant of £12.9m plus additional strategic funding of £5.5m. This funding enables the various scientific groups and service units within the Institute to carry out their research.

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

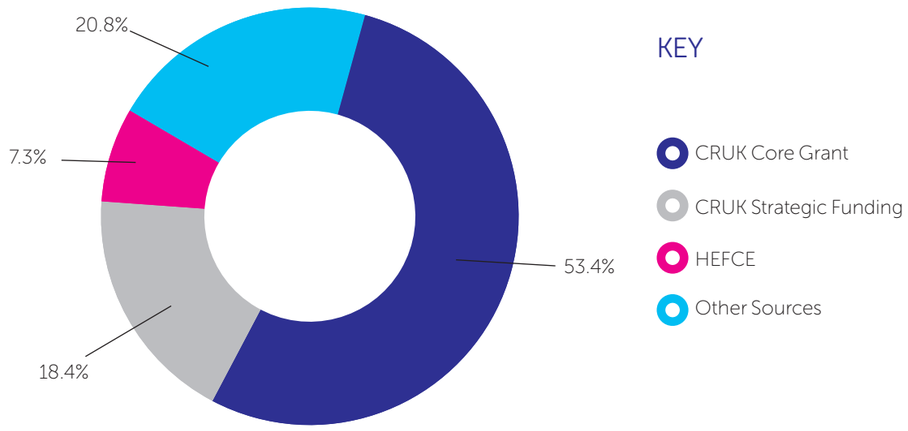
The balance of the Institute's funding is received from a number of additional sources. The research carried out through these additional projects enhances and supports the research undertaken by the core funding.

These sources are as follows:

- Amgen
- Angle Inc
- Astex Pharmaceuticals
- AstraZeneca
- Bioven
- Bloodwise
- Carrick Therapeutics
- CellCentric
- Christie Hospital NHS Foundation Trust
- Clearbridge Biomedicals
- CRT Pioneer Fund
- Euclises Pharmaceuticals Inc
- European Commission
- European Research Council
- Fondation ARC pour la Recherche sur le Cancer
- GlaxoSmithKline
- John Swallow Fellowship
- Kay Kendall Leukaemia Fund
- Leo Pharma Foundation
- Menarini Biomarkers Singapore
- Merck
- Moulton Charitable Trust
- National Institute of Health Research
- Pancreatic Cancer Research Fund
- Pickering Leukaemia Research
- Prostate Cancer UK
- Rosetrees Trust
- Taiho Oncology Inc
- The US Department of Health and Human Services
- Wellcome Trust
- Worldwide Cancer Research

We are immensely grateful to all our funders.

CRUK MANCHESTER INSTITUTE FUNDING 2017



CAREER OPPORTUNITIES AT THE CANCER RESEARCH UK MANCHESTER INSTITUTE

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

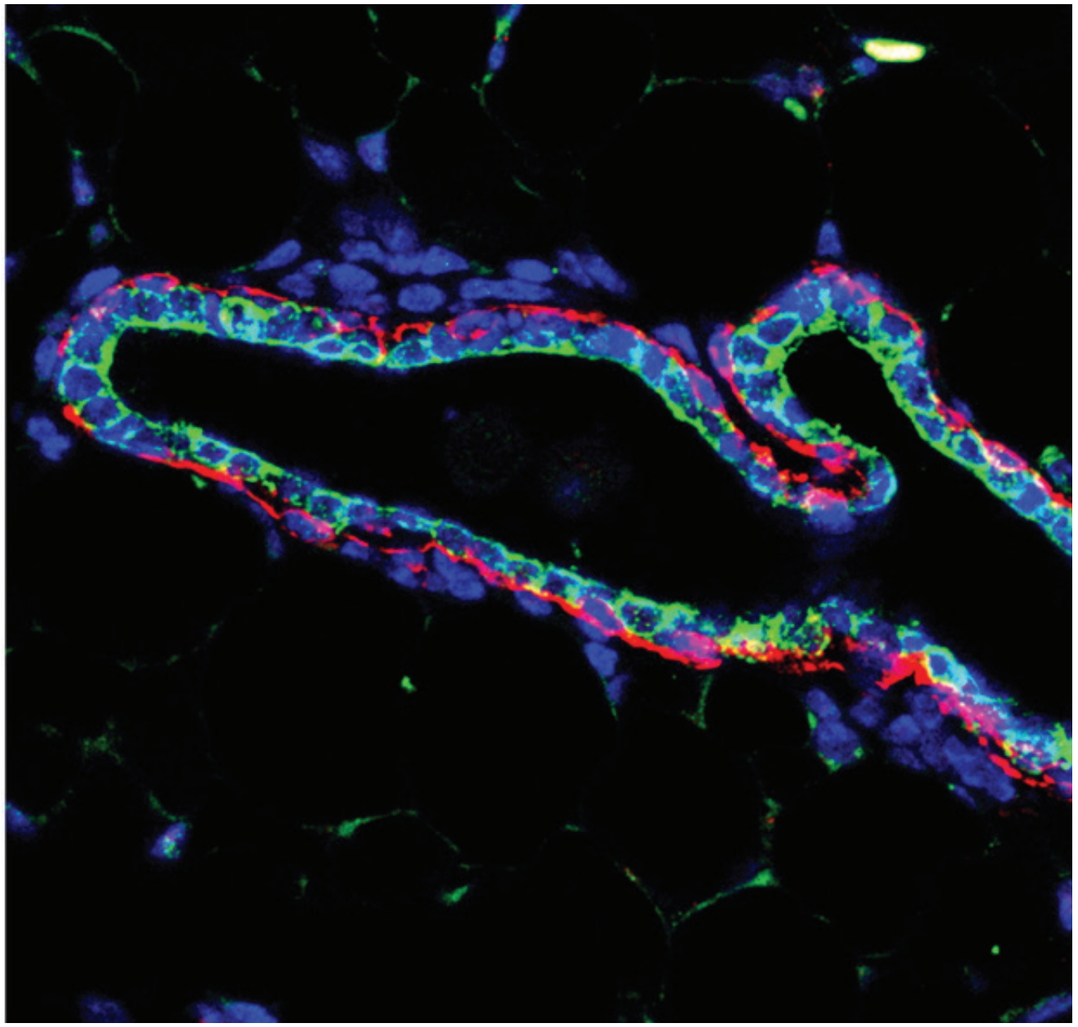
The Institute offers excellent laboratory facilities and outstanding core facilities, including molecular biology services, next generation sequencing, real-time PCR, mass spectrometry, flow cytometry, histology, advanced imaging, and a biological resources unit. Details of all groups and facilities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

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

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

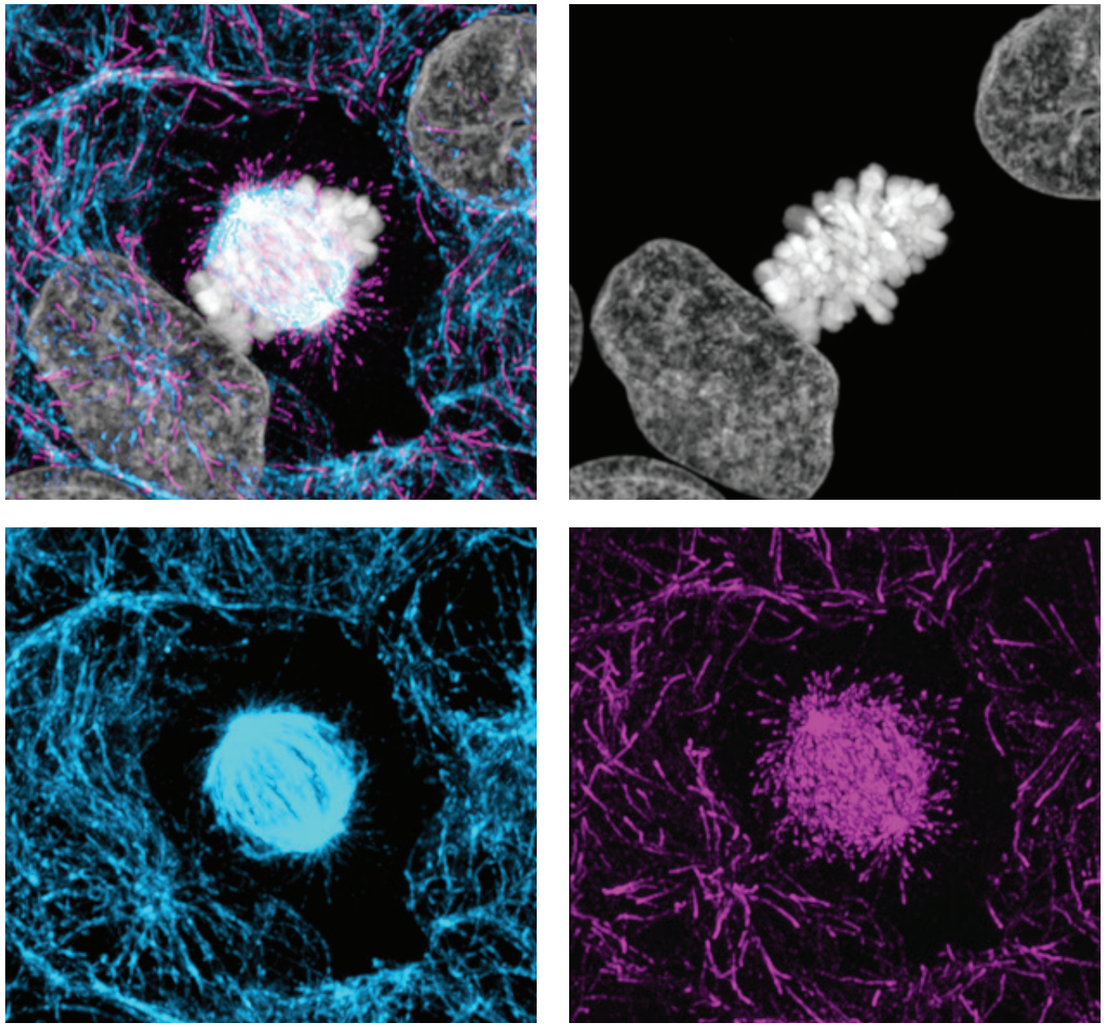
In addition to postgraduate and postdoctoral opportunities, the Institute is still seeking to recruit outstanding candidates to the positions of Junior and Senior Group Leaders. The packages provided are extremely attractive and commensurate with the experience of the applicant, with significant funding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

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



Confocal photomicrograph of a normal mouse mammary duct showing basal epithelial cells (Red), luminal epithelial cells (Green) and cell nuclei (Blue).

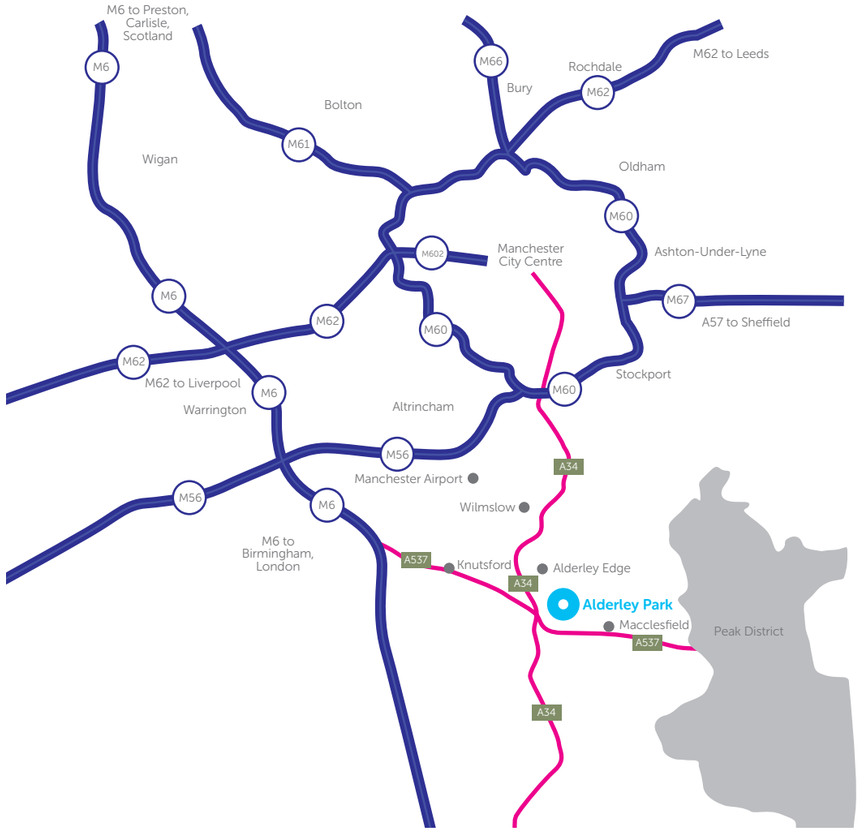
Image supplied by Haoran Tang (Molecular Oncology)



Panel of super-resolution images of a mitotic U2OS cell. Top left shows merged image of the condensed chromosomes (grey) being separated into two daughter cells by a scaffold of microtubules (cyan). EB1 is a protein that localises at the growing tips of microtubules (in magenta). The three individual channels of each component are shown to illustrate the imaging capacity of the Zeiss Airyscan microscope.

Image supplied by Andrew Porter (Cell Signalling)

CONTACT DETAILS



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