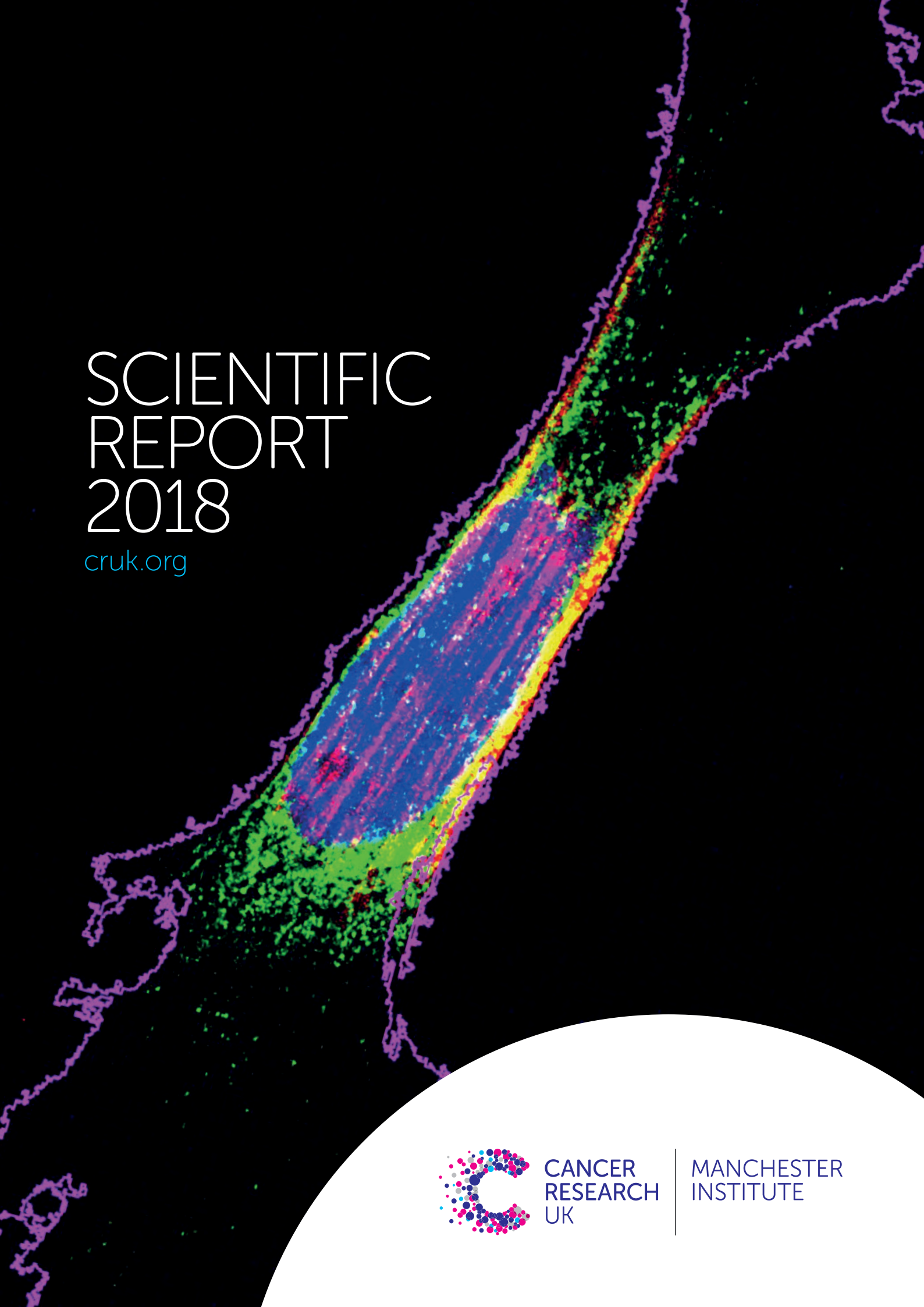

SCIENTIFIC REPORT 2018

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COVER IMAGE

STEF-depleted cell showing nucleus (blue) with active Rac1 (green) targeted to the nuclear membrane where it has restored the cables of the actin cap (red). Outline of the cell shown in magenta.

Image supplied by Andrew Porter (Cell Signalling)

SCIENTIFIC REPORT 2018

MANCHESTER INSTITUTE

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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 Oglesby Cancer Research Building.

The Oglesby Cancer Research Building

DIRECTOR'S INTRODUCTION

Professor
Richard Marais

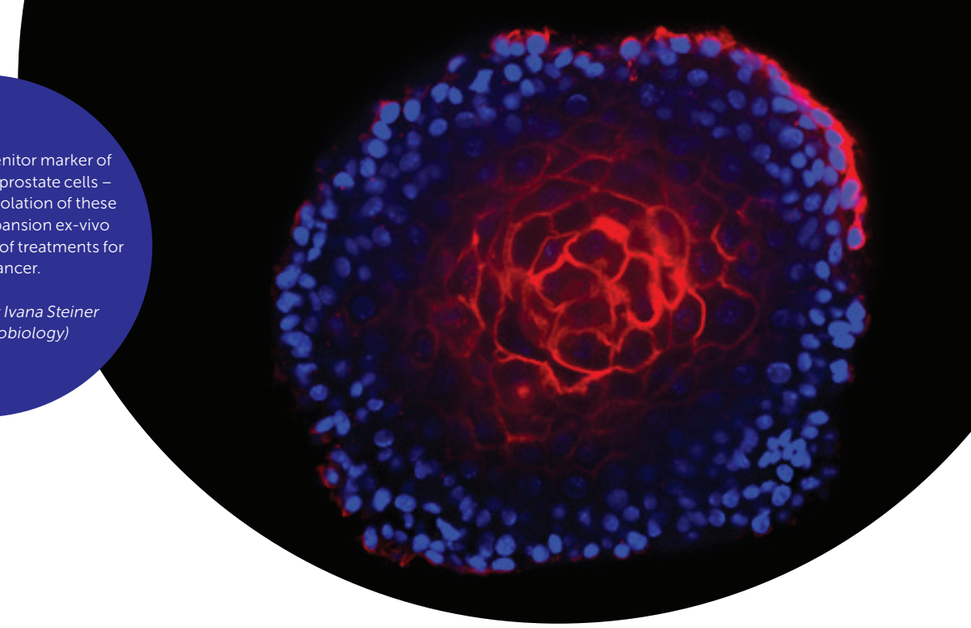
Director of the Cancer Research
UK Manchester Institute

Over the past year, we have continued to recover from the fire in April 2017 that caused so much damage to our main research facility, the Paterson Building, and by the spring of 2018, we had completed our relocation to our interim home at Alderley Park in Cheshire.

To mark the one-year anniversary of the fire, we held an event at Alderley Park to reflect on the year and to acknowledge the heroic collective effort that was required to complete our move so quickly. It was also an opportunity to celebrate what we have achieved scientifically despite the extensive disruption we endured. On that day, we were delighted to be joined by many of our colleagues from The University of Manchester and the other various external organisations who together played a key role in our recovery. It was an upbeat occasion that demonstrated the resilience and spirit of the Institute. Although temporary, we are making the most of our new home and seeking new opportunities that our environment offers, while working hard to maintain our vital relationships with our many academic and clinical colleagues on both the University main campus and the Christie site. I am pleased that our new location has not prevented us from inviting our supporters to visit the Institute, to tour our facilities and talk to our scientists. It has been a pleasure to meet so many supporters at our laboratories but also at the various other events that our staff and students have attended over the last year, and we look forward to many more occasions over the coming year where we will share our progress with those who make our research possible.

Institute Deputy Director Professor Caroline Dive proudly displays her CBE medal

We are proud of our scientific achievements of the year and the progress that we have continued to make despite the disruption. Scientific highlights from the year include a study in Nature Communications from the Tumour Suppressors' group who found that a combination of host p53 mutation status and tumour cell engulfment may promote genomic instability. The Leukaemia Biology group uncovered the surprising mechanism by which LSD1 inhibition leads to differentiation of acute myeloid leukaemic blast cells. Contrary to the assumption that the mechanism of action of the inhibitors would be via the abrogation of LSD1 histone demethylase activity, the group found that they disrupt an interaction with the GFI1 transcription factor suggesting the potential of further therapeutic options for this type of leukaemia. The Transcriptional Networks in Lung Cancer group identified micro-RNAs that modulate gene expression changes that promote lung tumorigenesis in a KRAS – dependent manner and determined that they act by turning off key tumour suppressor genes. The Prostate Oncobiology group found a way to identify prostate cancer cells that are resistant to hormone deprivation therapy. In a collaborative study with colleagues in Clinical and Experimental Pharmacology (CEP) and my Molecular Oncology group, and with clinicians at the Christie, we analysed the genomic characteristics of prostate lesions that can be identified by a commonly used MRI technique. Our results suggest that complex analysis is needed in order to not underestimate the complexity of a tumour and thus the risk of progression.



Novel luminal progenitor marker of castration-resistant prostate cells – LY6D (red) – allows isolation of these rare cells and by expansion ex-vivo produce a new range of treatments for prostate cancer.

Image supplied by Ivana Steiner (Prostate Oncobiology)

My group has also shown that DNA isolated from plasma can be used as an indicator of tumour burden and as a prognostic biomarker for overall survival of melanoma patients. The CEP group extended their work on the generation of xenograft models derived from tumour cells isolated from the blood of small cell lung cancer patients. Using this approach, they have identified the utility of Wee1 and PARP inhibition in certain genetic backgrounds that mimic loss of BRCA function; this approach has now entered clinical trials. In another study, they used short-term cultures from these models ex vivo to expedite the testing of novel therapeutic approaches and reduce the number of mice required to unravel new lung cancer biology.

The Drug Discovery Unit (DDU) is continuing to develop well under the Directorship of Caroline Springer who joined from The Institute of Cancer Research in autumn 2017. Several members of her team moved with her from London and, together with existing members of the group, they moved into specialised drug discovery laboratories in Alderley Park in early 2018. They are working with an exciting portfolio of projects, many of which have arisen from projects within the Institute. The DDU have also been part of two collaborative studies relating to their PARG inhibitor programme. The first with the Mitosis and Cancer Pharmacology group led by Stephen Taylor at The University of Manchester, demonstrating the potential for PARG inhibition as a therapeutic option for some ovarian cancers that do not display PARP inhibitor sensitivity. The second study with colleagues at the Netherlands Cancer Institute shows that loss of PARG is a potential mechanism of resistance to PARP inhibition in tumours that are deficient in homologous recombination. A partnership agreement to develop these PARG inhibitors has been signed with IDEAYA Biosciences, an oncology-focused biotechnology company, in early 2018.

Two of our Senior Group Leaders, Iain Hagan and Tim Somervaille underwent quinquennial reviews in September and despite the disruption following the fire, their programmes were rated very highly. Santiago Zelenay underwent a successful mid-term review having developed an exciting portfolio of projects. Earlier this year, The University of Manchester formed a new research institute – The Lydia Becker Institute of Immunology and Inflammation; Santiago is the co-lead for the Cancer Immunology section together with Rob Bristow. There is further representation from the Institute including Amaya Viros and Rob Metcalf and from Caroline Dive who supervises a PhD student together with Lydia Becker Director Tracy Hussell. Other developments in immune-oncology include the tumour inflammation and immunology monitoring laboratory (TIIML) which the CEP group have set up, led by Elaine Kilgour, to support biomarker sciences for immune-based therapeutics. CEP have also played a major part in the success of the first phase of TARGET which is a ctDNA-driven selection phase I trial. The molecular profiling of patients in TARGET is underpinned by ctDNA-based liquid biopsies performed by the CEP team and supported by the scientific computing infrastructure at CRUK MI. The overall project is a team approach with the Christie and The University of Manchester, including the clinical lead Matt Krebs who completed his PhD with CEP in 2011. The project is also supported by the work of the

digital Experimental Cancer Medicine Team who created eTARGET, a platform that allows researchers, scientists and clinicians to meet virtually and review patient details to support assessment and clinical decision-making. The ctDNA activity within CEP is also playing a key role in supporting the recently initiated PrecisionPanc project aimed at delivering personalised treatments for pancreatic cancer patients based on molecular profiling. Both TARGET and PrecisionPanc are exemplars of the personalised medicine agenda that is key to the mission of the Manchester Institute.

Certain indicators of our success such as funding applications and other awards have been affected this year as a result of the disruption caused by the fire and relocation. There have, however, been some notable achievements to celebrate. In May I was both delighted and honoured to join the ranks of so many of my scientific heroes by receiving the fellowship of the Royal Society. In June, Caroline Dive, Deputy Director of the Institute and Head of the CEP group visited Buckingham Palace to receive the CBE that she was awarded in the New Years' Honours list for services to cancer research. Rob Bristow who moved to Manchester in 2017 was awarded the Research Leadership Award from Prostate Cancer Canada. Senior Group Leaders Georges Lacaud and Angeliki Malliri were awarded Professorships by The University of Manchester and gave enjoyable inaugural addresses describing their careers to date at an event held at Alderley Park. Angeliki was also the recipient of the Philip Godfrey Fund Memorial Award from the Biochemical Society, while Zoi Diamantopoulou from her group received the BACR Chris Marshall Prize for Cell Signalling and her PhD student Joe Maltas won a poster prize at the International PhD Student Cancer Conference held at The Francis Crick Institute.

Stuart Williamson from CEP was awarded a prestigious CRUK-Fulbright Scholarship to spend six months at Stanford University developing his research into vasculogenic mimicry in the laboratory of Julien Sage. Rebecca Lee from my group was awarded the Association of Cancer Physicians McElwain Prize as well as the Institute's Dexter Prize for Young Scientists in recognition of her research demonstrating the utility of ctDNA analysis for predicting both disease-free and long term survival of stage II/III melanoma patients following resection. Melanie Galvin won the Andrew Blake Tribute Award for her work in CEP refining methods of tumour passage resulting in the use of fewer mice and eliminating the need for surgery. There was further success in CEP with an American Association for Cancer Research (AACR) Women in Cancer Research Scholar Award for Francesca Chemi and a poster prize awarded to Sam Humphrey at the CRUK Lung Cancer Centre of Excellence workshop.

Our Grants Committee continues to provide an invaluable service to the Institute's scientists in conducting a peer review of applications prior to submission. There were some notable successes, including Isabel Romero-Camarero from the Leukamia Biology group who was awarded a Kay Kendall Leukaemia Fund Junior Research Fellowship to understand the regulation of FOXC1 in Acute Myeloid Leukaemia. Eduardo Bonavita from the Cancer Inflammation and Immunity group was awarded an Advanced Fellowship from EMBO and Maria Roel from Prostate Oncobiology received an I2C Postdoctoral award from the Spanish government.

I am delighted to be part of a Grand Challenge consortium funded by CRUK and led by Stephen Elledge at Harvard Medical School that shall investigate why specific oncogenes are associated with particular cancers but not with others. Caroline Dive is part of two CRUK-funded consortia; the first is an Accelerator Award to find new personalised approached to treat bowel cancer; the second is an Early Detection Award to determine whether monitoring of patients' liquid biopsies collected in the community from patients with resected non-small cell lung cancer can predict early relapse. Sara Valpione from my group was awarded an EORTC Translational Research Grant to study the tumour and microenvironment transcriptomic landscape of melanoma.

There is much to look forward to in the coming year. We continue to make progress in the planning of the replacement for the Paterson Building. The ambition is to build a larger facility to allow for greater interaction and synergy with our clinical colleagues and to facilitate a multidisciplinary approach to our research. There are several new clinical trials starting in 2019 that are combined efforts between the Institute's scientists and our clinical colleagues. In July, many of our scientists will be participating in a conference hosted in Manchester which will highlight some of these interactions and their role in the development of phase 1 clinical trials. This promises to be an excellent showcase for the exciting developments taking place in our city and the collaborations that underpin them.

RESEARCH HIGHLIGHTS

In this section we highlight some research publications from 2018 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.

Mackay HL, Moore D, Hall C, Birkbak NJ, Jamal-Hanjani M, Karim SA, Phatak VM, Piñon L, Morton JP, Swanton C, Le Quesne J, Muller PAJ.
Genomic instability in mutant p53 cancer cells upon entotic engulfment.
Nature Communications 2018; 9(1):3070.

Cell-in-cell (CIC) structures in histopathological sections of tumours are defined by the presence of one whole cell enclosed in a tumour cell. How CIC are formed and how they contribute to cancer remains poorly understood. The Tumour Suppressor group investigated CIC structures in lung cancers and identified an association between the presence of mutant p53 proteins and CIC structures. In cancer cell lines these structures were formed by a process of entotic engulfment. Engulfed tumour cells either died or escaped regardless of their p53 status, but exerted a profound effect on the host cell. In p53 null host cells the engulfed cells most often caused replication stress and subsequent death of the host cell. In contrast, host mutant p53 cells were much more likely to survive this stress, but at the cost of aberrant cell divisions, multinucleation and genomic rearrangements. Xenograft injections of high-engulfing cells showed enhanced growth over less frequent engulfers. These findings suggest that pro-tumourigenic cell engulfment activity is associated with mutant p53 expression, and that the combination of cell engulfment and p53 mutant status may be a key factor in chromosomal aberrations in human tumours.

Maiques-Diaz A, Spencer GJ, Lynch JT, Ciceri F, Williams EL, Amaral FMR, Wiseman DH, Harris WJ, Li Y, Sahoo S, Hitchin JR, Mould DP, Fairweather EE, Waszkowycz B, Jordan AM, Smith DL, Somervaille TCP.
Enhancer activation by pharmacologic displacement of LSD1 from GFI1 induces differentiation in acute myeloid leukemia.
Cell Reports 2018; 22(13):3641-3659.

Lysine-specific Demethylase 1A (LSD1) has recently emerged as a candidate therapeutic target in cancer, at least in part due to its high expression in poor prognostic sub-groups of patients with the disease. Initially identified as a core component of an RCOR1 and histone deacetylase transcription corepressor complex called CoREST, LSD1 was later found to demethylate histone tails. A first-in-man phase 1 trial of the LSD1 inhibitor ORY1001 (from Oryzon Genomics) recently demonstrated that LSD1 inhibitors promote blast cell differentiation in patients with Acute Myeloid Leukaemia (AML) associated with translocations targeting the Mixed Lineage Leukaemia gene. The assumption had been that differentiation is induced through blockade of LSD1's histone demethylase activity. However, members of the Leukaemia Biology group observed that rapid, extensive drug-induced changes in transcription occurred without genome-wide accumulation of the histone modifications targeted for demethylation by LSD1 at sites of LSD1 binding. They also found that a demethylase-defective mutant rescued LSD1 knockdown AML cells as efficiently as the wild-type construct. Rather, LSD1 inhibitors disrupt the physical interaction of LSD1/CoREST with the SNAG-domain transcription factor GFI1, which is bound to a discrete set of enhancers located near to critical genes which regulate myeloid differentiation. The consequent inactivation of GFI1 leads to increased enhancer histone acetylation within hours which directly correlates with up regulation of nearby, subordinate genes.

Somerville TDD, Simeoni F, Chadwick JA, Williams EL, Spencer GJ, Boros K, Wirth C, Tholouli E, Byers RJ, Somervaille TCP.
Derepression of the iroquois homeodomain transcription factor gene IRX3 confers differentiation block in acute leukemia.
Cell Reports 2018; 22(3):638-652.

The acute myeloid leukaemias (AML) are a heterogeneous group of proliferative malignancies characterised by a hierarchically-organised cellular structure and accumulation of poorly differentiated myeloid blasts in bone marrow and blood. Through in silico and functional experiments, the Leukaemia Biology group identified a significant role for the iroquois homeodomain transcription factor gene IRX3 in the differentiation block of AML, which is the cardinal pathologic feature of the disease. IRX3 is normally expressed in the developing nervous system, limb buds and heart, whereas transcript levels are very low in normal human bone marrow cells. They observed high IRX3 expression in ~30% of patients with AML and ~50% and ~20% respectively of those with T-or B-acute lymphoblastic leukaemia. Forced expression of IRX3 alone was sufficient to immortalise normal bone marrow stem and progenitor cells in vitro and induce lymphoid leukaemias in vivo. Moreover, IRX3 knockdown induced terminal differentiation of IRX3 high AML cells. Combined expression of IRX3 and Hoxa9 in murine bone marrow stem and progenitor cells impeded normal T-progenitor differentiation in lymphoid culture and substantially enhanced the morphologic and phenotypic differentiation block of AML in myeloid leukaemia transplantation experiments, through suppression of a myelomonocytic differentiation program. Likewise, in cases of primary human AML, high IRX3 expression is strongly associated with reduced myelomonocytic differentiation. Overall, our results demonstrate that tissue-inappropriate derepression of IRX3 contributes to the block of myelomonocytic differentiation in AML.

Thambyrajah R, Fadlullah MZH, Proffitt M, Patel R, Cowley SM, Kouskoff V, Lacaud G.
HDAC1 and HDAC2 modulate TGF-β signaling during endothelial-to-haematopoietic transition.
Stem Cell Reports 2018; 10(4):1369-1383.

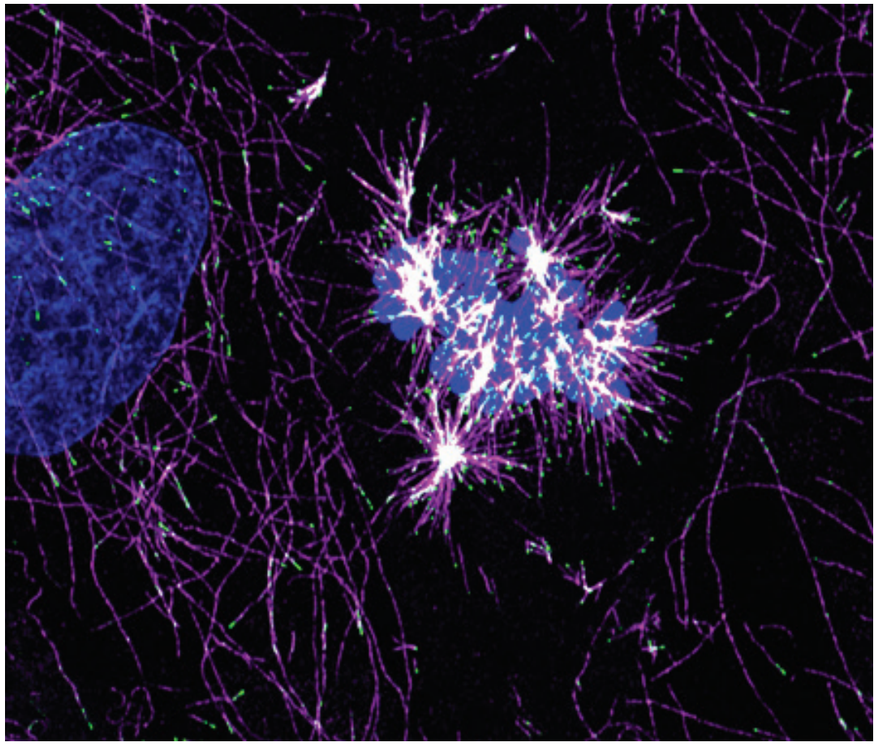
The first haematopoietic stem and progenitor cells are generated during development from a specific type of endothelium called haemogenic endothelium (HE) through a trans-differentiation process. The molecular and cellular mechanisms underlying this critical endothelial-to-haematopoietic transition (EHT) remain poorly understood. In this study, members of the Stem Cell Biology group investigated the role of histone deacetylases during EHT. They first pharmacologically inhibited HDAC activity with the pan HDAC inhibitor Trichostatin A and observed a significant impairment in haematopoietic cell generation from embryonic stem cell-derived HE cells in vitro and from embryo HE cells ex vivo. They then focused on HDAC1 and HDAC2 that are prevalent members of the HDAC family. Loss of either of these epigenetic silencers through conditional genetic deletion reduced haematopoietic transition from HE. Combined deletion of HDAC1 and HDAC2 was totally incompatible with blood generation. In order to define the molecular changes occurring in *Hdac1* and *Hdac2* knockout HE cells, they performed global transcriptomic analysis and determined the genome wide DNA binding patterns of HDAC1 and HDAC2. These analyses identified TGF-β signalling as one of the pathways controlled by HDAC1 and HDAC2. They experimentally demonstrated that activation of this pathway in HE cells reinforces haematopoietic development. Altogether these results establish that HDAC1 and HDAC2 are critical during EHT, modulate TGF-β signalling and that stimulation of this pathway in HE cells might therefore be beneficial for producing blood cells for regenerative therapies.

Shi L, Middleton J, Jeon YJ, Magee P, Veneziano D, Laganà A, Leong HS, Sahoo S, Fassan M, Booton R, Shah R, Crosbie PAJ, Garofalo M.
KRAS induces lung tumorigenesis through microRNAs modulation.
Cell Death and Disease 2018;9(2):219.

KRAS is one of the most mutated oncogenes in human cancers. MicroRNAs (miRNAs) are small non-coding RNAs of ~22 nucleotides in length which play a main role in cancer development and spread. By using inducible human and mouse cell lines and by overexpressing either wild-type or mutant KRAS ($KRAS^{G12D}$) the Transcriptional Networks in Lung Cancer group identified KRAS-modulated microRNAs in non-small cell lung cancer (NSCLC). They showed that miR-30c and miR-21 were significantly upregulated by both KRAS isoforms and induced drug resistance by silencing NF1, RASA1, BID and RASSF8. Systemic delivery of LNA-anti-miR-21 in combination with cisplatin in vivo suppressed the development of lung tumours in a mouse model of lung cancer ($KRAS^{LSLG12D}$). Furthermore, miR-30c and miR-21 were significantly elevated in plasma and in tumours from patients that underwent surgical resection of early stage NSCLC compared to normal lung. Mechanistically, we showed that ELK1 is responsible for miR-30c and miR-21 transcriptional activation through direct binding to the miRNA proximal promoter regions. Taken together, this study proves that miR-30c and miR-21 are important mediators of KRAS-driven tumorigenesis and could be valid biomarkers in NSCLC.

This mitotic cell (DNA stained in blue) has been left at 4°C for 30 minutes, which causes depolymerisation of microtubules (magenta), then briefly returned to 37°C, causing a burst of microtubule growth, marked by EB1 staining (green) at the growing tips.

Image supplied by Andrew Porter (Cell Signalling)



Woroniuk A, Porter A, White G, Newman DT, Diamantopoulou Z, Waring T, Rooney C, Strathdee D, Marston DJ, Hahn KM, Sansom OJ, Zech T, Malliri A.
STEF/TIAM2-mediated Rac1 activity at the nuclear envelope regulates the perinuclear actin cap.
Nature Communications 2018; 9(1):2124.

Cell migration is important for many physiological processes, such as embryo development, wound healing and immune responses, but is also required for the dissemination of cancer cells. When cancer cells invade, they often have to squeeze through tight spaces, which requires correct orientation of the nucleus, the largest organelle of the cell. An important cytoskeletal structure that regulates nuclear morphology and orientation during migration is the perinuclear actin cap, an array of actin cables above the nucleus that 'strap' the nucleus in place. In this study, scientists from the Cell Signalling group showed that the protein STEF, an activator of RAC, a protein which in turn controls actin fibres, is required for cell migration. Moreover, they showed that STEF localises to the outer nuclear membrane where it regulates perinuclear RAC1 activity. STEF depletion reduced apical perinuclear actin cables (a phenotype rescued by targeting active Rac1 to the nuclear envelope), increased nuclear height, consistent with the impaired constraint of the nucleus, and impeded nuclear orientation during migration. STEF down-regulation also decreased nuclear stiffness and reduced expression of TAZ-regulated genes, indicating an alteration in mechanosensing pathways as a consequence of disruption of the actin cap. This study therefore sheds light on the mechanisms regulating the perinuclear actin cap, a key regulator of nuclear morphology and hence cell migration.

Barros-Silva JD, Linn DE, Steiner I, Guo G, Ali A, Pakula H, Ashton G, Peset I, Brown M, Clarke NW, Bronson RT, Yuan GC, Orkin SH, Li Z, Baena E.
Single-cell analysis identifies LY6D as a marker linking castration-resistant prostate luminal cells to prostate progenitors and cancer.
Cell Reports 2018; 25(12):3504-3518.

Prostate cancer is one of the leading causes of morbidity and mortality in men. While early stage PCa patients can be treated and cured with local therapies (surgery/radiotherapy), treatment options for metastasised patients remains palliative. Antiandrogen therapy is the mainstay therapy for these patients; however, patients will inevitably develop resistance to these therapies. Notably, the exact identity of castrate resistant (CR) cells and their relation to CR prostate cancer (CRPC) is unresolved. In this paper, the Prostate Oncobiology group use single-cell gene profiling to analyse the molecular heterogeneity in basal and luminal-compartments. Within the luminal compartment, we identify a subset of cells intrinsically resistant to castration with a bi-lineage gene expression pattern. We discover LY6D as a marker of CR prostate progenitors with multipotent differentiation and enriched organoid-forming capacity. Lineage tracing further reveals that LY6D$^+$ CR luminal cells can produce LY6D$^-$ luminal cells. In contrast, in luminal cells lacking PTEN, LY6D$^+$ cells predominantly give rise to LY6D$^+$ tumour cells, contributing to high-grade PIN lesions. The team used gene expression analyses from patients' biopsies and identified that *LY6D* expression correlates with early disease progression, including progression to CRPC. Their work thus identifies a subpopulation of luminal progenitors characterised by LY6D expression and intrinsic castration resistance. These studies suggest that LY6D may serve as a prognostic marker for advanced prostate cancer, which will allow researchers to further stratify risk profiles for PCa patients and to help tailor more specific therapies.

Marina A. Parry, Shambhavi Srivastava, Adnan Ali, Alessio Cannistraci, Jenny Antonello, João Diogo Barros-Silva, Valentina Ubertini, Vijay Ramani, Maurice Lau, Jonathan Shanks, Daisuke Nonaka, Pedro Oliveira, Thomas Hambrock, Hui Sun Leong, Nathalie Dhomen, Crispin Miller, Gerard Brady, Caroline Dive, Noel W. Clarke, Richard Marais, Esther Baena.
Genomic evaluation of multiparametric magnetic resonance imaging-visible and -nonvisible lesions in clinically localised prostate cancer.
European Urology Oncology [Epub 18 September 2018]

The diagnosis of prostate cancer is based on imaging studies, followed by ultrasound-guided biopsies of suspicious lesions, which remain the gold-standard therapeutic procedure. However, more recently, there is a trend towards the use of multiparametric (mp) MRI to trigger biopsies in PCa patients, combined with genetic testing to refine risk stratification. mpMRI is supposed to provide superior images of higher resolution than ultrasound. Importantly, the study led by researchers in Prostate Oncobiology and Molecular Oncology, identified that even with this superior technique >10% potentially significant PCas are missed because they are not detected by mpMRI. They hypothesised that the genomic makeup of these "invisible" lesions could provide important insights into their metastatic capacity and help to assess their potential lethality. To address this question, the team have recently completed a study correlating genomics and mpMRI in men undergoing radical prostatectomy in order to elucidate the genomic characteristics of mpMRI visible and non-visible tumours and to assess the inter-relationship. They found that the intra-tumour heterogeneity within visible mpMRI lesions bears the risk of misclassifying patients when using genomic biomarkers from a single biopsy. These findings have practice changing implications as they indicate that restricting biopsies to mpMRI visible lesions underestimates the complexity of PCa, which bears the risk of misclassifying the patients' risk for progression.

Valpione S, Gremel G, Mundra P, Middlehurst P, Galvani E, Girotti MR, Lee RJ, Garner G, Dhomen N, Lorigan PC, Marais R.
Plasma total cell-free DNA (cfDNA) is a surrogate biomarker for tumour burden and a prognostic biomarker for survival in metastatic melanoma patients.
European Journal of Cancer 2018; 88:1-9.

The total metastatic tumour volume or burden is prognostic in melanoma. However, determining the tumour burden is extremely time-consuming and not feasible in clinical practice. The aim of the study undertaken by members of the Molecular Oncology group was to assess the potential of plasma total cell free DNA as surrogate biomarker of tumour burden and prognosis in metastatic melanoma patients. With this purpose, they measured tumour burden in melanoma patients by calculating the total volumes (the sum of single metastases) visualised by standard scans and correlated that to total plasma circulating cell-free DNA (cfDNA) concentration, before and during treatment. The team found that baseline cfDNA concentration correlated with pre-treatment tumour burden and the correlation was maintained during treatment. Additionally, higher levels of baseline cfDNA levels were associated with worse prognosis. In particular, a cut-off value of baseline cfDNA=89pg/µL identified two distinct prognostic groups (HR=2.22 for high cfDNA, P=0.004). Patients with cfDNA ≥89pg/µL had shorter survival (10.0 versus 22.7 months, P=0.009; HR=2.22 for high cfDNA, P=0.004) and the significance was maintained when compared to the standard melanoma prognostic biomarker (LDH) in a multivariate analysis. Thus, the group have demonstrated that cfDNA is a surrogate biomarker of tumour burden in metastatic melanoma patients, and moreover it is prognostic for overall survival.

Lallo A, Gulati S, Schenk MW, Khandelwal G, Berglund UW, Pateras IS, Chester CPE, Pham TM, Kalderen C, Frese KK, Gorgoulis VG, Miller C, Blackhall F, Helleday T, Dive C.
Ex vivo culture of cells derived from circulating tumour cell xenograft to support small cell lung cancer research and experimental therapeutics.
British Journal of Pharmacology [Epub 14 November 2018]

In 2014, the Clinical and Experimental Pharmacology group pioneered a new approach to generate clinically relevant models of small cell lung cancer (SCLC) using a patient's 10ml blood sample from which they enriched circulating tumour cells and explanted them into immune compromised mice (Hodgkinson et al, Nature Medicine). The resultant so called 'CDX' models were a landmark for SCLC research. However, these in vivo models take several months to develop and need to be passaged up to three times before experiments can commence. To both reduce the number of animals required (in line with the 3Rs' principles) and to accelerate their studies testing novel therapeutics for this aggressive lung cancer, the group developed ex vivo short-term cultures from established CDX tumours. They defined the reversible and irreversible molecular changes that occur during short term culture and showed that they can genetically manipulate CDX cultures and return CDX cells to mice for in vivo function testing and cell tracking experiments without distorting their tumour growth dynamics. They reported studies using CDX cultures to screen novel therapeutics before selecting promising candidates to validate in vivo, providing data to support early clinical trials. In summary, short-term CDX cultures have advantages over long established cell lines that have undergone irreversible drift in culture and add a new tool to understand the biology of SCLC and test targeted therapies.

Lallo A, Frese KK, Morrow CJ, Sloane R, Gulati S, Schenk MW, Trapani F, Simms N, Galvin M, Brown S, Hodgkinson CL, Priest L, Hughes A, Lai Z, Cadogan E, Khandelwal G, Simpson KL, Miller C, Blackhall F, O'Connor MJ, Dive C.
The combination of the PARP inhibitor Olaparib and the WEE1 inhibitor AZD1775 as a new therapeutic option for small cell lung cancer.
Clinical Cancer Research 2018; 24(20):5153-5164.

Small cell lung cancer (SCLC) is a highly aggressive lung cancer that metastasises early with poor prognosis. For the past three decades, standard of care chemotherapy has resulted in temporary tumour responses followed by disease relapse in the majority of patients and novel therapies are urgently required. In this study and in collaboration with AstraZeneca, members of the Clinical and Experimental Pharmacology group exploited their SCLC circulating tumour cell patient derived explant models (CDX) to test the new drug combination of a DNA damage repair inhibitor (PARP) and a cell cycle regulator inhibitor (WEE1 kinase). The rationale for this combination in SCLC is centred on high levels of endogenous DNA damage, rapid proliferation rate, absence of G1 checkpoint function due to mutant *TP53* and oncogene driven replication stress. The combination efficacy was variable across CDX models derived from patients with differing degrees of initial chemosensitivity, but superior to standard of care chemotherapy (cisplatin/etoposide). In one CDX model (with a *PALB2* mutation driving 'BRCAness'), the combination resulted in cures. Of importance, they tested the novel combination in a pair of CDX models made from the same patient prior to treatment and again post treatment at disease relapse. Here the combination showed promise in the pre-treatment model but was ineffective at disease progression. These data have informed early clinical trials of Olaparib and AZD1775 that are currently underway.

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RESEARCH GROUPS

Section of healthy skin highlighting the different cell types that develop into melanoma (pink) or squamous cell carcinoma (green and red). Image taken on the Olympus VS120 Virtual Slide Microscope.

Image supplied by Candelaria Bracalente (Molecular Oncology)

CANCER INFLAMMATION AND IMMUNITY

Group Leader
Santiago Zelenay

Postdoctoral Fellows
Eduardo Bonavita
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Scientific Officer
Shih-Chieh Chiang[1]

Graduate Students
Charlotte Bell
Christian Bromley
Eimear Flanagan

[1]Joined in 2018

Immunotherapies have led to remarkable clinical responses across a wide range of cancer types. The development of these cancer treatments builds on more than one hundred years of basic research in immunology. Under the premise that further fundamental research will provide insights into how to make immunotherapies more efficient and safer, our group studies the principles that determine the susceptibility of tumours to spontaneous or therapy-induced cancer immunity. Combining the use of preclinical cancer models with bioinformatic analysis of cancer patient samples, we have uncovered key cellular and molecular regulators of cancer immunity. Targeting these offers promising therapeutic avenues to enhance the efficacy of current cancer therapies.

Immunotherapy shares a podium of oncology treatments available alongside more conventional options such as surgery, chemotherapy and radiotherapy. Checkpoint inhibitors in particular constitute the most promising pan-cancer drugs promoting significant patient benefit across multiple malignancies. Numerous recent clinical trials have shown that these drugs can induce responses that frequently outperform the mainstream treatments. Nonetheless, the fraction of patients experiencing profound and durable responses is still limited and restricted to certain tumour types. Likewise, these treatments are associated with adverse, and often life threatening, effects. Critically, we currently have no reliable means to predict which patients will derive benefit from treatment and whether a patient will develop toxicities. In the Cancer Inflammation and Immunity group we believe that increasing our understanding of the principles and rules that define the immunogenicity of cancer will help answer these major open questions. We carry out basic and translational research mining cancer patient datasets and simultaneously study genetically engineered cancer models to establish cause-and-effect relationships in relevant in vivo settings.

Relying on murine cancer models, we have made great progress characterising and comparing the cellular and molecular composition of tumours with unequivocal progressive or regressive fates. In doing so, we have uncovered an essential role for natural killer cells in spontaneous and immune-checkpoint blockade-induced cancer immunity. Eduardo Bonavita gave a talk on this work during the 5th European Congress of Immunology held in Amsterdam in September 2018. Through in silico analysis of cancer patient datasets, we have also found a remarkable conservation in the inflammatory profile of our cancer mouse models and multiple human malignancies. Moreover, we have derived an inflammatory gene signature that predicts overall survival in a wide range of cancer types. This signature also associates with the outcome from immune checkpoint blockade in various patient datasets, outperforming previously published immune signatures. Encouraged by these findings and in collaboration with Cancer Research Commercial Partnerships, we have filed an application to patent our method for predicting outcome from these immunotherapies.

In parallel, we have been evaluating the therapeutical significance of our findings and found that the efficacy of immune checkpoint inhibitors can be greatly enhanced by co-administering anti-inflammatory drugs in various preclinical models. Victoria Pelly gave a talk at the ISREC-SCC Symposium 2018: Horizons of Cancer Biology and Therapy in Lausanne describing our progress in this highly clinically relevant line of research. This work has also been vital for the design of a soon to open clinical trial. In this study, led by Anne Armstrong, a Consultant Medical Oncologist at the Christie NHS Foundation Trust specialising in breast cancer, we will soon start testing in the clinic some of our more exciting preclinical findings.

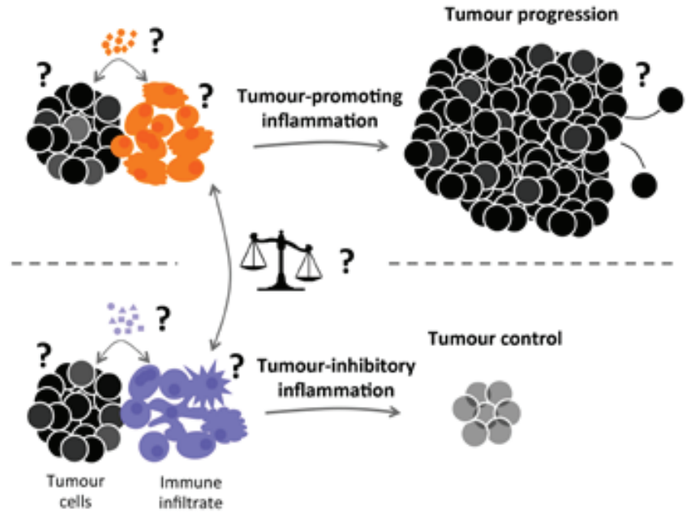

Figure 1
Our working model postulates that the tumour inflammatory profile at baseline and on-treatment influences tumour outgrowth and spread. Within this framework, the current basic research questions of the Cancer Inflammation and Immunity group are centred on identifying cellular and molecular mediators that regulate the type of inflammatory response at the tumour bed.

In another project, we continue our efforts to identify dominant mediators of immune evasion. We argue that these mechanisms, unlike recessive mechanisms of immune escape, such as loss of tumour antigens or downregulation of the antigen-presentation machinery by cancer cells, constitute attractive therapeutic targets to boost the efficacy of current immunotherapies. Screening a large and heterogeneous panel of murine cancer lines of diverse tissue origins and genetic makeup, we have uncovered conserved cancer cell intrinsic features that selectively define cancer lines with unique and profound ability to regulate multiple immune cell types.

Despite recent breakthroughs with many of these treatment modalities aimed at harnessing the anti-tumour function of the immune system, cytotoxic therapy remains the standard of care for most unresectable malignancies. Tumour shrinkage following chemotherapy or radiotherapy is largely attributed to the damaging effects of these treatments on rapidly proliferating cells. In addition to their direct killing effect on cancer cells, growing evidence also points to a major role for the immune system in regulating the mid and long-term efficacy of these mainstream clinical practices. These observations are consistent with the notion that certain cell death modalities, by virtue of the release of the so-called damage-associated molecular patterns, can stimulate innate immune antigen-presenting cells, drive T cell responses against antigens contained within the dying cells and promote tumour growth control. However, this concept is highly controversial, with an equally vast amount of literature suggesting that treatment induced cancer cell death is associated with the induction of immunological tolerance against dead-cell associated antigens and/or pro-tumourigenic inflammatory responses that instead fuel tumour progression and spread. The underlying basis for these opposing outcomes is largely unknown. We have thus embarked upon a new project intended to shed light onto this issue by exploiting our expertise on the analysis of the immune and inflammatory landscape of tumours.

Overall our recent findings support a model in which the tumour inflammatory profile at baseline and early on-treatment largely dictates cancer progression and treatment outcome. Therefore, to fully characterise the inflammatory and immune landscape of tumours, over the last year we have significantly increased our use of multiplex immunoassays and next generation sequencing techniques. The use of bioinformatics has thus become an essential and regular component of our research toolkit.

Finally, our on-going research projects have emphasised how much there is still to learn about key aspects of the immune response to cancer and have triggered new fundamental questions. To start answering them our group has expanded and embarked upon further collaborations with industry and prominent cancer biologists and renowned immunologists, including Alberto Mantovani (Humanitas University), Carlos Caldas (CRUK Cambridge Institute), Daniel Davies (University of Manchester) and Nadia Guerra (Imperial College London). Some of these collaborations have already resulted in high-profile collaborative research articles published in Nature and Cell (Molgora et al. Nature 2017; Bottcher et al Cell 2018).

Publications listed on page 62

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 how the diverse cues are integrated to co-ordinate cell division and death is therefore critical to understanding the biology of cancer.

Indeed, DNA damaging and anti-mitotic therapies owe much of their success to the checkpoint pathways that ensure transition through the cell division cycle only occurs when genome integrity is guaranteed. We therefore study the targets of two of these therapeutically important checkpoint pathways: (1) the commitment to; and (2) the exit from, mitosis – the physical process of genome segregation. Because the regulatory networks that control cell division are highly conserved, we complement our studies of cell division in human cell lines with manipulation of the simple, unicellular, fission yeast in order to identify the key questions to ask of the analogous controls in the complex context of human cell division cycle control.

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 control the rate of proliferation by determining the timing of progression through both the point of commitment to the cell cycle in G1 phase, known as the "restriction point", and the transition from G2 into M. Passage through these key transitions is driven by the activation of distinct CDK-Cyclin protein kinase complexes. The G2/M transition is a critical safeguard of genome integrity; incomplete DNA replication or DNA damage triggers checkpoint pathways that block the G2/M transition, to ensure that chromosomes are not segregated when incomplete or damaged. The G2/M transition is driven by activation of the Cdk1-Cyclin B protein kinase. Wee1 related kinases phosphorylate the catalytic subunit, Cdk1, to inhibit the complex during interphase. This phosphate is removed by Cdc25 phosphatases to promote mitotic entry. Cdk1-Cyclin B activation promotes a positive feedback loop that boosts Cdc25 and inhibits Wee1 activities to ensure that mitotic commitment is a rapid and irreversible bi-stable switch (Figure 1).

We study three core aspects of cell cycle control: (1) the role played by the centrosome in determining when cells commit to genome segregation; (2) the biology of the Wee1 family of kinases that restrain entrance into division in response to genome stress; and (3) the regulation of the mitotic exit protein phosphatases whose activities counteract those of the pro-mitotic kinases in order to drive cells out of division.

The initial appearance of active Cdk1-Cyclin B on human centrosomes, before propagating throughout the cell, suggests that the centrosome provides a specific microenvironment to trigger the G2/M transition. Our studies of the fission yeast centrosome equivalent, the spindle pole body (SPB), provide molecular insight into how and why this switch may operate.

We were able to drive fission yeast cells into division by releasing Cdk1-Cyclin B or Polo kinase activity at the SPB. In contrast, release at any other location around the cell had no impact upon division timing. Our attempts to define the molecular basis for such a striking impact have been guided by lessons from the SPB scaffold Cut12. Simply blocking the recruitment of protein phosphatase 1 (PP1) to Cut12 enabled us to delete the *cdc25*$^{+}$ gene without compromising viability. This bypass of the requirement for an otherwise essential mitotic inducer arose from the impact of the Cut12/PP1 axis on Polo kinase activity. Polo activity was inappropriately elevated by the abolition of PP1 recruitment to Cut12. Enhanced Polo activity probably overcomes the need for Cdc25 because it boosts Polo's ability to inhibit Wee1 to such a degree that it completely silences Wee1. Then, without the kinase putting the phosphate onto Cdk1, there is no need for the phosphatase that normally reverses the missing phosphorylation.

SPB/centrosomal control of cell cycle progression extends beyond Cut12. The signalling network that controls the timing and execution of mitotic exit events also relies upon the anchorage of a signalling network to an SPB associated scaffold. In this case it is the anchorage of the Septum Initiation Network (SIN) to the scaffold Sid4 that is essential for the correct control of mitotic exit under normal conditions and for the ability to restrain division when mitotic spindle function or cytokinesis is perturbed. Unexpectedly, we identified a second signal transduction network on Sid4 that determines the level of Cut12/PP1/Polo signalling required to promote mitotic commitment. Such confluence of signalling networks at the centrosome provides a plausible rationale for the use of the centrosome as a signalling hub: signals from multiple pathways, to a limited number of neighbouring scaffolds on the SPB, can set the flux through the outgoing signalling cascades that determine the timing of mitotic progression.

Figure 1: Feedback control by Polo kinase in Cdk1-Cyclin B activation at the G2/M transition
Cdk1-Cyclin B activity is held in check in interphase as a consequence of phosphorylation of Cdk1 by Wee1. Cdc25 removes the inhibitory phosphate to trigger mitosis. This trigger level of Cdk1-Cyclin B then activates polo kinase to further boost Cdc25 activity and inhibit Wee1 to make this transition a bi-stable switch between two distinct states. Our work shows that signalling events on the fission yeast equivalent of the centrosome, the spindle pole body, determine when this switch is flipped to trigger division.

We are applying the philosophies acquired during these studies of fission yeast cell cycle control to address the role/s played by Wee1 family kinases in human cell cycle progression. While the development of a Wee1 inhibitor has rapidly progressed through to clinical trials, we still know remarkably little about the basic biology and use of the three Wee1 family kinases in the cancer cell cycle. As a full understanding of Wee1 biology is key to identifying the optimal context and full spectrum of opportunity for Wee1 inhibition in the clinic, we are addressing when, where and how, these kinases are used in human cancer cells.

Cdk1-Cyclin B activation promotes the activities of an array of mitotic protein kinases that phosphorylate a broad spectrum of targets to drive chromosome condensation and the formation and function of the division apparatus. Once each phase of mitosis is complete, each phosphorylation event is reversed to support transition to the next phase of division. As a protracted mitotic arrest can trigger apoptosis, there is great interest in learning more about the biology of protein phosphatases to identify routes that could enhance the mitotic delay that naturally arises as a consequence of the abnormal karyotype of tumour cells.

PP1 and the protein phosphatase 2A isoforms PP2A-B55 and PP2A-B56 play key roles in mitotic control. PP1 is recruited to docking sites from where it dephosphorylates targets. Hetero-trimeric PP2A enzymes comprise single scaffolding and catalytic subunits, alongside one of four different types of regulatory subunit. Multiple, alternatively spliced, genes give the potential for hundreds of variants of PP2A-B55 and PP2A-B56 in humans, whereas fission yeast can live on one of each, or in the case of PP2A-B55, none.

After confirming that PP1, PP2A-B55 and PP2A-B56 activities decline upon mitotic commitment in fission yeast, we found that direct recruitment of PP1 to PP2A-B55 and PP2A-B56 reactivates these PP2A phosphatases to support appropriate mitotic progression/exit. Mitotic inhibition of PP1 arises from direct phosphorylation by Cdk1-Cyclin B. The destruction of Cyclin B subsequently allows PP1 to auto-dephosphorylate and restore its own phosphatase activity. Reactivated PP1 then reactivates PP2A-B55. Polo phosphorylation of the PP1 docking site of PP2A-B56 initially blocks PP1 binding to PP2A-B56. When Polo activity declines in mitotic exit, PP2A-B55 dephosphorylates the Polo phosphorylation site on B56 to allow PP1 to reactivate PP2A-B56 (Figure 2). We are now assessing the impact of phosphorylation on PP2A-B55 function and seeking the phosphorylation events on PP2A complexes that are reversed by PP1 recruitment to restore PP2A-B55 and PP2A-B56 activities.

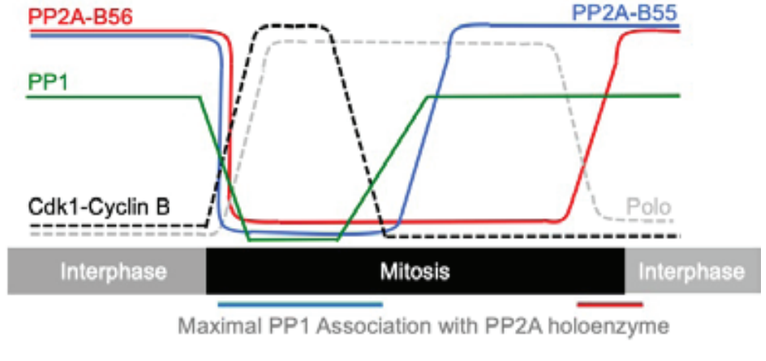

Figure 2: The mitotic PP1-PP2A phosphatase relay
PP1 and PP2A activities are all repressed upon entry into mitosis. The mode of PP2A repression is unclear however, it is well established that Cdk1-Cyclin B phosphorylation represses PP1 activity. Cyclin B destruction then allows PP1 itself to auto-catalytically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

CELL PLASTICITY & EPIGENETICS

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During neoplastic development cancer cells are constantly exposed to ever-changing conditions, first within their niche of origin and further by the ecosystems encountered whilst colonising other tissues. The fast-paced nature of the environment drives micro-evolutionary processes that give rise to different sub-clones of cancer cells within a tumour leading to increased heterogeneity during the course of the disease. This heterogeneity can arise either as a result of somatic mutations acquired through the development of the tumour or due to the ability of the same genotype to produce many discrete, sometimes dramatically different, phenotypes. The latter phenomenon is called phenotypic plasticity and provides a dynamic source of diversity that may support rapid adaptation during various environmental perturbations. Our group is interested in unravelling the molecular details underlying cellular plasticity in order to understand the molecular mechanisms that cancer cells use to escape from therapeutic paradigms.

The past year was one of settlement and growth for our group. We successfully completed a first round of recruitments and rapidly established a working environment within CRUK MI. We initiated successful pilot experiments and established many of the bioinformatic pipelines required to analyse our output data. Particularly, we focused our attention onto setting up appropriate model systems to answer our queries and got everything up and running swiftly.

The model system

Our group is interested in exploring the mechanistic details underlying cellular plasticity and in particular its relevance in drug tolerance. To do so, we take advantage of the exquisite response exhibited by cancer cells when challenged with TRAIL (Tumor-necrosis factor Related Apoptosis Inducing Ligand). Briefly, TRAIL triggers apoptosis by a clearly defined molecular route and, though breakthrough evidence supports the use of TRAIL as a therapeutic agent for many cancer types, fractional killing and reversible resistance have been observed in many cases. Notably, the primary mode of action of many currently used therapeutic agents is to directly induce DNA damage, thereby being mutagenic by default. In net contrast, TRAIL promotes cell death by first activating apoptotic cascades that will only lead to DNA damage at the cellular dismantling stage. This, together with the reversible nature of TRAIL tolerance observed in clonal cell populations, strongly suggests that the phenomenon of fractional killing is mainly epigenetically encoded rather than based on the acquisition of permanent genetic alterations. This particularity singles out the TRAIL system as an ideal model to study the inheritance of epigenetically encoded traits as the TRAIL-tolerant phenotype is propagated throughout several cell divisions after TRAIL withdrawal and fades away gradually until full reversion is reinstated.

The data

Our initial experiments demonstrate that a variety of hRAS(G12V) in vitro transformed cell lines display fractional killing and reversible resistance to TRAIL. We were able to map the precise moment where the resistant state is acquired within the clonal population, the duration of the molecular "memory" and its propagation in the absence of the challenging agent. Interestingly, we were able to recapitulate the acquisition, propagation and reversion of the resistant phenotype sequentially, thus not only suggesting that the process is reversible and epigenetically encoded but also demonstrating the robustness of the model system.

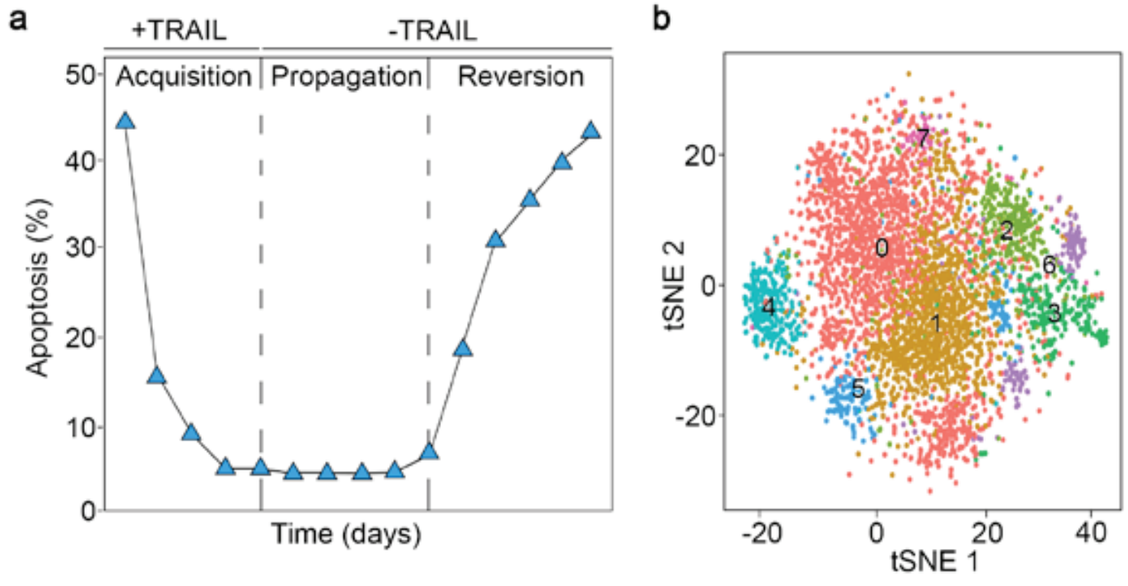

Figure 1: Our model system.
a) Line graph representing the response of hRAS(G12V) transformed clonal cell populations when challenged with TRAIL for a given period of time and then released from the selective pressure. Apoptosis was daily induced by TRAIL (maximum dose) and determined by PARP-cleavage 4hs after induction (maximum PARP activity). The main biological phases observed are depicted.
b) tSNE plot representing the identified 8 subpopulations observed in hRAS(G12V) transformed clonal cell lines as determined by single-cell RNA sequencing.

An outstanding question in the cancer field relates to whether the observed resistance to a given treatment is an inherent feature of a subpopulation of cells contained within the naïve population or whether this feature arises "de novo" in response to treatment. In an effort to discriminate between these possibilities, we found that hRAS(G12V)-transformed clonal populations harbour up to 8 discrete and transcriptionally divergent subpopulations that co-exist within treatment naïve cells. Strikingly, each individual subpopulation responds to TRAIL treatment by re-adjusting their gene expression profile generating once again up to 8 discrete divergent subpopulations. This result indicates that cellular plasticity may play a major role in determining the outcome of the apoptotic response and suggests that resistance to treatment can be sustained concomitantly by several discrete transcriptional and/or epigenetic states.

Moreover, our results show that resistant cells are dramatically different when compared to naïve populations at the signal transduction level. Indeed, a handful of tyrosine phosphorylation modulated pathways are solely activated in the resistant state and we are now exploring the immediate consequences of such events in our system. Thus, we are currently in the process of establishing experimental validation pipelines that will enable us to identify in a robust manner which pathways are indeed fundamental to maintain the resistant state and to describe their role in the acquisition and/or propagation of the TRAIL-tolerant phenotype.

Following our initial observations, the main goal of our work is to unravel the key molecular players and mechanisms that orchestrate the establishment, maintenance and temporal propagation of stable phenotypic states within isogenic populations of cancer cells that are exposed to a cytotoxic challenge. In those lines, we hypothesise that differential RNA expression and/or epigenetic landscapes within isogenic populations determine phenotypic lineages that can significantly vary in their response to the cytotoxic agent; yet all may eventually lead to the establishment of resistance. Moreover, our data supports the notion that the ability of a cell to change its molecular properties and convert to a different stable phenotype – known as lineage switching – lies at the basis of non-genetic adaptation. Overall, our research will begin to shed light onto the molecular mechanisms orchestrating acquisition of "temporal molecular memory" and will provide a first insight into the machineries that grant the inheritance of complex traits during adaptation to a new environment.

CELL SIGNALLING

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Recurrent mutations and overexpression in tumours implicate the small GTPase RAC and its activators, the guanine nucleotide exchange factors (GEFs), in the formation and progression of cancers. Furthermore, the effects of deleting genes encoding RAC proteins or RAC GEFs in mouse, or of inhibiting RAC-GEF/RAC interactions with drugs, strongly suggest that targeting RAC signalling could constitute a cancer treatment.

However, 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 RHO-like family of GTPases, cycles between a GDP- and a GTP-bound state. When GTP-bound, it interacts with various effector molecules that regulate several cellular processes including proliferation and migration. Multiple mechanisms control RAC activity, including control of nucleotide binding and hydrolysis by GEFs and GTPase Activating Proteins (GAPs) respectively, regulation of subcellular localisation and modulation of RAC protein levels (reviewed in Porter et al., Small GTPases 2017; 7: 123). As mentioned above, several studies using recombinant RAC and RAC GEF mice have shown that RAC is required for the formation and growth of tumours. However, the role of RAC in the malignant progression of tumours is not always positive. There are cases where deletion of RAC GEFs leads to tumours with increased malignancy and there are reports suggesting that reduced RAC levels correlate with more aggressive tumours (Porter *et al.*, Small GTPases 2017; 7: 123). In line with these studies, in vitro data have shown that activation of RAC may lead to opposing migratory phenotypes raising the possibility that targeting RAC in a clinical setting could exacerbate tumour progression. For these reasons it is important to identify the factors that influence the selection of RAC-driven cellular processes. We hypothesised that RAC GEFs may be critical determinants of downstream RAC signalling and that activation of RAC by different GEFs might determine whether it has a pro- or anti-migratory phenotype. GEFs are typically large proteins harbouring multiple protein-protein interaction domains and, besides stimulating guanine nucleotide exchange, they act as molecular scaffolds targeting active RAC to particular subcellular locations and potentially increasing the local concentration of selective effector molecules influencing downstream processes. In a recent study (Marei et al., Nat Commun. 2016; 7: 10664), we provided evidence that such a hypothesis is correct. We demonstrated that two RAC GEFs, TIAM1 and P-REX1, promote RAC-driven inhibition and RAC-driven promotion of cell migration respectively, through regulating the RAC interactome. We showed that TIAM1 promotes the association of RAC with proteins known to be important for the formation and maintenance of cell-cell adhesions, while P-REX1 enhances the interaction between RAC and proteins promoting cell migration, such as 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.

Role of RAC and its regulators in inhibiting migration and antagonising malignant progression

Mice deficient for the RAC activator TIAM1 are resistant in the formation and growth of RAS-induced skin tumours. However, tumours arising in *Tiam1*-deficient mice progressed more frequently to malignancy (Malliri et al., Nature 2002; 417: 867). This can be explained by the role of TIAM1 and RAC in the regulation of cell-cell adhesion; TIAM1 is required for both the formation as well as the maintenance of cadherin-based adhesions (Malliri et al., J Biol Chem 2004; 279: 30092). Consistent with these early findings, optimal cell-cell adhesion

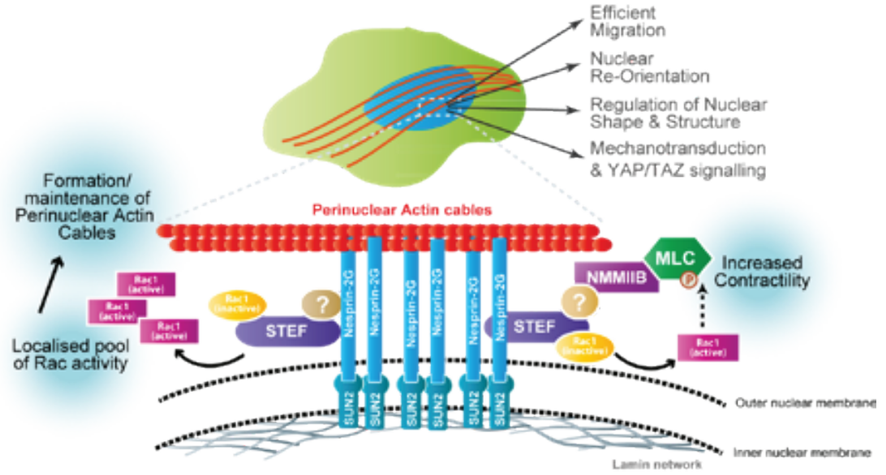

Figure 1. Model describing the role of perinuclear STEF.
STEF at the nuclear envelope generates a localised pool of active Rac1 which is required for the formation and/or stabilisation of perinuclear actin cables and for perinuclear actomyosin contractility. Disruption of the perinuclear actin cap in STEF depleted cells alters the structural and morphological properties of the nucleus, with consequences for directed cell migration and mechanotransduction.

disassembly and scattering of epithelial cells by the oncoprotein SRC requires phosphorylation and consequent cleavage and depletion of TIAM1 from cell-cell adhesions (Woodcock et al., Mol Cell 2009; 33: 639). Moreover, in another study from our laboratory (Vaughan et al., Cell Rep 2015; 10: 88), we found that TIAM1 is ubiquitylated and degraded specifically from the cell-cell adhesions upon treatment of cells with hepatocyte growth factor (HGF), a cytokine that is abundant in cancer and promotes invasion of cancer cells.

Apart from these studies showing that TIAM1 inhibits migration by promoting cell-cell adhesion we recently identified another mechanism by which TIAM1 hinders migration. We demonstrated that TIAM1 localises in the nucleus of several colorectal cancer cell lines and that nuclear TIAM1 inhibits their migration via suppressing the interaction of the transcriptional co-activator TAZ with its cognate transcription factor TEAD. Suppression of this interaction by TIAM1 inhibited expression of TAZ/YAP target genes implicated in epithelial-mesenchymal transition, cell migration, and invasion. Consistent with these in vitro data, we showed that TIAM1 localised not only in the cytoplasm, but also in nuclei of tumours of a colorectal cancer microarray. We also showed that nuclear staining intensity significantly decreased with advancing Dukes stage and that patients with high nuclear TIAM1 had significantly better survival than those with low nuclear TIAM1 (Diamantopoulou et al., Cancer Cell 2017; 31:621).

Role of RAC and its regulators in promoting cell migration

As mentioned above, the RAC activator P-REX1 promotes cell migration and invasion. 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., EMBO Rep. 2010; 11: 292). In a recent study, we established that this RAC activator is a critical regulator of the perinuclear actin cap, a contractile structure composed of thick, aligned actomyosin filaments that interact with the apical surface of the interphase nucleus via the Linker of the Nucleus and Cytoskeleton (LINC) complex. The actin cap functions to constrain the nucleus and is required for efficient re-orientation of the nucleus in polarising cells. The tension force required to constrain and anchor the nucleus is dependent on actomyosin contractility generated by the actin motor Non-muscle myosin IIB (NMMIIB); depletion of NMMIIB causes nuclear expansion and an over-rotation phenotype. There is mounting evidence that the actin cap works to bridge the extracellular environment and the nucleus via its connection with actin cap associated focal adhesions and the LINC complex, providing a direct, rapid pathway for mechanotransduction. We showed that STEF localises to the nuclear envelope, co-localising with the key perinuclear proteins Nesprin-2G and NMMIIB, where it regulates the activity of perinuclear Rac1. We also showed that STEF depletion reduces apical perinuclear actin cables (a phenotype rescued by targeting active Rac1 to the nuclear envelope), increases nuclear height and impairs nuclear re-orientation during front-rear polarisation. Moreover, we observed a decrease in pMLC and myosin-generated tension at the nuclear envelope in STEF-depleted cells, indicating that localised STEF-mediated Rac1 activity might regulate NMMIIB activity to promote stabilisation of the actin cap. Consistent with these data, STEF depletion resulted in a decrease in nuclear stiffness and reduced expression of TAZ-regulated genes, indicating that mechanosensing pathways are altered as a consequence of disruption of the actin cap (Figure 1 and Woroniuk et al., Nat Commun. 2018; 9: 2124). 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.

Publications listed on page 62

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In 2018 the Clinical and Experimental Pharmacology group had three major goals: 1) to discover, develop, validate and implement biomarkers to support personalised medicine for cancer patients; 2) to characterise and exploit our expanding panel of circulating tumour cell derived explant models of small cell lung cancer (SCLC) for discovery of new targets and to test novel therapies for this aggressive cancer of unmet clinical need; and 3) to develop new teams with broadened scope as we continue to develop the Manchester Centre for Cancer Biomarker Sciences. New initiatives include: (i) development of a community-based project for frequent monitoring of minimal residual disease using liquid biopsies from patients whose lung cancer was resected with curative intent; (ii) following our feasibility study, the roll out of the second phase of our TARGET trial where circulating tumour DNA profiling assists selection of the most appropriate trial for patients entering The Christie NHS Foundation Trust Phase I Trials Unit; (iii) biomarker analysis on immunotherapy trials in our tumour immunology and inflammation monitoring laboratory; (iv) initiation of studies that exploit nanoparticles aimed to increase liquid biopsy sensitivity; and (v) establishment of our Bioinformatics and Biostatistics Team.

The CEP group is subdivided into nine teams; Preclinical Pharmacology (PP), Nucleic Acids Biomarkers (NAB), Cells and Proteins (CAP) incorporating the Tumour Immunology and Inflammation Laboratory (TIIML), Tissue Biomarkers (TB), Biomarker Bioinformatics and Statistics (BBS), Quality Assurance (QA), the digital Experimental Cancer Medicine Team (digital ECMT), CEP operations (OPS) and CEP administration (AD). In the past 12 months, our biomarkers' portfolio supported 22 clinical trials (16 academic sponsored, 6 pharmaceutical company sponsored and 6 NIHR badged), and 20 experimental medicine studies. During 2018, we initiated several exciting new collaborations with AstraZeneca, Bristol Myers Squibb, Merck, Novartis and PsiOxus Therapeutics. Our PP team has also set up collaborative studies on SCLC with Amgen, Epigene Therapeutics, FLXBio and AstraZeneca.

Highlights

Understanding SCLC biology and the search for new therapies

Despite multiple clinical trials, the standard of care for small cell lung cancer remained unchanged for over three decades with only the recent introduction of immunotherapy in third line therapy benefitting a minority of patients. Although SCLC phenotypic heterogeneity was reported 30 years ago, the current World Health Organization classification of lung tumours recognises a single morphological classification. Due in part to the failure to identify novel treatments for SCLC, there have been recent attempts to develop a molecular classification system that can aid in the implementation of precision medicine. To this end, the PP team applied RNAseq to our panel of 45 patient circulating tumour cell derived explant (CDX) models and the data are being used to hierarchically cluster tumours into subgroups that may provide insight to SCLC biology. Principal component analysis using the 1000 most variable transcripts reveals at least four groups, suggesting that there may be functionally distinct subtypes. Several transcriptional drivers including the proneural basic helix-loop-helix transcription factors ASCL1 and NEUROD1, as well as the POU family member POU2F3, have been shown to promote neuroendocrine and non-neuroendocrine differentiation states, respectively. These transcription factors define three of the four transcriptional groups, and we are currently investigating both their role in tumour maintenance and progression and seeking druggable downstream targets.

The majority of SCLC patients are diagnosed with aggressive metastatic disease. We have developed SCLC CDX models that metastasise to clinically relevant sites including the liver and brain that we can now use to interrogate the underlying mechanisms (Figure 1).

Genetically related models from the same patient can demonstrate different metastatic capabilities, thus facilitating our ability to discover genomic regulators of dissemination. Furthermore, our new brain metastasis models offer a unique opportunity to interrogate this biology and investigate potential therapeutic opportunities.

Liquid biopsies for SCLC

Targeted therapies are now entering clinical trials for SCLC but serial tissue biopsies are challenging. With this in mind, the NAB team continue to develop and test liquid biopsies for this disease. We have developed a novel genome wide ctDNA copy number aberration (CNA) approach and targeted NGS of ctDNA on 110 genes relevant to the SCLC genomic landscape and novel therapeutics. Having completed a pilot study of 69 extensive and limited stage SCLC patients, we are extending analysis to include longitudinal profiles of over 130 SCLC patients to establish if these approaches can be used as a routine patient monitoring tool for assessing tumour load, treatment response and patient outcomes. Initial data indicates that CNA readouts can be used to measure both treatment response and disease relapse including in limited stage SCLC. The NAB team is also building upon our observation reported last year that CNA patterns identified in SCLC circulating tumour cells obtained prior to patient 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 through national and international collaborations, as well as streamlining single CTC analysis.

The utility of non-small cell lung cancer CTCs

Within the CRUK Lung Cancer Centre of Excellence-led TRACERx (TRAcking non-small cell lung Cancer Evolution through therapy [Rx]) consortium (PI, Professor Charles Swanton, UCL), we have continued to examine the genetic status of single CTCs compared to tumour profiles from patients with resectable NSCLC. We have applied both CNA and whole exome sequencing (WES) to single pulmonary vein circulating tumour cells (PV-CTCs) detected in early stage NSCLC at surgical resection with curative intent. An examination of 103 patients from TRACERx confirmed findings from our previous pilot study that PV-CTC detection is associated with lung cancer relapse, independently of tumour stage. In a patient where we compared primary tumour, PV-CTCs and a pleural metastasis detected 10 months after surgery, 50% of PV-CTC private mutations

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METASTATIC CELLS ARE DETECTED IN MULTIPLE ORGANS OF CDX17P, INCLUDING BRAIN

Micrometastases and DTCs were detected in lungs, liver, brain, ovary, and bone of CDX17P animals, whereas no micrometastases were observed in the analysed organs of CDX17 animals.

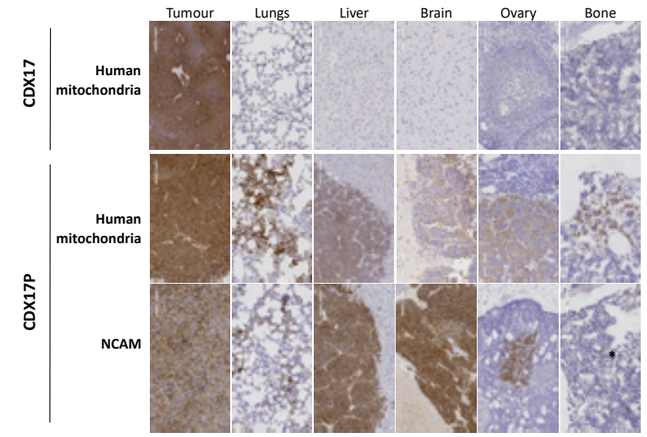

Figure 1. Immunohistochemistry showing examples of brain and liver metastases from a subcutaneous CDX tumour stained with Haematoxylin and Eosin (H&E). NCAM (CD56) staining identifies neuroendocrine cells and human cells stain with a human specific antibody to a mitochondrial protein.

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undetected in the primary tumour or circulating tumour DNA at surgery were detected in the metastasis. The data from this patient formally link CTCs to metastasis and demonstrate a case where PV-CTCs represented a minor subclone disseminating early that was responsible for disease relapse.

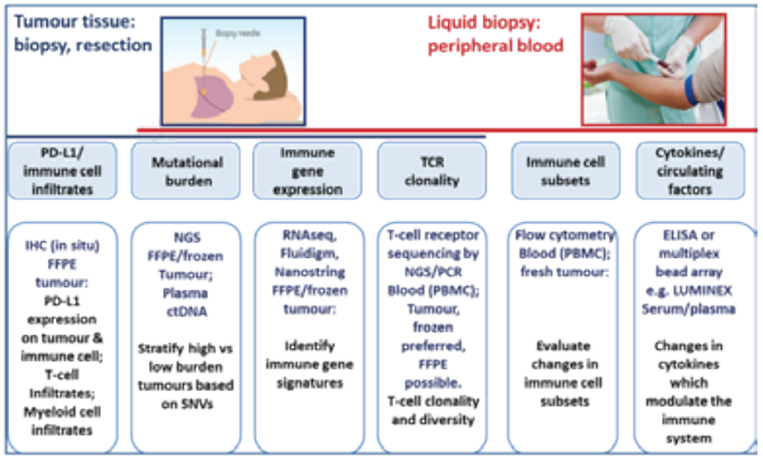
Liquid biopsies for earlier detection and minimal residual disease monitoring in NSCLC

We continue to collaborate with Dr Phil Crosbie and his clinical colleagues at Manchester University NHS Foundation Trust (MFT) having processed and banked blood samples from 748 "high risk, hard to reach" subjects who have taken part in a community-based lung health check study which offered low dose CT scanning. We are now evaluating blood biomarkers that may identify those with early stage lung cancer using both plasma based assays aimed at identifying DNA (ctDNA), microRNA and proteins that are linked to cancer, as well as cell based assays, including the "no cell is left behind" High Definition Single Cell Analysis (HDSCA) platform for detecting CTCs. Evaluation of HDSCA will be carried out in a continued collaboration with the system developer Professor Peter Kuhn (University South California), and plasma assays will include established published assays and novel assays in development within CEP. We were recently awarded additional CRUK funding and with this we are now setting up a minimal residual disease monitoring study in partnership with Lloyds Pharmacies where, after their surgery, patients will give blood samples on a monthly basis in their community pharmacy, for collection and transport to CEP with an aim to develop a liquid biopsy that picks up relapsing disease earlier.

ctDNA applicable to clinical trials

The TARGET Trial (Tumour chARacterisation to Guide Experimental Targeted therapy), was initiated in 2015 and is led from the clinic by CEP alumnus Dr Matthew Krebs (The Christie NHS Foundation Trust). We have regular Molecular Tumour Board meetings to integrate clinical data, tumour mutation profiling (archival and/or fresh biopsies) and contemporaneous CEP ctDNA data utilising our visualisation tool eTARGET, developed by the digital Experimental Cancer Medicine Team (digital ECMT). We completed the feasibility stage A for 100 patients where ctDNA analysis by NGS of 641 cancer-associated genes and a comparison to matched tumour data revealed a 79% concordance (*Rothwell et al, in press Nature Medicine*). We have initiated TARGET stage B and ctDNA analysis has now been successfully applied to over 300 patients with improved sample processing and ctDNA CNA analysis. A ctDNA based measure of tumour mutational burden (TMB) is currently being developed. Ongoing studies are expanding the TARGET biomarkers to include tissue based immune markers, which along with ctDNA TMB will be assessed as a means of identifying patients who may benefit from immune checkpoint therapies.
In collaboration with Professor Richard Marais (Molecular Oncology group) and Professor Paul Lorigan (The Christie NHS Foundation Trust), we developed a GCP validated droplet digital PCR (ddPCR) ctDNA assay which is ready for deployment in the upcoming advanced cutaneous melanoma phase II trial (CACTUS), to define a switch in treatment from targeted to immunotherapy.

Figure 2.
The TIMML biomarker 'toolkit'.



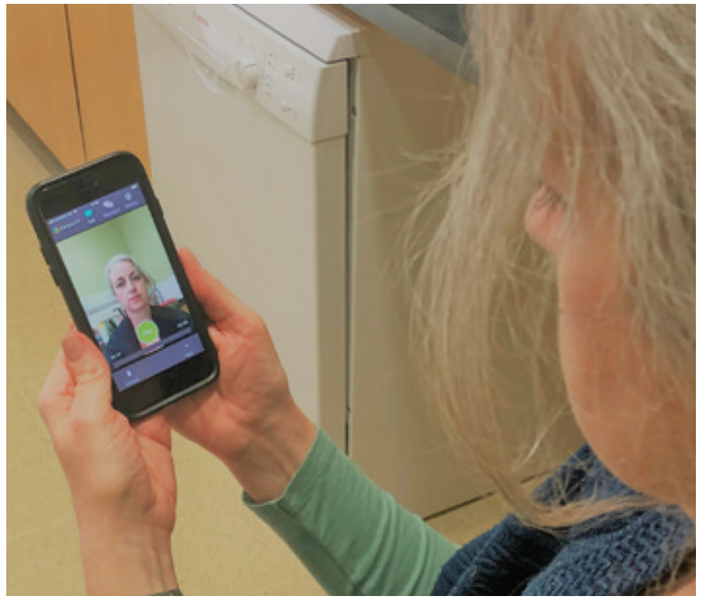


Figure 3.
The handheld device that allows patients to input their own data whilst on a clinical trial.

In order to increase assay sensitivity, the NAB team are examining approaches to specifically enrich plasma tumour DNA using established ctDNA methylation assays and, in collaboration with Professor Kostas Kostarelos (The National Graphene Centre), asking whether nanoparticles (lipid- and graphene-based) or 'nanonets' can be used to scavenge nucleic acids in the blood and be 'tuned' to preferentially enrich different tumour derived particles and nucleic acids.

Biomarkers to inform immunotherapy trials

The Tissue Biomarker and Nucleic Acid Biomarker teams are working closely with the TIIML to build and expand our immune-oncology 'biomarker toolkit' for analysis of tumour tissue and blood samples to support immune-oncology trials (Figure 2).

Liquid biopsy assays are now available including lymphoid and myeloid flow cytometry panels, multiplex ELISAs for cytokine analysis and an assay for assessment of PD-L1 expression on CTCs enriched by CellSearch. For analysis of the immunogenic status of the tumour microenvironment, we developed immunohistochemistry (IHC) assays for a range of immune markers and are currently developing IHC assays for the Mismatch Repair Deficiency proteins MSH2, MSH6, MLH1 and PMS1. We have also established a robust and reproducible workflow for RNA extraction and immune gene expression analysis from Formalin Fixed Paraffin Embedded tissue using the nanostring platform. Implementation of these assays within clinical studies is beginning in blood samples from the TRIBE study (Tyrosine Kinase Inhibitor Therapy In Renal Cell Carcinoma Immune Biomarker Evaluation) undergoing analysis for activated, exhausted and memory T-cell subsets. The PD-L1 CTC assay is being developed as an exploratory endpoint in a SCLC clinical trial. As part of the iMATCH (Innovate Manchester Advanced Therapies Centre Hub) project, validation of a multiplex ELISA assay for monitoring cytokine storms in patients receiving advanced T-cell therapies is nearing completion. Immunotherapy associated markers are also being developed for the TARGET study, including analysis of infiltrating lymphocytes and PD-L1 expression in tumour tissue alongside measurement of tumour mutation burden by analysis of ctDNA.

Digital clinical trials

The digital Experimental Cancer Medicine Team (www.digitalecmt.org) has the capability to test new technology, devices, data-science techniques and new ways of working under clinical trial conditions that brings researchers, clinicians, technology and patients together to innovate in early clinical trials. One example of this approach is eTARGET, a decision support tool that integrates clinical and genomic NGS data to facilitate decision-making for matching patients with clinical trials and has transformed the way that the Manchester Molecular Tumour Board for the TARGET trial works.

As another example of an on-going digital clinical trial, PROACT (Patient Reported Opinions About Clinical Trial Tolerability) that was developed with patients, is a mobile phone app or website tool that allows patients to say how they are feeling on an early clinical trial (Figure 3). Figure 3. The handheld device that allows patients to input their own data whilst on a clinical trial.

Video, voice or text messages are sent between patients and their medical teams. Initial feedback has been very positive with over 90% of patients offered PROACT taking part. The digital ECMT has also developed a digital clinical trial for patients to be able to monitor their kidney function at home, which is planned to start in 2019.

Publications listed on page 62

DRUG DISCOVERY

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The last year has continued to be a time of advancement for the Drug Discovery Unit, under new leadership and direction with the appointment of Caroline Springer as Director, and moving to temporary laboratories at Alderley Park in early 2018. Caroline also brought with her a number of new cancer drug target projects which are being progressed in tandem with ongoing DDU projects by the integrated team.

Our pan-RAF drug has now completed its Phase I clinical trial at The Christie and Royal Marsden NHS Foundation Trusts, and we look forward to the commencement of Phase 2 trials. This orally bioavailable, well-tolerated panRAF/SRC drug was discovered in collaboration with Professor Richard Marais and is designed to treat mutant BRAF or mutant RAS melanoma and other tumour types driven by these mutant oncogenes.

Our DNMT-1 and RET pre-clinical candidate development programmes also continue to make good progress and we anticipate these compounds will enter first-in-human trials in the near future.

Our partnership with IDEAYA Bioscience on our Poly(ADP-ribose) glycohydrolase (PARG) programme continues to develop. Following on from an out-licencing agreement, IDEAYA gained access to multiple proprietary series of our small molecule inhibitors of PARG. In this collaboration we are now assessing in vivo activity. The goal is to nominate a pre-clinical candidate shortly.

Metastasis is the main cause of death in cancer, and we are excited to assess the potential of our lysyl oxidase (LOX) inhibitors to treat primary tumours and metastases in patients. LOX is an enzyme that regulates cross-linking of structural proteins in the extracellular matrix and plays a critical role in metastasis and in tumour growth in many cancers. LOX is a validated therapeutic target, and our aim, in collaboration with Professor Marais, is to discover first-in-class, orally bioavailable small molecule LOX inhibitors. Despite being a protein that cannot be purified or crystallised, with funding from Wellcome and CRUK we have discovered LOX inhibitors with good pharmacokinetics properties and have progressed our LOX drug discovery programme to late lead optimisation. Our advanced inhibitors have demonstrated significant therapeutic activity against a number of primary tumour models and pronounced anti-metastatic efficacy in a metastatic tumour model. Professor Marais' group has discovered new biology concerning LOX interaction with signalling pathways, which provides good biological rationale for combining targeted agents with our LOX inhibitors. 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, as monotherapy and in combinations.

Cancer stem cells (CSCs) are a subset of tumour cells with the ability to perpetuate cancer growth indefinitely. CSCs are involved in tumour progression, resistance to treatment and recurrence in many cancers. Current therapies target bulk tumour cells, but CSCs escape resulting in tumour regrowth and treatment failure. Thus there is a need for drugs to target the CSC subpopulation within tumours, to use in combination with the standard of care. We are pursuing a drug discovery programme to target cancer stem cells that has progressed rapidly to lead optimisation. We have discovered potent and selective inhibitors of our CSC target, which have promising pharmacokinetic profiles (Figure 1). Our medicinal chemistry has been greatly supported by internal computational chemistry and by crystallography in collaboration with Leicester University (funded by a CRUK Accelerator Award). We are collaborating with Professor Richard Marais and The University of Manchester based expert Dr Robert Clarke, in elucidating the biology of our target.

Our gene-directed enzyme prodrug therapy (GDEPT) programme uses engineered vaccinia viral vectors to target tumours selectively and produce a unique bacterial enzyme locally, which is able to convert subsequently administered prodrugs to cytotoxic drugs thus killing the tumour cells. In collaboration with Professor Marais we have demonstrated selective tumour targeting with our viral vectors and long-term tumour xenograft regressions in mice, and are currently working to generate improved vaccinia vectors. In addition, we will be looking to assess the potentially beneficial immune responses to our GDEPT system, in collaboration with Institute colleague Dr Santiago Zelenay.

Originally initiated somewhat earlier in our portfolio, and in collaboration with AstraZeneca, we have recently initiated a new project aimed at reversing hormone therapy resistance in prostate cancer. Drawing upon the disease expertise of the Manchester Institute in this area, with experts such as Professor Rob Bristow, we believe this approach has the potential to offer significant benefit to men with castrate-resistant prostate cancer. This emerging project also integrates our new Structural Biology alliance with Professor Richard Bayliss at the University of Leeds, and will allow us to understand, on a molecular level, how our compounds interact with their target at the atomic level. We will exploit this information to design improved compounds and, in doing so, increase the rate of progression of this project. These efforts will be greatly enhanced through the work of Dr Rae Lawrence, our new Principal Computational Chemist, who joined the team earlier this year.

In the longer term, our portfolio is being repopulated from the impressive number of early targets which have been sourced from our Senior Group Leader collaborators, such as Professors Richard Marais, Caroline Dive, Iain Hagan, Dr Claus Jorgensen 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 NHS Foundation Trust and the CRUK Lung Cancer Centre of Excellence.

Across all our projects, we will ensure that 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.

Following our move in 2018, we have greatly valued the opportunity to work together in the new laboratories at Alderley Park 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.

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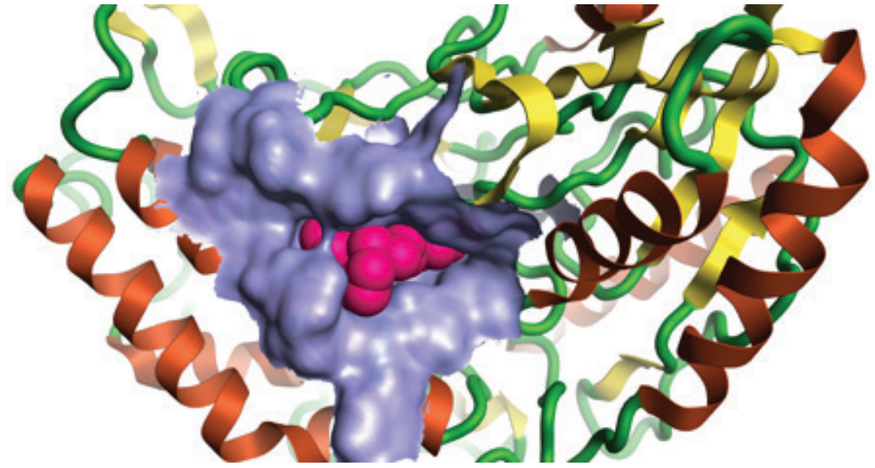

Figure 1.
A potent and selective CRUK MI designed ligand bound to a target involved in inhibiting CSC growth.

HEAD AND NECK CANCER BIOLOGY

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The particular focus of the Head and Neck Cancer Biology group is on salivary gland cancers because there is a significant unmet need in this patient group. Genomic profiles in head and neck tumour samples will be examined to identify alterations that can be targeted by drug therapies. Our primary aim is to develop a new biological understanding of how changes at the gene and protein level result in tumour growth and metastasis. Novel insights provided by this approach will allow drugs to be screened using cell lines and mouse models. Those drugs which are found to be effective in laboratory studies will be taken forwards to clinical trials in patients with the goal of improving patient survival.

Initial research will focus on adenoid cystic carcinoma (ACC), working in collaboration with Professor Caroline Dive and the Clinical and Experimental Pharmacology group. ACC is a salivary gland cancer, which metastasises in over 50% of patients leading to a median survival of 3 years, with no effective drug therapies. Due to chromosomal rearrangements, 90% of ACC tumours are driven by overexpression of the myeloblastosis (MYB) transcription factor gene family members: a-MYB and c-MYB. The overall goal is to develop new therapies for ACC by identifying mechanisms through which MYB drives tumour growth and metastasis, and to develop predictive and prognostic biomarkers to optimise patient treatment.

Chromatin immunoprecipitation sequencing has identified MYB downstream targets that are enriched for genes controlling cell cycle regulation. As the cell cycle is dysregulated through multiple mechanisms in most human tumours and offers avenues for therapeutic targeting, our initial focus is to determine whether and how MYB overexpression impacts on cell cycle control in adenoid cystic carcinoma.

For functional studies to identify critical effector genes downstream of MYB, we are developing isogenic MYB knock-down/knock-out ACC models using shRNA and CRISPR/Cas9 or dCas9-KRAB followed by lentiviral mediated MYB rescue. To validate candidates identified through this approach as therapeutic targets, in vitro and in vivo drug screening will be performed in models of ACC. As metastasis is a defining feature of ACC, in vivo and ex vivo fluorescence/luminescence imaging studies in isogenic models will be used to identify functional predictors of metastasis. Fundamental discoveries in ACC biology will be translated to patients using tumour/liquid biopsies to identify and validate prognostic and predictive biomarkers.

Findings can be rapdly translated from this research to develop biomarker led clinical trials for this patient group. As there is a high risk of metastasis and no standard drug therapies, if this research is successful it would have immediate global impact in this patient population. The aim is to develop a platform to broaden research focus across other rare sub-types of head and neck cancers. In addition, understanding of MYB biology in ACC provided by this study may provide insight into other solid tumours where MYB is frequently overexpressed including sub-groups of patients with breast and colorectal cancers.

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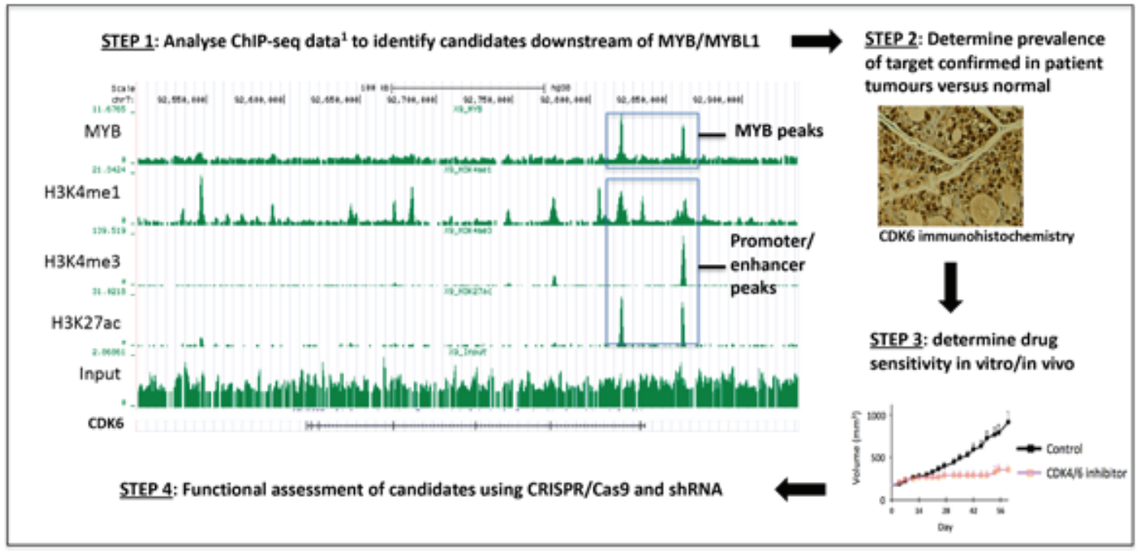

Figure 1. Approach to identify and validate candidate therapeutic targets in adenoid cystic carcinoma.
Step 1: ChIP-Seq data using ACC tumours to determine MYB binding (downloaded from ENCODE 1 Drier et al., Nature Genetics 2016) was analysed to identify MYB binding peaks coinciding with promoter and enhancer histone marks (histone 3 lysine 4 mono-methylation (H3K4me1) and tri-methylation (H3K4me3) and lysine 27 acetylation (H3K27ac)) in the regions of transcriptional start sites of biologically plausible genes. Example shown is in the region of CDK6. Step 2: ACC patient samples are analysed and to determine the prevalence of candidates in tumour compared with normal salivary gland. Step 3: ACC cells are screened in vitro and in vivo for evidence of sensitivity/resistance. In the example shown, ACC patient derived tumour xenografts show tumour growth inhibition with pharmacological inhibition of CDK4/6. In parallel, in Step 4: CRISPR-Cas9 and shRNA approaches are undertaken to knock-down/knock-out MYB and MYL1 to determine the functional significance of MYB/MYBL1 bound genes on tumour growth and metastasis.

LEUKAEMIA BIOLOGY

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A core focus of the team is to identify new therapeutic targets for development through to the clinic, for longer term patient benefit. Along these lines, over the last seven years we have had a significant investment in the story of LSD1. Lysine Specific Demethylase 1 is one of a number of epigenetic regulators which have recently emerged as candidate therapeutic targets in cancer.

It was initially identified as a core component of an RCOR1 (CoREST) histone deacetylase (HDAC) transcription corepressor complex and later found to have lysine-specific demethylase activity. With regard to its enzymatic function, LSD1 is a flavin adenine dinucleotide (FAD) dependent homologue of the amine oxidase family with an ability to demethylate monomethyl or dimethyl lysine 4 (K4) of histone H3 as well as a number of other targets such as DNMT1 and TP53.

The interest in LSD1 as a therapeutic target in cancer arose from the observation of its high level expression in poor prognosis sub-groups of prostate, lung, brain and breast cancer, as well as in certain haematologic malignancies. In 2010 William Harris, then a graduate student in the lab, noticed that expression of LSD1 was very high in murine leukaemia stem cells driven by an MLL-AF9 fusion oncogene, but was down regulated as those cells underwent differentiation. This prompted him to perform a knockdown experiment and he discovered that LSD1 transcript depletion resulted in leukaemia cell differentiation. The first drug found to inhibit LSD1 was tranylcypromine, a monoamine oxidase inhibitor previously used in the treatment of depression. We found that this drug, as well as some derivative compounds synthesised for us by James Hitchin, Donald Ogilvie and Allan Jordan in the Drug Discovery Unit, induced murine and primary human leukaemia cells to differentiate whether tested in vitro or in vivo. These data were published in *Cancer Cell* in 2012 and led to our establishing a collaboration with Tamara Maes and Carlos Buesa at Oryzon Genomics in Barcelona to set up a Phase 1 trial of their highly potent and selective orally-active LSD1 inhibitor ORY-1001, now called iadademstat.

We won funding through a commercial-academic European Union funding scheme called EUROSTARS to run this first-in-class, first-in-man trial which opened in 2014 in the UK at The Christie NHS Foundation Trust and at sites across Spain and France. The trial completed its recruitment in late 2016 with evaluation of the results currently being finalised for publication in early 2019. This study in patients with relapsed or refractory acute leukaemia evaluated the safety, pharmacokinetics, and preliminary activity of iadademstat and determined the recommended dose as a single agent. We found iadademstat to be well tolerated with most adverse events consistent with transient myelosuppression. Encouragingly, in terms of responses we observed one complete remission and a number of haematological improvements with induction of blast cell morphological and molecular differentiation, in keeping with expectations from our prior pre-clinical data. These data have led on to a Phase 2a study called ALICE, which is a pilot study to assess the safety, tolerability, dose finding and efficacy of ORY-1001 (iadademstat) in combination with azacitidine as first line therapy in older patients with acute myeloid leukaemia; the first patient was recruited to this protocol just before Christmas 2018. More generally, interest in LSD1 as a therapeutic target continues to increase worldwide with multiple pharmaceutical companies currently conducting early phase clinical trials with related compounds in diverse clinical settings such as myelofibrosis, Ewing sarcoma and small cell lung cancer.

With LSD1 inhibitors in early phase clinical trials, an appreciation of their mechanism of action is essential. Given the well-established activity of LSD1 as a histone demethylase, the assumption has been that LSD1 contributes to gene repression by removing monomethyl and dimethyl histone marks from lysine 4 of histone H3, and that this is the key activity targeted for potential therapeutic effect. However, LSD1 also interacts with multiple transcription factors

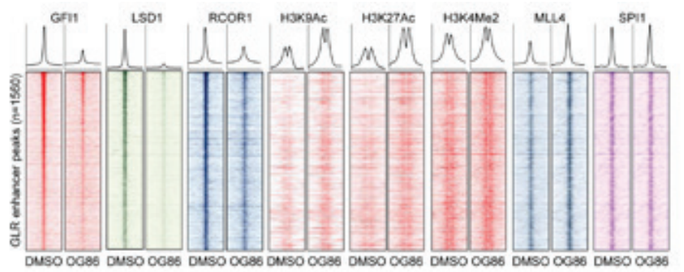
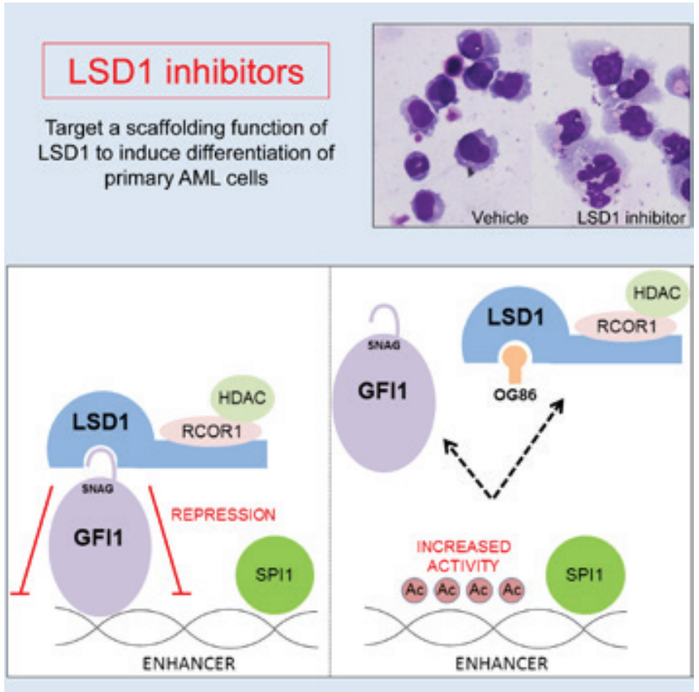


Figure 1:
Heat maps show ChIP signal for the indicated proteins or histone marks at 1,560 GFI1-bound enhancers, ranked top to bottom according to GFI1 peak strength, in THP1 AML cells treated with DMSO control or OG86, a tranylcypromine derivative inhibitor of LSD1, for 24 hours.

Figure 2:
Summary of mechanism by which LSD1 inhibitors promote differentiation in AML.



raising the possibility that other mechanisms may be important. To begin to address this question some years back James Lynch, former Postdoctoral Fellow in the lab, treated AML cells with a potent LSD1 inhibitor and performed ChIP sequencing and RNA sequencing analyses. Our prediction based on the literature was that we would see accumulation of histone methylation marks genome wide at sites of LSD1 binding close to genes whose expression changed significantly. To our surprise we did not find this at all, even though drug treatment very strongly promoted significant changes in gene expression, with concomitant cellular differentiation. This critical observation led to a series of experiments led jointly by Gary Spencer, James Lynch and Alba Maiques-Diaz over several years, which have revealed the actual mechanism by which LSD1 inhibition promotes differentiation in AML. We reported our findings in *Cell Reports* in 2018.

We first noted using comparative transcriptomic techniques that pharmacologic inhibition of LSD1 mimics knockdown of the transcription factor gene *GFI1*. Given that physical association of LSD1 with the N-terminal SNAG domain of GFI1 is essential for the function of GFI1 as a transcription repressor, we evaluated whether OG86 disrupts this interaction and found in immunoprecipitation experiments that it did. In fact, using ChIP sequencing we discovered that GFI1, LSD1 and its CoREST complex binding partner RCOR1 strongly associate one with another on chromatin at enhancer regions genome-wide. Treatment of AML cells with an LSD1 inhibitor targets GFI1/CoREST chromatin-bound complexes for disruption and release to the nucleoplasm. Through generation and expression of conditional expression constructs, where LSD1 was fused directly to the DNA binding domain of GFI1, Gary Spencer was able to show that inhibitor-induced myeloid differentiation in AML cells is entirely dependent upon the physical separation of LSD1 from GFI1. To determine the consequences of LSD1:GFI1 physical separation and concomitant inactivation of GFI1 at GFI1-bound enhancers, Alba Maiques-Diaz performed additional ChIP sequencing experiments. She found that most GFI1-bound enhancers were pre-loaded with two myeloid lineage transcription factors called SPI1 and CEBPA, and that following treatment of cells with LSD1 inhibitor, there was loss of CoREST complex histone deacetylases HDAC1 and HDAC2 and gain of the histone acetyltransferase CBP and the methyltransferase MLL4. In keeping with this we observed increased histone acetylation at GFI1-bound enhancers within hours of drug treatment (Figure 1) and a slower latency accumulation of histone methylation by 48 hours. Interestingly, in contrast to histone acetylation, the accumulation of histone methylation at GFI1-bound enhancers did not correlate well with the increased expression of the subordinate network of genes controlled by these enhancers.

Thus using a tractable experimental system and confirmatory analyses in patient cells, we have shown that both irreversible and reversible inhibitors of LSD1 promote differentiation through disruption of the protein:protein interaction of GFI1 with LSD1 (Figure 2), rather than impairment of histone demethylation. Our studies reveal a critical role for both GFI1 and LSD1 as key contributors to the cardinal pathologic feature of *MLL*-translocated AML, the differentiation block of immature blast cells. An intriguing final point is that drug discovery programs focusing on inhibition of demethylase activity as a target have generated compounds that function through an unexpected mechanism. The important possibility is raised that a search for compounds that maximally disrupt SNAG domain:LSD1 interactions might yield molecules with higher potential therapeutic efficacy.

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MOLECULAR ONCOLOGY

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We study cancer biology and work with clinicians so that the new knowledge we generate can be used to improve cancer patient care, primarily in melanoma but also in other diseases including breast and pancreatic cancers. Our multi-disciplinary approach allows scientists and clinicians to work closely together. Our aim is to improve our understanding of cancer biology and develop new drugs and diagnostic tools that can be used to optimise cancer treatment and improve patient care.

Developing more effective prevention, diagnosis and treatment of cancer, begins with an increased understanding of cancer biology, and in particular the mechanisms that drive cancer initiation. To this end, we established a programme to determine how ultraviolet radiation (UVR) causes melanoma. It is widely accepted that exposure to UVR, either naturally from sunlight or artificially from tanning beds, is associated with increased melanoma incidence. However, the relevance of the genomic changes that result from different components and patterns of UVR exposure to the progression of this disease is poorly understood. In a recent study, we examined the genomic alterations in tumours from our mouse melanoma model, following exposure to different wavelengths, patterns and quantities of UVR. We found that exposure to short wavelength UVR accelerates melanoma development and increases mutation burden compared to long wavelength UVR. We also found that only a few exposures to short wavelength UVR were enough to accelerate melanoma development (Figure 1A). We found that the type of genomic changes in short-wavelength UVR-exposed tumours had a predominance of a pattern of mutations known as signature 7, which has previously been associated with UVR-induced DNA damage; this pattern was absent in the tumours from the mice that did not receive short-wavelength UVR. Strikingly, we found a similar dichotomy in human melanoma, with ~85% of cases showing a predominance of signature 7 and ~15% where signature 7 was not predominant. Thus, although patients generally present with clinically similar disease, our data suggests at least two distinct mutation processes drive this disease. Notably these two groups show differences in overall- and disease-free survival (Figure 1B). Our data suggests that there are at least two types of cutaneous melanoma with distinct aetiologies leading to different disease courses and outcomes.

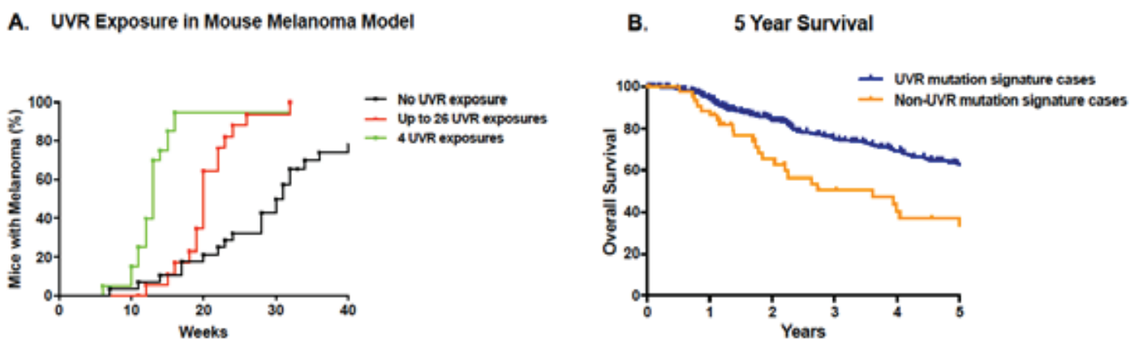

Figure 1: UVR exposure accelerates melanomagenesis and can imprint specific mutation signatures on tumours
(A) Rate at which melanomas develop in a physiologically relevant mouse model of melanoma either in the absence of UVR exposure, or after 4 or up to 26 UVR exposures. (B) Five-year overall survival of patients with cutaneous melanoma segregated by a predominance of the UVR mutation signature (signature 7) in the tumour genomes.

Our studies reveal a complex interaction between UVR and melanoma and have shown that even a few incidences of UVR exposure increase the risk of melanoma. Further, we provide insight into the correlation between the tumour's genomic landscape and disease progression. Going forward, we will study the clinical features of patients in the two groups to understand the relationship between UVR-induced genomic alterations and survival.

Better understanding of melanoma biology that emerges from the type of study described above underpins our efforts to translate our findings into the clinic. To that end, we recently initiated a clinical trial based on laboratory discoveries, following our recent reports that circulating tumour DNA (ctDNA) in the blood of high-risk (stage III) melanoma patients (so-called "liquid biopsies") can be a surrogate of patient tumour burden. We hypothesised that ctDNA could reveal how patients are responding to treatment and so developed CAcTUS (**C**ircul**A**ting **T**umour DNA g**U**ided therapy **S**witch), a biomarker-driven phase II trial to investigate if ctDNA can guide a switch between targeted and immune therapy in patients with advanced cutaneous melanoma. CAcTUS is due to open in the next few months with the aim of maximising response in melanoma by using ctDNA to determine when to switch between first and second line therapies (Figure 2). This trial is a testament to our extraordinarily successful relationship between laboratory and clinic, representing a collaborative effort between ourselves and the Clinical and Experimental Pharmacology group at CRUK MI and the clinicians of the Melanoma Group at the Christie NHS Foundation Trust.

In the past year, we also collaborated with and supported leading researchers in the fields of melanoma epidemiology and histopathology to characterise further and understand better distinct melanoma subtypes. In one study, we provide new insights into naevoid melanomas, which we propose can be further classified into two types, with distinct clinical and histopathological features. In another study, we presented data that suggests nodular melanoma (NM), a histopathologically distinct subtype of cutaneous melanoma, is a distinct fast-growing entity from the outset. These two studies have implications for the prognosis of these rare melanoma subtypes. Finally, in addition to our work in melanoma, in the past year we also harnessed our expertise in genomic and transcriptomic tumour analysis in a collaborative study with the Prostate Oncobiology group at CRUK MI. We characterised the genomic, epigenomic and transcriptomic features of clinically localised prostate cancer, and in particular assessed these in the context of tumour visibility by multiparametric MRI.

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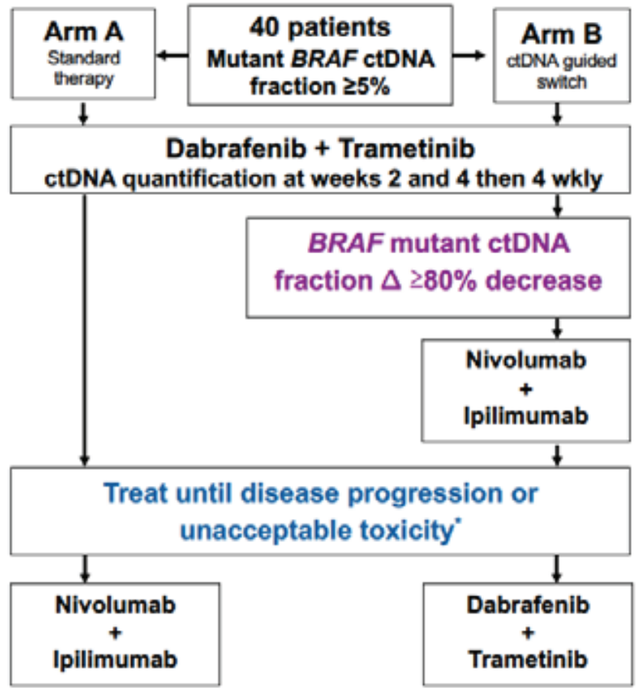
Figure 2:
Trial schema for CAcTUS.



www.cruk.manchester.ac.uk/Our-Research/Prostate-Oncobiology

PROSTATE ONCOBIOLOGY

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Personalised treatment for prostate cancer (PCa), the most commonly diagnosed cancer in men in the UK, remains a challenge because there are yet no clearly defined molecular subtypes to predict patient response. In recent years, deep sequencing and single cell studies have significantly advanced our understanding of the cellular composition and gene expression programs that shape tumour architecture, opening new routes to tackle cancer. The goal of the Prostate Oncobiology group is to apply these techniques to understand the prostate cellular landscape and its evolution during neoplastic transformation to facilitate the development of better therapies.

It has now become evident that tumours are composed of co-evolving heterogeneous sub-clones, which have fundamental implications for the response to treatment. Ultrasound-guided biopsies of PCa-suspicious lesions remain the gold-standard therapeutic procedure. However, there is a trend towards the use of multiparametric (mp)MRI to trigger biopsies in PCa patients combined with genetic testing to refine risk stratification. Importantly, >10% potentially significant PCas are missed by this approach because they are not detected by mpMRI; the genomic makeup of these "invisible" lesions could provide important insights into their metastatic capacity and help to assess their potential lethality.

To address this question, we have recently completed a study correlating genomics and mpMRI in men undergoing radical prostatectomy in order to elucidate the genomic characteristics of mpMRI visible and non-visible tumours and to assess the inter-relationship. We found that the intra-tumour heterogeneity within visible mpMRI lesions bears the risk of misclassifying patients when using genomic biomarkers from a single biopsy. For instance, considering a previously validated threshold for the percentage of genomic aberrations (PGA≥7.49%), of the two cores obtained from the same visible lesion, one core can classify the patient as low risk, while the other core can classify the patient as high risk. Similarly, intra-tumour transcriptomic heterogeneity within visible mpMRI lesions can also lead to misclassification. As a result, single mpMRI targeted biopsy poorly reflects the genomic heterogeneity of PCas and may therefore be unsuitable to assess the patients' risk based on genomic analyses. On the contrary, limiting biopsies to mpMRI visible-only lesions may underestimate the patients' individual risk for disease progression and metastasis.

Our study showed that PCa tumours undetected by mpMRI can harbour genomic alterations, which are commonly seen in metastatic castration resistant prostate cancer (mCRPC), including RB1 and TP53 loss, and MYC amplification. In our recently published work mpMRI non-visible tumours could be regarded as genomically aggressive and could potentially give rise to lethal clones. Thus, our study further emphasises the complexities of diagnosis in clinically localised PCa. Importantly, it highlights the shortcomings of this new diagnostic method, using mpMRI on its own as a triage test, and the need of additional biomarkers to inform patient diagnosis and prediction of treatment response. Thus, our study has the potential of being practice changing by refining diagnostic testing in PCa.

In a complimentary study focused on the heterogeneity of PCa, we assessed the cellular composition within the prostate epithelium in the context of response to therapy (i.e. androgen-deprivation) by combining single cell profiling and functional characterisation. For this, we compared the mouse prostate of intact mice with those that underwent androgen-deprivation upon surgical castration. Our single cell expression analysis revealed an unexpected molecular heterogeneity in the prostate luminal lineage and to a lesser degree also in the basal lineage. We found that a subset of prostate cells in the luminal lineage co-express multiple basal (e.g. *Krt5, Krt14, Trp63*) and luminal (e.g. *Krt8, Krt18,* and *Ar*) markers together with prostate stem/progenitor marker genes (e.g., *Ly6d, Trop2, Sca1, Cd133, Cd166*). Many of these stem/progenitor genes are found within a bi-lineage gene set expressed in both basal cells and a portion of luminal cells. An important observation from our study is that many castration-resistant (CR) prostate cells in the luminal lineage exhibit a bi-lineage expression signature similar to that of intermediate cells, raising a possibility that such intermediate cells may be intrinsically CR.

Our studies identified LY6D as a novel marker linking intermediate cells to prostate stem/progenitor cells and CR prostate cells. LY6D is a gene with yet no established role in prostate development or cancer. It is a member of the Ly6/uPAR family, characterised by their roles in cell proliferation, cell-cell interaction, immune cell maturation, and cytokine production, which are all essential components of tumour initiation and progression. However, in PCa, the functional role of LY6D for tumorigenesis and tumour maintenance remains unknown. Our in vitro and in vivo data showed that LY6D$^+$ cells in the luminal lineage represent luminal progenitors inherently resistant to androgen deprivation and with regenerative capacity. We found that LY6D$^+$ prostate cells from the SCA1^{high} and SCA1$^{low/-}$ gates were enriched with organoid-forming multipotent luminal progenitors and formed increasing numbers of multipotent organoids in the absence of androgen. Taken together, these findings suggest that LY6D expression correlates with PCa initiation and progression to castration-resistant growth from the luminal lineage (Figure 1). Importantly, in support of this hypothesis, analysis of human PCa cohorts revealed that higher LY6D expression levels are associated with more aggressive disease and worse outcome, suggesting that LY6D may serve as a prognostic biomarker for advanced PCa.

Further studies are warranted to determine the cellular composition of tumours during progression and their association with mpMRI visibility, as well as the precise role of LY6D in prostate epithelial heterogeneity, PCa initiation and progression to adenocarcinoma, to validate its utility as a novel biomarker for patient stratification, and to assess the impact of CR LY6D$^+$ cells as novel therapeutic target for patients with lethal PCa.

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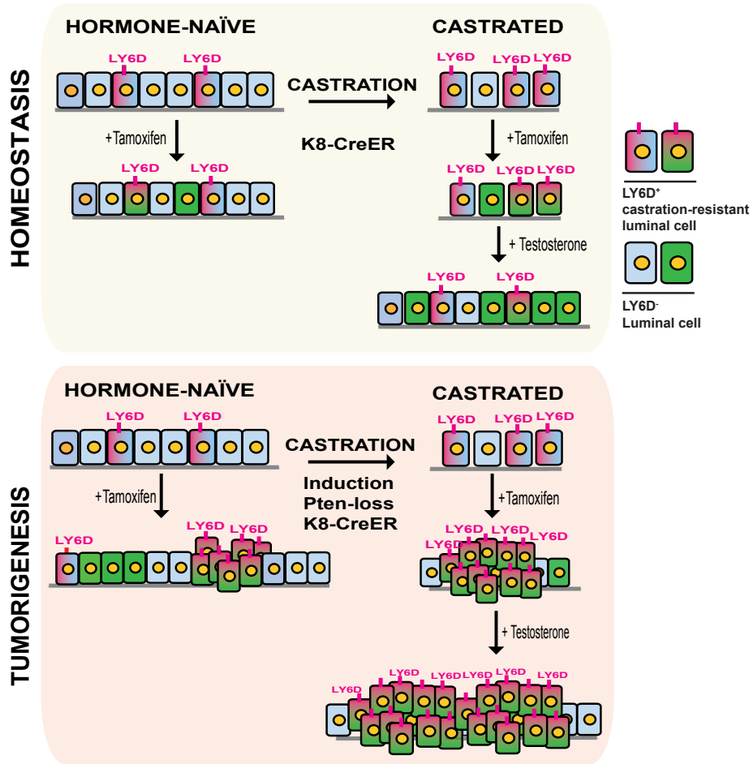

Figure 1:
Schematic diagram showing LY6D$^+$ luminal progenitors in homeostasis and PCa tumorigenesis (upon tamoxifen induced Pten-loss in *K8-CreER;Pten$^{-/-}$* mice) derived from putative CR LY6D$^+$ luminal cells (with red bars), or LY6D$^-$ luminal cells in hormone-naïve or castrated mice.

RNA BIOLOGY

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The RNA Biology group is interested in how changes in transcription drive oncogenic transformation, how sets of genes work together to regulate the behaviour of normal cells, and how these patterns of gene activity change in tumours. We are particularly interested in long noncoding RNAs (lncRNAs). These are transcripts that are never translated into proteins, but are increasingly being shown to act as part of the regulatory systems within a cell.

Advances in nucleotide sequencing have led to the discovery of thousands of human genes that express RNA molecules that do not encode proteins. Despite their lack of coding potential, these noncoding RNAs typically feature similar patterns of histone marks to protein coding genes, produce RNA molecules that are often processed to incorporate 5' caps and poly(a) tails, are often alternatively spliced, and are consistently differentially expressed between different conditions. These observations together suggest that noncoding genes are under regulatory control, and are thus unlikely to be transcriptional artefacts. A key question therefore, is the extent to which noncoding RNAs exert an important and previously underappreciated role within a tumour cell.

The RNA Biology group is particularly interested in noncoding RNAs greater than 200 nucleotides in length. These long noncoding RNAs (lncRNAs) are now known to outnumber protein coding genes, but their relatively recent discovery means that only a small number have been assigned a function. Those that have been studied however, have been shown to perform a diversity of roles, driven by their ability to hybridise to specific nucleotide sequences through complementary base-pairing. This allows them to target specific DNA and RNA molecules, and to fold into secondary structures that define their interactions with particular proteins. As a consequence, lncRNAs perform a wide range of targeting and scaffolding roles throughout the cell.

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, the implication is that there will be similar types of lncRNAs acting in human cells. We are seeking these lncRNAs.

To do this we have been exploiting the availability of large cohorts of RNA sequencing data derived from patient tumours; using these to search for noncoding RNAs that are differentially expressed in tumour cells, and then applying computational approaches to infer potential functions for these transcripts. Our goal therefore, is to identify previously uncharacterised lncRNAs expected to have an important role in driving tumour growth and maintenance. These 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

Much of the work in the group makes use of large volumes of DNA and RNA sequencing data derived from human tumours. We have developed our own de novo annotation strategies, making it possible to discover previously unannotated lncRNAs, and then to ask how their patterns of expression change in tumours. This requires substantial computing power. The RNA Biology group is highly interdisciplinary, and makes use of techniques from computer science and Artificial Intelligence. The CRUK MI has a large High Performance Computing (HPC) cluster allowing us to employ big data techniques to cluster datasets, to seek patterns in these data, and to ask how these patterns change in tumour cells.

Polygenic mini-drivers

Large-scale tumour genome sequencing projects have made it possible to identify specific genes that are mutated more often than would be expected by chance, and are thus likely to be 'driver' genes that perform a critical role in the processes that govern oncogenic transformation. This has rapidly advanced the field.

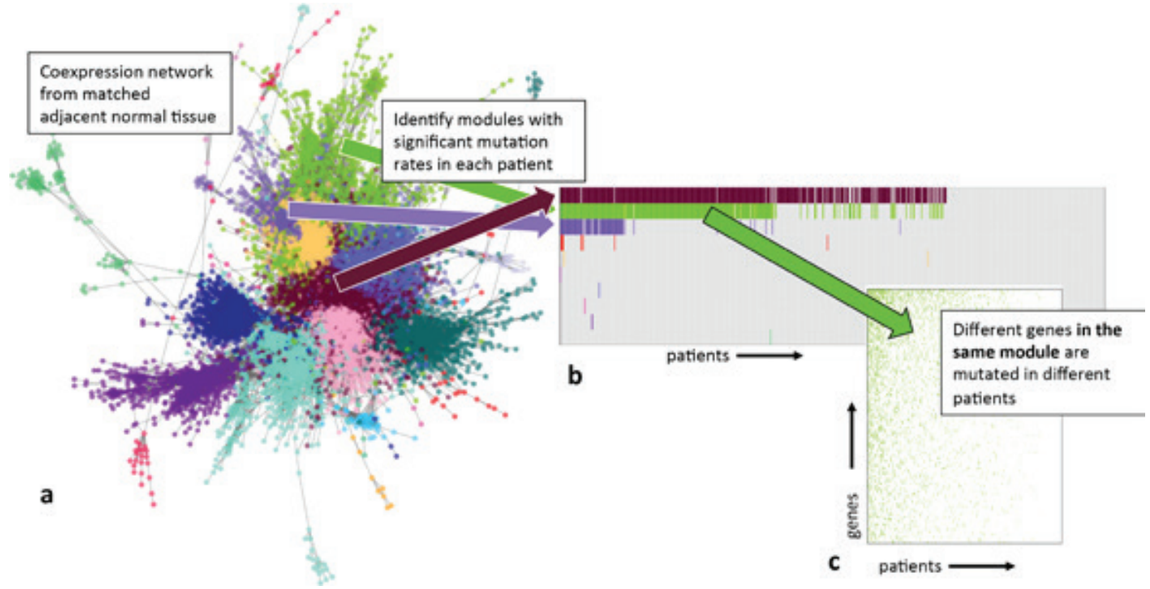

Figure 1: Identifying polygenic mini-driver signatures
(a) A coexpression network built from RNA seq data derived from matched adjacent normal tissue in LUAD patients. (b) Mutations from independent tumours are mapped onto the network, and modules with a disproportionate number of mutations are identified. (c) While a significant number of genes are mutated in a module, different genes in the module are mutated in different patients. Figure derived from data used in Bennett et al., 2018.

One of the most striking results from these sequencing projects has been the degree of heterogeneity in mutation patterns – to the extent that there is often little or no overlap between the sets of mutations identified in different tumours. A consequence of this is that even the most commonly occurring driver mutations do not occur in every patient. For example, the most frequently mutated gene in lung adenocarcinomas (LUAD), TP53, is altered in only half of all LUAD tumours (Imielinski et al., 2012; Bennett et al., 2018). This is surprising, given that all cancers share a set of hallmark phenotypes (Hanahan and Weinberg, 2000), irrespective of their particular mutational profiles. It thus raises a fundamental question: how do two tumours share the same canonical hallmark phenotypes when there is no overlap between mutational profiles?

We set out to address this apparent paradox by building models that integrate somatic mutation data with gene expression profiles derived from LUAD patients (Figure 1, Bennett et al., 2018). One possibility is that in tumours that lack a canonical driver mutation, oncogenesis might instead be driven by the accumulation of multiple weak-effect mutations congregating around the same cellular subsystems. Our analyses provided significant support for this 'mini-driver model' of oncogenesis, and led to the identification of a novel mutation signature predictive of disease-specific survival. They thus provide a potential explanation of how the same hallmark phenotypes can emerge in different tumours with a high degree of inter-tumour heterogeneity.

In silico error correction improves cfDNA mutation calling

The possibility of characterising a tumour by the mutations detected in the circulating free DNA (cfDNA) found in peripheral blood has the potential to revolutionise cancer genomics through the development of novel 'liquid biopsies' that support tumour profiling. This is challenging because circulating tumour DNA (ctDNA) accounts for only ~0.1% of the total cfDNA. Reliable profiling therefore requires high sequencing depth and multiple rounds of PCR amplification. This leads to elevated PCR error rates, and loss of mutation calling accuracy.

In collaboration with the CEP group we have developed a novel algorithm for PCR error correction that exploits the redundancy that occurs when sequencing many PCR amplicons arising from the same initial cfDNA fragment (Kim et al., 2018). Our purely in silico approach achieves similar levels of performance to more complex strategies that use additional protocol steps and calibration samples. Our approach is useful because these alternative strategies add cost and complexity to the protocol, and may also reduce the efficiency of library preparation. This is particularly undesirable when dealing with limited amounts of ctDNA material.

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SKIN CANCER AND AGEING

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The primary focus of our studies is melanoma and skin cancer in the elderly population. Melanoma affects over 12,000 people and causes over 2,000 premature deaths each year in the UK, and 85% of deaths occur in patients older than 60. Age is the most powerful predictor of outcome together with primary tumour thickness.

One factor underpinning the increased incidence of melanoma in the elderly is that as we age we acquire more ultraviolet (UV)-driven damage. However, although more melanomas appear in the elderly population and more frequently occur at anatomic sites that have accumulated sun damage over the years, they can also arise at rarely exposed sites (Figure 1). The aged skin anatomy and function varies greatly depending on the amount of sun damage that has accumulated over the years, with chronically damaged sites presenting greater tissue decay, more wrinkles and more UV-driven mutations in the aged cells. Our lab aims to understand how differences to the pattern of skin ageing (or accumulated sun damage) and differences in the tumour are linked to greater incidence and mortality in the aged subset of the population. We also aim to identify new strategies to improve the outcome of old patients with early stage, primary high-risk disease.

An important focus of our research is the tumour biology of melanoma in the elderly patient. The genomic characteristics driving melanoma specifically in the aged population have not been investigated. This year we have made progress towards describing the molecular aspects that define melanoma in the elderly patient. For this major aim of our lab, we have looked at the mutation rates, mutation types and patterns of DNA damage accumulated in melanoma cells during ageing. Additionally, we are performing a comprehensive genomic analysis of age-specific melanoma molecular characteristics linked to poor outcome in the elderly population. We are identifying the unique features that define melanoma in elderly patients and are investigating the specific changes that can reliably identify old patients at high risk of melanoma-specific death. We aim to identify the DNA and/or RNA changes that can be used as a predictor tool in clinical practice, and we are working to test genetic markers that can stratify aged patients better than the current guidelines of staging. Because checkpoint inhibitors are showing promising results in the adjuvant setting to treat early stage melanoma patients, and the cost burden to health care limits access to these drugs, we are additionally investigating if certain genetic markers linked to high risk of progression can be used to identify patients with a higher rate of response to immunotherapy.

In addition to the age-specific tumour biology, we are studying the role of the aged skin microenvironment in melanoma progression in the elderly patient. Previous work has shown that multiple cells composing the melanoma tumour stroma contribute to disease severity. For example, certain macrophage subtypes and fibroblasts alter the immune and metabolic milieu at the site of melanoma metastases and promote disease spread. With ageing there is an increase in melanoma incidence at all anatomic sites, but there is a specific increase in incidence particularly at anatomic sites with chronic sun damage. Some melanoma subtypes that arise at sites of chronic sun exposure, over severely sun-aged skin, have a better outcome; but paradoxically, overall melanoma deaths increase in parallel to melanoma incidence in the elderly. Our lab is addressing the role of stroma in promoting or inhibiting melanoma progression at the earliest stages of disease. We are particularly interested in the earliest primary stage, when melanomas that arise predominantly from intra-epidermal melanocytes breach the dermo-epidermal basal membrane to invade the deeper cutaneous tissue and prepare for lymphatic, local and neurovascular spread.

One intriguing aspect of melanoma progression in the elderly is the higher likelihood of spreading via the vascular system, and one possibility could be that structural and functional changes in the aged cutaneous microenvironment are responsible for this phenomenon. Thus, we are first examining how skin degrades and how this affects the main physiological functions. One important feature of human skin is that structural and functional decay depend greatly on the environmental pressure. Thus, skin from sun-protected sites becomes paucicellular across the three layers, and there is less connective tissue in the dermis, leading to thinning across all the layers of the skin. In contrast, skin from sun-exposed sites present a predominant pattern of dermal connective tissue degradation, where collagen fibre networks disappear to form globules of degraded collagen and other fibres that form the connective extracellular matrix. We are investigating how UV damage versus sun-protected, chronological cutaneous ageing affects homeostasis. Human skin affords the opportunity to study the cellular and acellular differences in an organ subject to different levels of extrinsic or environmental pressure. Finally, this year has given us a significant opportunity to establish clinical collaborations that will allow us to explore these questions in human tissue.

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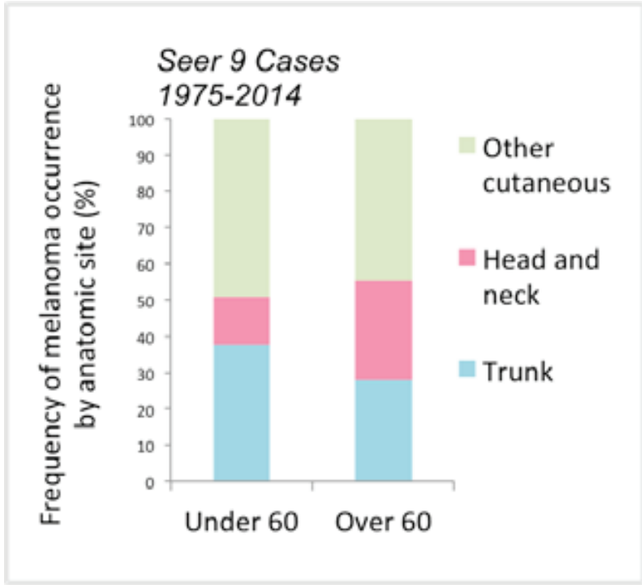

Figure 1:
The proportion of primary cutaneous melanomas arising at specific anatomic sites varies depending on age. Old patients have more melanomas in heavily sun-damaged skin.

STEM CELL BIOLOGY

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[1] Joined in 2018
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The genes encoding for core binding factors AML1/RUNX1 and 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, the transcription factor RUNX1 has also been shown to be critical for haematopoietic development. Our group studies the function of RUNX1 in haematopoietic development, maintenance and malignancies in order to better understand how alterations of these functions might lead to cancer.

RUNX1 dosage in haematopoietic development

Alterations in RUNX1 dosage or activity in the blood system have been associated with several blood disorders. Both reduction (thrombocytopenia, myelodysplastic syndrome) and gain (Down syndrome haematopoietic disorders) of functional *Runx1* alleles lead to haematological abnormalities and imbalances. In addition, RUNX1 dosage plays a crucial role in the maintenance of leukaemias harbouring core-binding factor related translocations. Several studies have clearly established that during the ontogeny of the blood system *Runx1* haploinsufficiency or mutations result in a decreased generation of haematopoietic stem and/or progenitor cells. At this early stage in development, the first blood cells arise from a specialised endothelium (haemogenic endothelium or HE), via a process termed endothelial-to-haematopoietic transition (EHT). Although we and others have shown that RUNX1 is critical for EHT and consequently the emergence of blood cells from HE, little is known about the precise role of RUNX1 dosage in this process. We therefore sought to define the role and importance of RUNX1 dosage in HE and EHT.

At the transcriptional level *Runx1* is controlled by two alternative promoters that generate the transcripts encoding for the two main RUNX1 isoforms. The P1, or distal promoter, controls the expression of the distal RUNX1 isoform RUNX1C, and the P2, or proximal promoter, controls the proximal isoform RUNX1B. In mice, *Runx1* P2 promoter activity starts early during haematopoietic development and is detected in HE in which it is the sole active *Runx1* promoter, indicating that the RUNX1B isoform is responsible for the initiation of EHT. In contrast to P2, the *Runx1* P1 promoter is activated later in development during EHT in committed CD41^{+} haematopoietic progenitors and is the main active promoter in the adult haematopoietic system. At a functional level, various proteins have been shown to be able to modulate RUNX1 activity including CBFβ. CBFβ is a critical RUNX1 cofactor that heterodimerises with RUNX1, enhances its DNA-binding affinity and protects it from degradation. RUNX1 protein is barely detectable in *Cbfb*$^{-/-}$ cells and the mouse *Cbfb* knockout model is embryonic lethal and shows an almost identical phenotype to the *Runx1* knockout model, suggesting that the phenotype is mainly caused by a reduction in RUNX1 activity levels. More recently, SOXF transcription factor family members SOX7 and SOX17 have emerged as novel important regulators of RUNX1 activity. These transcription factors have established roles in vasculogenesis and angiogenesis, suggesting that they are potentially able to interact with RUNX1 in the context of HE development. Indeed both SOX7 and SOX17 are co-expressed with RUNX1 during a narrow temporal window of haematopoietic development that encompasses the HE stage. Furthermore, both factors have been proposed to be negative regulators of RUNX1 transcriptional activity and their overexpression blocks haematopoietic development.

Studying the role of RUNX1 dosage in detail during the initiation of the haematopoietic system from HE is particularly challenging in vivo as temporal and stage specific modulation of gene dosage in embryos is difficult to achieve. To circumvent this limitation, we took advantage of the in vitro mouse embryonic stem cell (mESC) differentiation system. Indeed, mouse ESCs have been shown to recapitulate key events of early embryonic yolk sac haematopoiesis including HE formation and

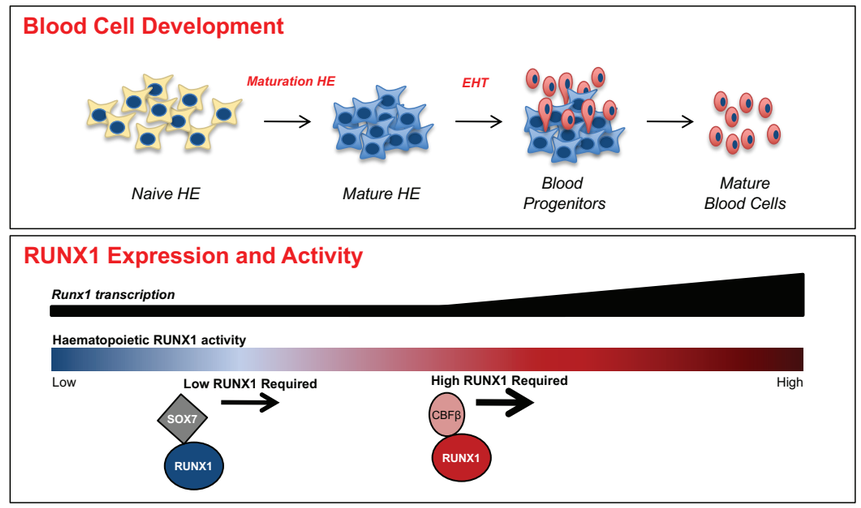

Figure 1:
RUNX1 levels control both the initiation and completion of blood formation from haemogenic endothelium (HE). In naive HE, RUNX1 transcription and protein levels are low. Low RUNX1 activity is required to mature the naive HE population and initiate a productive EHT. Once EHT is initiated a higher RUNX1 dose is subsequently required in order to produce mature haematopoietic cells. This shift in RUNX1 dosage and/or activity may rely on an increase in Runx1 transcription, the switch from the Runx1b to Runx1c isoform and potentially also by a change in RUNX1 binding partners. SOXF transcription factor family members like SOX7 may play a role in the latter process. SOX7 can interact with RUNX1 in HE and has previously been shown to compete with RUNX1 co-activator CBFβ.

differentiation. To evaluate the effects of different RUNX1B expression levels on HE and EHT, we utilised a mESC line that we previously generated in which *Runx1b* transcription is under control of a doxycycline inducible tet-on system and the endogenous alleles have been deleted. Analyses of this line in the presence of either a wildtype or a disrupted *Cbfb* locus allowed us to evaluate the effects of a wide range of RUNX1B levels on blood cell development from HE. We found that at the earliest stages, low levels of RUNX1B in HE are crucial for the maturation of the HE and the initiation of EHT. Circumventing HE maturation by increasing RUNX1 levels resulted in abortive differentiation, highlighting the need for a phase of low level RUNX1 expression in the HE to initiate EHT successfully. However once EHT was initiated, an increase in RUNX1 activity was required to successfully exit EHT and complete the blood formation process. We further demonstrated that SOX7 and RUNX1 interact in HE and generated data, suggesting that SOX7 might modulate RUNX1 activity by protecting it from degradation and sequestering it from its activator CBFβ. Altogether, our study indicates that RUNX1B is essential not only for the initiation of the EHT but also for its completion and that these two events require different levels of RUNX1B.

The CDK4/6 inhibitor palbociclib inhibits expansion of AML1/ETO leukaemia in vivo

Human acute leukaemias are characterised by the presence of recurrent chromosomal abnormalities, which encode the formation of chimeric transcription factors. The core binding factors AML1/RUNX1 and CBFβ are the most frequent targets of these genetic alterations. The t(8;21) translocation, resulting in AML1-ETO fusion and the inv(16) generating the SHMMC-CBFβ fusion, account for more than 20% of all the AML cases. In mice models, full length AML-ETO on its own, expressed from a viral vector or a targeted integration, is not able to induce leukaemia. However, *AML1-ETO9a (AE9a)*, an alternatively spliced form of AML1-ETO, which is thought to act as a dominant inhibitor of AML1/RUNX1, has been shown to cause rapid development of leukaemia in mice upon retroviral transfer. Seeking to establish an inducible AE9a-based mouse leukaemia model in our lab, we first established a mESC line containing a doxycycline *AE9a IRES GFP* inducible cassette. Doxycycline induced expression of AE9a in this mESC line was able to block the generation of haematopoietic cells during in vitro differentiation. Based on these results, we subsequently generated a mouse line from these inducible AE9a mESC. The mice expressing *AML1-ETO9a* developed extra medullary haematopoiesis followed by the development of acute myeloid leukaemia with a latency of around 6 months. The disease latency could be significantly shortened to 3 months when *AML1-ETO9a* was induced on a P53$^{-/-}$ background. We collaborated with the groups of Contanze Bonifer (Birmingham University) and Olaf Heidenreich (Newcastle University) to use this model to evaluate new therapeutic strategies for AML. We demonstrated that AML1-ETO drives leukaemia by directly promoting cell cycle progression and that palbociclib, a clinically approved inhibitor of CCND-CDK4/6 complexes, hampers leukaemic growth in vivo. These results establish that inhibition of G1 CCND-CDK complexes are a promising therapeutic strategy for the treatment of AML1-ETO AML.

Publications listed on page 66

SYSTEMS ONCOLOGY

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[1] Left in 2018
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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 host 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 group 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, Cell 2016). Using our recently implemented and optimised system for long term cell-specific labelling (Tape et al, Mol Cell Proteomics 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.

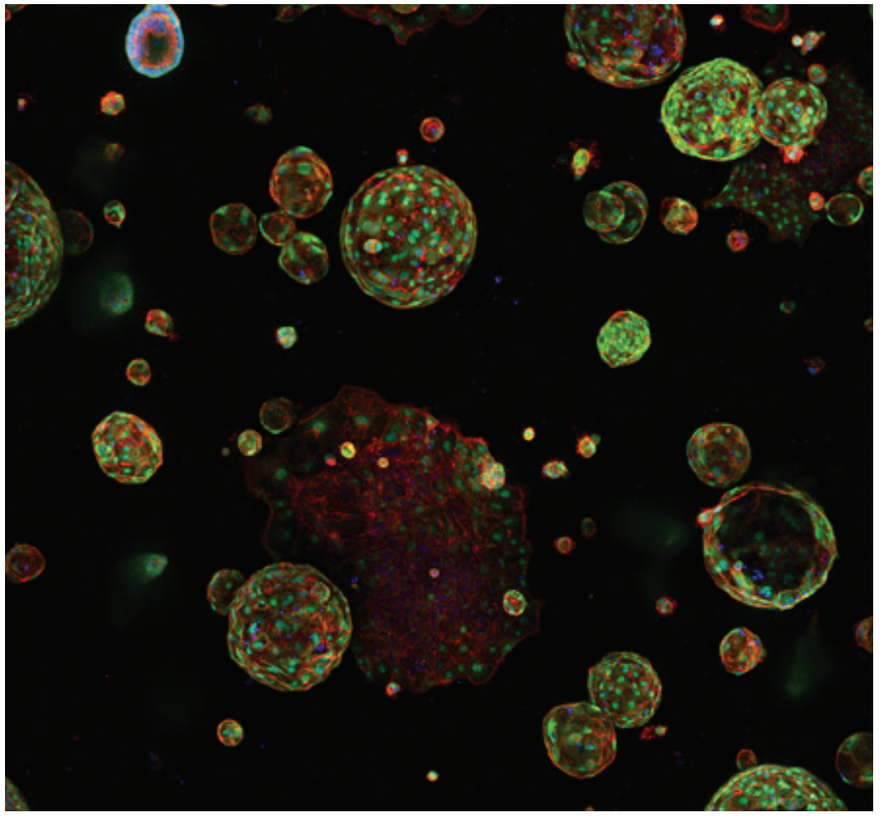

Figure 1:
Pancreatic Ductal Adenocarcinoma (PDA) is characterised by extensive stromal reaction and desmoplasia. Immunohistochemistry for epithelia (pan-cytokeratine), activated fibroblasts (alpha smooth muscle actin, αSMA) and collagen (Massons Trichrome) shown on pancreatic tissue isolated from Genetic Engineered Mouse model of Pancreatic Cancer. Shown is normal wild type (WT), KRas expressing early stages pancreatic ductal neoplasia (KC) or KRas/P53R172H expressing PDA. Notably, the epithelia lose 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 subscription 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, enabling patients to 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.

TRANSCRIPTIONAL NETWORKS IN LUNG CANCER

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Lung cancer causes the most cancer-related deaths in the world and the main obstacles to a cure are late diagnosis and chemoresistance. The major interest in our group is to identify the causes behind lung cancer spread 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. MicroRNAs (miRNAs) are single stranded RNAs of 19-25nt in length, that 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.

To investigate whether KRAS, one of the most mutated oncogenes in lung adenocarcinoma, was able to modulate miRNAs expression, we overexpressed wild type and mutant KRAS ($KRAS^{G12D}$) in non-small cell lung cancer (NSCLC) cells. We identified two miRNAs, miR-30c and miR-21, significantly upregulated in wild type and mutant forms and showed that miR-30c and miR-21 induce cell proliferation and enhance migration/invasion by inhibiting crucial tumour suppressor genes. Systemic delivery of anti-miR-21 in combination with cisplatin in vivo suppressed the initiation of lung tumours in a mouse model of lung cancer.

A subset of lung adenocarcinomas is driven by the EML4-ALK translocation. While ALK inhibitors in the clinic lead to excellent initial responses, acquired resistance to these inhibitors due to on-target mutations, or parallel pathway alterations, represents a major clinical challenge. We discovered that EML4-ALK cells with acquired resistance to crizotinib, ceritinib or alectinib overexpress specific Cyclin Dependent Kinases (CDKs) and CDK inhibitors halt tumour growth and robustly induce apoptosis in this setting.

KRAS and non-coding RNAs

The proto-oncongene 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, although so far no targeted drug has demonstrated efficacy, at least not in the clinical setting. One of our goals was to identify K-RAS-modulated miRNAs that, by targeting molecules involved in the RAS pathway, can be employed 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 miRNAs in NSCLC. We showed that miR-30c and miR-21 are significantly upregulated by both KRAS isoforms and induce drug resistance and enhance cell migration/invasion through the inhibition of important tumour suppressor genes, such as RASA1 and RASSF8. 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 in plasma from the same patients. Systemic delivery of LNA-anti-miR-21 in combination with cisplatin in vivo suppressed the development of lung tumours in a $KRAS^{G12D}$-driven genetic mouse model of lung cancer. Mechanistically, we demonstrated that ELK1 is responsible for miR-30c and miR-21 transcriptional activation by direct binding to the miRNA proximal promoter regions (Figure 1). 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., 2018).

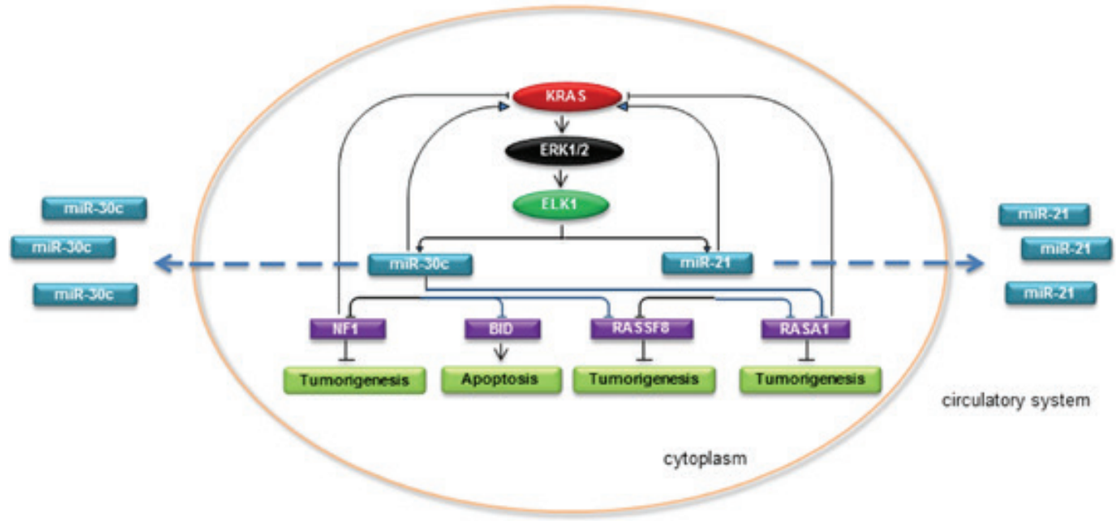

Figure 1. Working Model.
KRAS, through the transcription factor ELK1, activates miR-30c and miR-21, which in turn, by downregulating NF1, RASA1, RASSF8 and BID, regulates KRAS, NF-kB and the intrinsic apoptotic pathway, inducing lung tumorigenesis and inhibiting apoptosis in NSCLC. MiR-30c and miR-21 are released into the bloodstream and could be potential biomarkers for early NSCLC detection.

ALK-EML4 lung tumours

In NSCLC small molecule inhibitors for mutant kinases have offered unprecedented success in the management of disease. One of the most successful examples is Echinoderm Microtubule Like-4-Anaplastic Lymphoma Kinase (EML4-ALK)-mutant NSCLC, which affects 4-5% of lung cancer patients (Gainor et al., 2013). Several EML4-ALK inhibitors have already been approved by the FDA, namely crizotinib, ceritinib, alectinib, brigatinib and lorlatinib. Even though the objective response rate for the ALK inhibitors crizotinib and alectinib in the clinic surpasses 60%, patients typically develop resistance to these inhibitors and relapse soon thereafter (Hida et al., 2017).

In order to mimic the context of acquired resistance to ALK inhibitors in vitro, we utilised cell lines with acquired resistance to crizotinib (CrizR), ceritinib (CeritR) and alectinib (AlecR) by long-term exposure to these drugs. RNA-seq identified a cell cycle dysregulation in crizotinib-resistant cells, evidenced by an upregulation of CDKs and their partner Cyclins. Following this observation, we treated EML4-ALK drug-resistant cells with different CDK inhibitors which led to a rapid induction of apoptosis, while sparing normal epithelial cells, and exhibiting specificity towards EML4-ALK-mutant cells. We are further investigating whether these CDK inhibitors are well tolerated in vivo in mouse models.

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http://www.cruk.manchester.ac.uk/Our-Research/Translational-Oncogenomics

TRANSLATIONAL ONCOGENOMICS

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[1]Joined in 2018

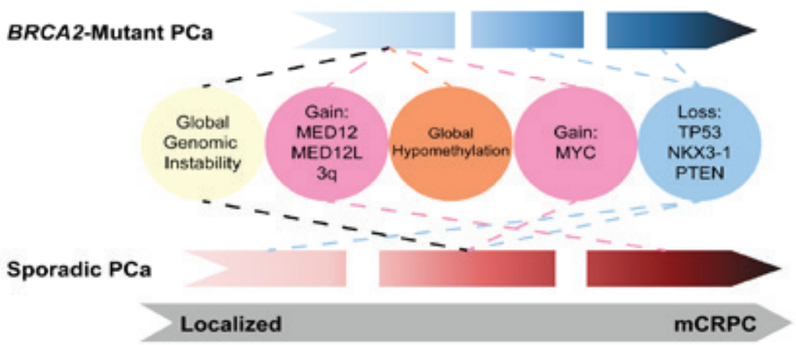
Defining aggressive features of localised prostate cancer

Control of genome stability requires careful coordination between cell cycle checkpoint control and DNA repair mechanisms. Defects in the repair of DNA double-stranded breaks have been associated with acquiring genetic instability, particularly in genes responsible for homologous recombination such as BRCA1 and BRCA2. Although germline mutations in DNA repair genes are rare in localised prostate cancer (PCa); mutations in the Breast Cancer susceptibility-2 (BRCA2) gene is the most frequent DNA repair defect observed and this confers an 8-9 fold increased risk of developing lethal prostate cancer and subsequent failure of standard of care treatment, shortening the overall cancer-specific survival to 5-8 years post diagnosis for 50% of patients, compared with >90% for stage-matched at diagnosis non-carriers (Castro et al. JCO, 2013).

Germline mutations in BRCA2: A model for localised aggression

Recently, our group, in collaboration with scientists in Melbourne, used whole genome sequencing to explore the genetic defects in tissues derived from untreated prostate cancers arising in men with familial BRCA2 mutations (Fraser et al. 2017, Taylor et al. 2017). We compared germline BRCA2-associated mutations to those found in sporadic prostate cancers and found a number of genetic changes associated with germline BRCA2 mutation. Surprisingly, these changes are generally not observed in untreated localised prostate cancer, but do occur in to metastatic castrate-resistant disease These included altered beta catenin-WNT signalling, defective mitotic control and DNA repair and altered androgen signalling. These findings suggest that in untreated BRCA2-associated prostate cancers, pathways are already upregulated that herald resistance to hormone therapy and genetic instability.

We now aim to determine the cancer cell signalling program of BRCA2-deficient PCa using non-malignant prostate epithelium and understand the cellular pathogenesis which gives rise to this disease. During the genesis of our lab this year we have employed CRISPR-Cas9 targeting of BRCA2 in human immortalised non-tumorigenic and prostate cancer cell lines and to this end have generated models of BRCA2 heterozygosity with isogenic controls. Since men with germline BRCA2 mutations are at high risk of developing a lethal disease we want to understand the role of BRCA2 loss in driving genetic instability and tumourigenesis. At present, there are very few cell lines that lack functional BRCA2, a fact that has hampered research into BRCA2 generally. This probably stems from essential roles of BRCA2 in DNA replication and mitosis. Our aim is to develop a panel of BRCA2-null prostate cell lines, using a strategy based on CRISPR-Cas9 technology to reversibly knockout the BRCA2 gene by means of a gene trap. We will build on this by generating murine and human prostate epithelium which are BRCA2 function-compromised and/or co-express secondary and tertiary mutations of interest to understand the molecular pathology of this disease and identify key vulnerabilities.



Taylor et al; Nature Comm, 2017

Figure 1:
Features of germline mutant BRCA2-associated prostate cancer.
Early in the development of BRCA2-Mutant PCa, the disease is characterised by amplifications in 3q, MED12, MED12L and MYC, global genomic instability, and a global hypomethylation profile. In comparison, these features are restricted to relatively advanced sporadic prostate cancers. Taylor et al., Nat Commun, 2017.

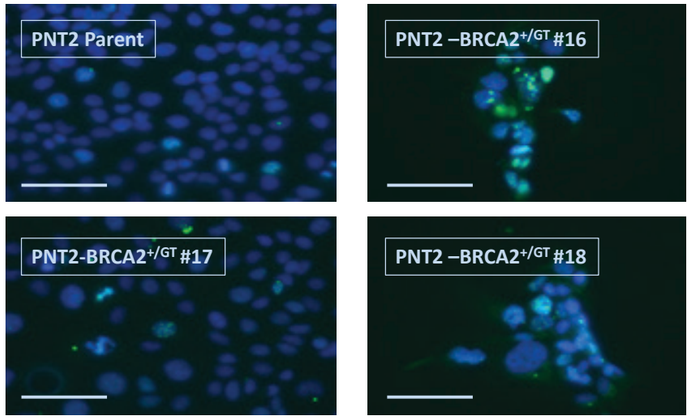


Figure 2:
Accumulation of DNA damage in cells heterozygous for BRCA2.
CRISPR/Cas9 gene editing technology was used to target a gene-trapping cassette to intron 7 of the BRCA2 gene in the prostate epithelial cell line, PNT2. Clones with integration of the gene trap were isolated by selection on Puromycin-containing medium. Inactivation of one BRCA2 allele by gene trapping (BRCA2+/GT) was associated with spontaneous accumulation of DNA damage during culture, as revealed by increased levels of gamma-H2AX (green signal), in three independent clones compared to the parental PNT2 cells. Magnification x400, bar 75 microns. Blue signal, DAPI.

Aggression in sporadic prostate cancer: clonal evolution and outcome

In a cohort of close to 300 localised prostate tumours, we detected multiple subclones in two thirds of patients and this specific subclonal architecture is associated with adverse clinicopathological features (Espiritu et al.Cell, 2018). Early tumour development is characterised by point mutations and deletions followed by later subclonal amplifications and changes in trinucleotide mutational signatures. Patients with low-risk monoclonal tumours rarely relapse after primary therapy (l7%), while those with high-risk polyclonal tumours frequently do (61%). In further bioinformatics analyses, we quantified hypoxia in 8,006 tumours across 19 tumour types using TCGA and ICGC datasets and observed that in ten tumour types, hypoxia was associated with elevated genomic instability. In aggressive localised PCa, hypoxia was associated with elevated rates of chromothripsis and genetic instability, particularly in polyclonal tumours. Further work using pan-cancer approaches showed that elevated hypoxia is associated with increased mutational load across cancers, irrespective of the underlying mutational class. Importantly, we observed an association between hypoxia and signatures associated with defective homologous recombination, DNA mismatch repair and base excision repair. This provides evidence that aggression is associated with a constellation of features that we term tumour *nimbosus*– an aggressive cellular phenotype in which co-incident hypoxia, genetic instability and aggressive sub-pathologies co-occur (Chua et al. Eur Urol, 2017). We are currently exploring whether nimbosus is operational in high-risk, locally advanced ad oligometastatic prostate cancers using both pre-clinical models (e.g. isogenic prostate cancer cells lines with DNA repair defects) and by characterising the genomes in clinical specimens derived from patients with aggressive PCa.

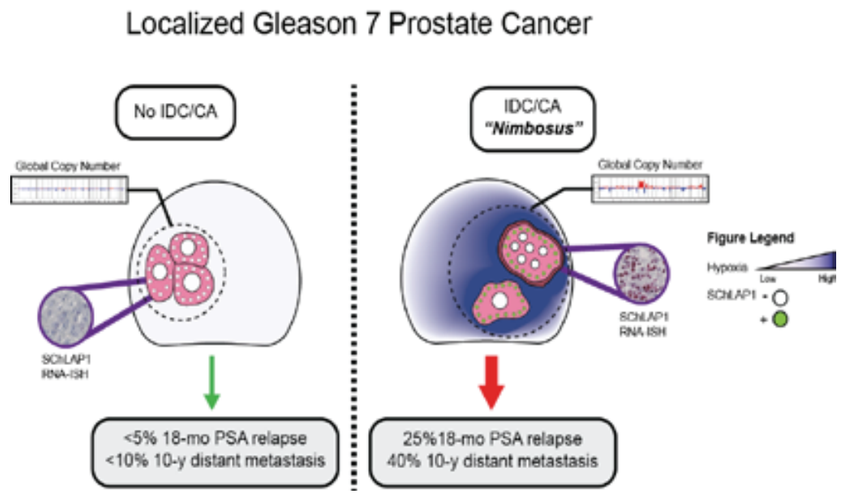


Figure 3:
Features of localised, aggressive prostate cancers.
A prostate cancer nimbosus, is associated with intraductal (IDC) and cribriform subpathologies, hypoxia and elevated SChLAP1. These patients are at high risk of disease recurrence and metastases compared to those without these features. CA = cribriform architecture; IDC = intraductal carcinoma; PSA = prostate-specific antigen; RNA-ISH = RNA in situ hybridisation. Chua et al, Eur Urology, 2017.

TUMOUR SUPPRESSORS

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The most frequently mutated gene in all cancers is the tumour suppressor p53 *(TP53)*. In our lab, we are interested in the different mutations in *TP53* and the functional consequences of these mutations in lung cancers. The mutation frequency for p53 in all cancers is about 60%, but can be variable in individual cancers. In non-small cell lung cancer (NSCLC) the frequency is about 30%, but in small cell lung cancer (SCLC) p53 is mutated in nearly all cases (90% or more). The vast majority of p53 mutations occur in the DNA binding domain of p53 (Figure 1). When looking at the mutation frequency in SCLC compared to all cancers, there are clear differences (Figure 1) that cannot be solely attributed to the 'smokers' mutation signature. In particular, mutations in the N-terminus, the C-terminus and at certain regions in the DNA binding domain of p53 are more prone to mutate in SCLC compared to all cancers (Figure 1). These data suggest selective advantages for these mutations to occur in SCLC.

Mutations in p53 can result in loss of p53 expression, or expression of mutant p53 proteins. Previous data from our lab has indicated that although the mutant p53 proteins often lose wildtype function, these are more harmful than a simple loss of p53 function/activity. Mutant p53 proteins gain the ability to inhibit the p53 family members p63 and p73 and the microRNA machine protein Dicer to promote invasion and metastasis. This inhibition leads to an enhanced recycling of integrins and growth factor receptors to the cell membrane, mediated by RCP (Rab Coupling Protein). We are following up on these data and are investigating a role for these proteins in mediating mutant p53-dependent chemoresistance.

Most recently we noted that mutant p53 proteins also gain the capacity to promote engulfment of neighbouring tumour cells (Mackay et al 2018) (Figure 2a). This was most often observed in heterogeneous populations of cells comprising p53 null and mutant p53 cells. Mechanistically, it mostly resembled the process of entosis, requiring ROCK activity and cell-cell adhesion molecules such as E-cadherin and beta-catenin. Upon engulfment of neighbours, p53 null cells were more likely to die as a result of replication stress. Mutant p53 cells were able to survive this stress, albeit at the cost of aberrant divisions and multinucleation. When implanted as co-cultures in recipient immune-compromised mice, the heterogeneous populations of cells, in which the highest numbers of engulfing cells were detected, grew faster. This suggests a tumour promoting role for cell engulfment. We investigated cell engulfment in tumours of about 300 lung cancer patients. In histology, engulfment can be seen as CIC (cell-in-cell) structures that resemble the appearance of engulfed cells in tissue culture (Figure 2b): an outside larger cell with a half-moon shaped nucleus that contains a 'rounded up inside cell' in a vacuole. As mutant p53 is often overexpressed in cancers, we stained the tissues for p53 and identified an association between mutant p53 staining and the presence of CIC, which was particularly apparent in tumours that had heterogeneous p53 staining. In this patient cohort, we could also identify a correlation between the presence of CIC and decreased survival (Figure 2c). In patients, we also noted that CIC was associated with multinucleation and aberrant mitotic structures, which generally are indicative of chromosomal changes. In collaboration with TracerX, we determined that CIC was indeed associated with genomic instability. Together these data demonstrate a role for CIC in promoting tumourigenesis. Future research will focus on further elucidating this role.

While analysing CIC, we found that in the same cell type, different GOF mutations of p53 have different potencies of cell engulfment. Similarly, when we provided our cell lines with these different mutants to the lab of H. Vakifametoglu-Norberg, Eriksson et al discovered differences in metabolism between p53 mutants regulating glycolysis (Eriksson, Mol Cell Biol, 2017). These data suggest that while many consider GOF mutants as similar, they can actually have dramatically different functional consequences to a cell. In our lab we are interested in these differences and are focussing on the different p53 mutations seen in SCLC. We have generated a library of frequent and less frequent SCLC p53 mutations and are testing these in functional assays in lung cancer cell lines. Pilot data revealed a number of differences that comprise loss of certain wildtype p53 functions as well as gain of other functions. We therefore aim to systematically characterise and categorise these mutations and compare these to SCLC patient characteristics. Several of the mutations we selected are present in CDXs (circulating tumour cell derived explants) of SCLC patients, which we plan to use to validate our results. These data could therefore provide evidence of functional differences in p53 mutations in SCLC.

Finally, we identified that specific metals can change the function of wildtype p53 and make it behave like a mutant p53 by promoting invasion and metastasis or interacting with mutant-specific interactant proteins. Smokers' lungs are exposed to a variety of metals and in many cancers increased metal levels can be detected. We characterised the mechanistical changes in p53 that occur upon metal exposure and are currently validating our findings in patients and mice. For the mouse studies, we will use iRFP (infra-red fluorescent protein)-expressing stable cell lines for heterotopic xenografts, with which we can monitor and count micrometastases to the lung (Figure 3). These data will provide important information between p53 functions and changes in extracellular environments, influencing/promoting tumorigenesis.

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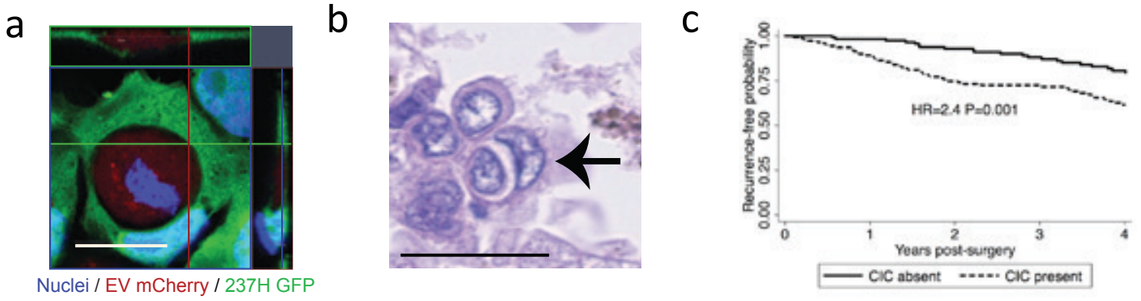


Figure 2.
a) Z-stack image of a cell-in-cell structure in H1299 cells; green: H1299 cells stably expressing mutant p53 and GFP, red: H1299 cells stably expressing an empty (EV) control plasmid and mCherry, blue: dapi. Top and left panels are showing 3D vertical and horizontal cross sections of the Z-stack.
b) Cell-in-cell structure in non-small cell lung cancer.
c) Survival of lung cancer patients based on the presence of CIC structures.

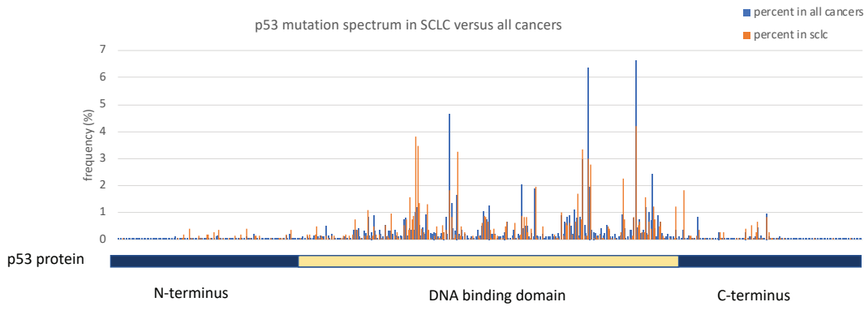


Figure 1.
p53 mutations frequency in SCLC compared to the frequency of all cancers (based on TCGA data)

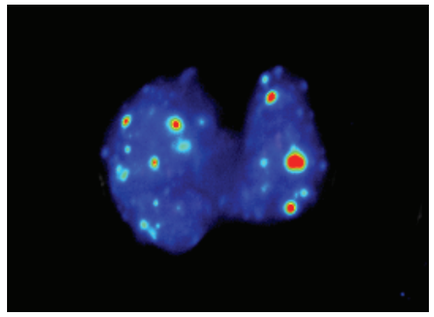


Figure 3.
Infra-red fluorescent image of micrometastases in the lung of iRFP H1299 cells injected intravenously in NSG mice

CANCER RESEARCH UK MANCHESTER INSTITUTE

RESEARCH SERVICES

RESEARCH SERVICES

Chief Laboratory Officer
Stuart Pepper

During the last year there have been several significant investments in the core facilities and it has been great to see the continued development of new services as the core facilities have settled into new laboratory spaces at Alderley Park.

Chief Laboratory Officer
Stuart Pepper

The recent purchase of a CyTOF system in the Advanced Imaging and FACS facility has been a great example of close partnership between the Systems Oncology group and the core facility, leading to the establishment of this exciting technology as a service available to all groups. Two other new platforms have also been procured to support our Biological Mass Spectrometry facility. Our older Orbitrap instrument did not survive the fire in the Paterson Building and so needed to be replaced, and a strategic decision was made to also upgrade the Q-Exactive. The addition of two new instruments to our Mass Spec facility means that we now have state of the art equipment to support a broad range of protein analysis applications. A final new technology to mention is the 10X Genomics Chromium platform which was installed in our Molecular Biology Core Facility this year. This was bought primarily to support single cell RNA sequencing, but will also be used for single cell CNV mapping and ATAC sequencing.

The ever increasing range of equipment generating large and complex data sets puts greater demands on our Scientific Computing Facility. Following a successful relocation of our HPC infrastructure, much of the focus over the last year has been on increasing the stability of all aspects of the system not just to benefit all the users of the service, but such that a growing number of clinical pipelines can be hosted and operated to the standard required for GCP compliance. As part of this work an extra 1.4PByte of storage to enhance our backup capacity has been added to the system.

Across the Institute there are three teams supporting in vivo research that include production of novel transgenic mice, breeding of a wide range of transgenic strains and a facility to support experimental procedures. All three teams have adapted to new working arrangements with the breeding facility delivering mice on a twice weekly basis to the Alderley Park site. With all in vivo work the Institute is highly committed to the principles of 3Rs, and we were very pleased when one of our staff was invited to speak at the NC3Rs oncology workshop this year.

Laboratory services have adapted to working across two sites, with the main function based at the Oglesby Cancer Research Building and some reagents transferred to Alderley Park by our Logistics team.

Looking back at 2018 it is good to see how quickly the various specialist facilities have settled into the new space at Alderley Park, and have maintained momentum for continual enhancement of the technology platforms available to support the research programs of the Institute.

Advanced Imaging and Flow Cytometry

Steve Bagley, Jeff Barry, Michele Fresneda Alarcon[1], Antonia Banyard, Helen Carlin, Jack Eastham[1], Isabel Peset Martin, Heather Woodhouse, Kang Zeng

[1]Joined in 2018

The Imaging and Cytometry facility's remit is to provide state-of-the-art tools for both the fundamental and translational study of cancer – from molecular interactions in primary cells through to tissue-wide responses. Taking our lead from the requirements of the group leaders and their research, the facility is able to adapt as the team responds by introducing new equipment, methods and workflows at both Alderley Park and at the Oglesby Cancer Research Building (formerly the MCRC Building). Technologies include microscopy (confocal and super resolution), high content screening, histological imaging and flow cytometry sorting and analysis.

The introduction of Mass Cytometry (CyTOF) has been a major initiative this year; the technology is based on both flow cytometry coupled with time of flight mass spectrometry, which permits the inquiry of protein expression on a single cell basis. Antibodies are labelled with rare earth heavy metals rather than light emitting fluorochromes in traditional flow cytometry – consequently crossover of signals and detection of discrete labels becomes easier to achieve. Working alongside the Systems Oncology group, panels have been formed for human and murine to assist researchers in adopting this technology. The introduction of this technology has been quite an undertaking, however the advantage of being to be able detect over 50 distinct events per cell leads to functional and phenotypic assays of the tumour niche.

Histological whole slide scanning and analysis was a process first introduced into the facility in 2008. Over this time an array of instruments and software have been developed to provide data at the single cell or across the whole tissue. These efforts have been made in conjunction with the Histology facility – both facilities working together can identify the correct tools for the whole histological workflow. In the last year the software HALO has been introduced for histological image analysis; this permits a range of topographical and single cell analysis techniques such as those enumerating RNA and gene expression, cell and object-based analysis, along with bioinformatics for histology data. Research potential is to be extended in the coming year with the purchase of a replacement whole slide imaging system, which will permit brightfield, darkfield, polarised and fluorescence imaging.

The facility data load year on year is on the increase and so with the laboratory's relocation this has provided an opportunity to investigate all of our workflows. Consequently, we are working closer with the Scientific Computing team who handle all of our live data.

In the next year we will also see a major change to the facility as Imaging and Flow Cytometry become separate facilities. Jeff Barry will be heading the Flow Cytometry facility whilst the Imaging facility will develop new modalities. Initiatives are planned for analysis and distribution of data along with increased support of assisting in preclinical image analysis.

Biological Mass Spectrometry Facility

Duncan Smith, Yvonne Connolly

2018 has been a year of change for the Mass Spectrometry facility. In Q1 2018, we moved into Alderley Park tasked with relocation of the three tandem Mass Spectrometers previously situated in the Paterson Building. This has involved re-location, installation, testing, repair and instrument performance testing to ensure the hardware could perform at their previous levels. After extensive efforts, one system was found to be beyond repair and another is in the final stages of testing. It has been necessary to outsource MS provision for the vast majority of 2018. The Institute submitted a tender for two new Mass Spectrometers in Q3 2018 and successfully purchased an Orbitrap Lumos and Q-Exactive HFX, two state-of-the-art proteomics platforms with superior performance capabilities to anything CRUK MI has had access to previously. Excitingly, both systems were installed at AP in November 2018. The new systems represent the very best platforms available for proteomics. The first service provision samples were run on the new systems in late December 2018. We are now establishing our portfolio of workflows on the new hardware that will bring an end to the need to outsource MS provision over the course of Q1 2019.

Biological Resources Unit

Transgenic Breeding
Team Leader: Jen Hughes[1], Kim Acton[2]

[1]Joined in 2018 [2] Left in 2018

The BRU Transgenic Breeding Team breeds mice to meet the requirements of CRUK MI researchers. Ten staff members currently provide day-to-day care for 133 different transgenic mouse lines spread across approximately 2,400 cages in a facility located within the main University campus. Services offered include rederivations using fresh and frozen sperm and embryos, pairing mice for breeding, monitoring timed matings, recording and weaning litters, ear snipping for identification and genotyping, managing the genotyping service (using an external service provider), translating and transferring genotyping results, monitoring tumour prone lines for onset of symptoms and cryopreservation of lines that can be archived. In accordance with Home Office requirements the mice are closely monitored in order to ensure high welfare standards. The 3Rs (Replacement,

Reduction and Refinement) initiatives undertaken in the last year include adapting and standardising the ear snipping process and grouping male mice at time of weaning where possible, so that the numbers of singly housed males are reduced.

The last year has been a busy one with 39 new breeding lines being started and 19 lines being closed. The breeding facility is housed in a clean unit with a high health status and is kept free from common mouse pathogens, which means that new transgenic mouse lines from external sources cannot be brought in directly as live mice. New lines coming from external sources instead have to be transferred in as either embryos or sperm and thoroughly health screened in order to ensure that the resulting offspring are pathogen free. When live mice are sent they are housed in quarantine facilities during the rederivation process.

Five of the 39 new lines have been rederived, using embryos and live mice sent from Singapore, USA, London, Birmingham and Manchester. Another 8 of the new lines have been transferred from the Transgenic Production Facility and the remainder have been generated by crossing mice from existing lines.

Mice are transferred on request in twice weekly shipments to the BRU Experimental Team at Alderley Park. After transfer a minimum of 1 week acclimatisation is required before mice can be enrolled in experiments. As well as shipping to Alderley Park, this year we have also shipped mice to USA, Spain and the University BSF facility.

One of our main aims for 2018 was to source specialist mouse management software in order to improve work flow, reduce errors and allow information to be accessed more easily. The procurement process has now been completed and we are beginning to implement actions necessary for the changeover which will be completed in 2019.

Biological Resources Unit

Experimental Services
Team Leader: Lisa Doar

Moving to Alderley Park and having everyone back together was a great morale boost for the team. Throughout the year our workload has increased steadily and by Q3 was back to pre-fire levels – this is quite an achievement and testament to how hard the team have worked.

The cages at Alderley Park are smaller than the ones we previously used, meaning we have to clean the mice out every week rather than fortnightly as we were doing in the past. We have therefore had to recruit an extra two temporary posts to match the increasing workload.

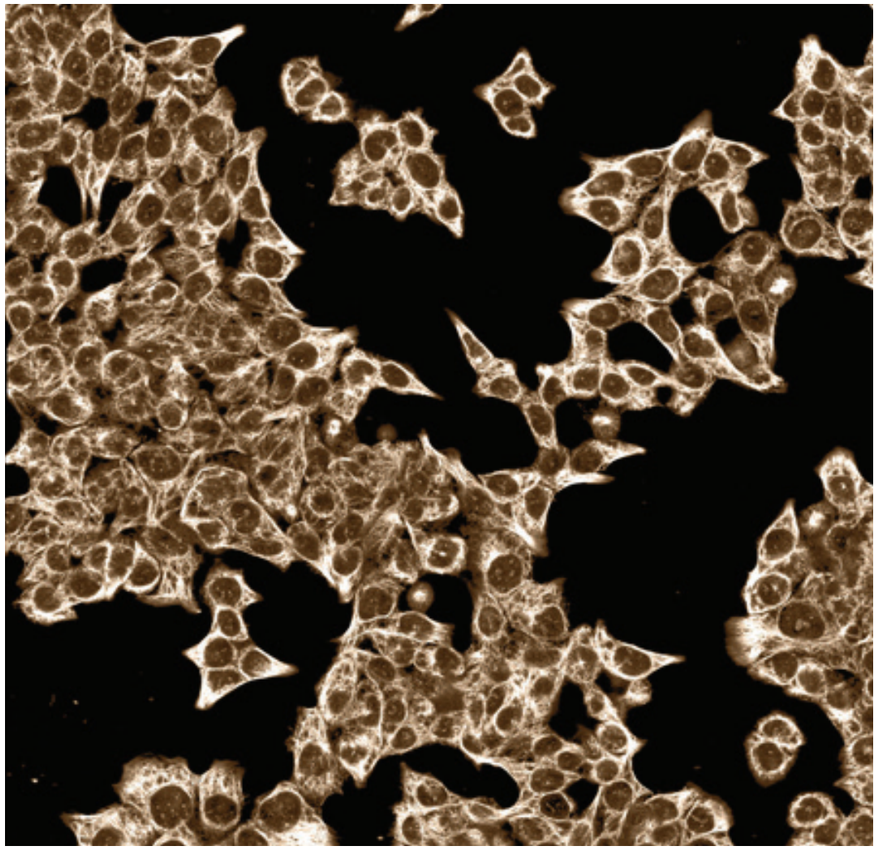
Our ultrasound imaging machine was water damaged during the fire so we have purchased a newer model which is more user friendly than the original one and with better image quality and the ability to produce 3D images much faster. Consequently demand for ultrasound work has increased greatly and is now used by a range of research groups to monitor pancreas, bladder and prostate tumours, as well as looking at spleen inflammation and liver metastasis. We have also used the kit to successfully carry out image guided injections into tumours in the bladder for the first time ever.

Another piece of equipment we had on trial in December 2018 is the 'Pearl' by LiCor – this is an optical imaging modality which uses near-infra-red fluorescence to monitor tumour development. The issue with general fluorescence imaging is that many of the tissues in the mice auto-fluoresce at the same wavelength we are trying to look at, generating more background noise. This system uses a slightly different wavelength so we should get a much better signal. We are comparing the three systems we have available so the results should be really interesting.

Surgical work has been a big focus this year and we have spent a lot of time implementing improvements and training licensees in these techniques. One key area has been looking at alternative forms of wound closure which are more comfortable for the mouse. We have been moving towards using glue or sutures rather than wound clips as they can very easily be put in too tight and cause skin-healing issues. We have set up several new surgical models across the research groups this year, including an orthotopic eye model and an orthotopic bladder model. We have also introduced refinements to our orthotopic mammary fat pad model so we can now carry this out non-surgically. In September, the NC3Rs invited us to talk at a symposium they were running in collaboration with CRUK where the focus was on setting up new cancer models and skill-sharing between institutes. Joanne Roberts and Janet Watson presented the talk and discussed these particular three models, generating much interest and discussion points.

The Zeiss AiryScan microscope can capture many more cells at high resolution simultaneously. Here, HCT116 colon cancer cells are stained for their microtubule cytoskeleton. Cells are undergoing division, migration and clustering, allowing cell-by-cell examination of the effects of individual proteins or drug targets.

Image supplied by Andrew Porter (Cell Signalling)



Transgenic Production Facility

Natalia Moncaut, Mark Willington, Athina Papaemmanouil

The Transgenic Production Facility (TPF) offers a comprehensive service in the generation of genetically modified mouse lines using the CRISPR technology. The Facility is responsible for the design of the best targeting strategy and for the production of all the reagents required for the process.

Generating mouse models remains instrumental in revealing the complexities of human cancer biology. Working together with different research groups at the Institute, this last year we have successfully produced new mouse lines including the conditional and constitutive knock-outs of specific genes involved in different aspects of the human disease. Also we generated new strains carrying patient-specific point mutations and larger knock-ins. New projects have been started involving different transgenic approaches like targeting mouse embryonic stem cells or random transgenesis. The TPF is also responsible for the design of targeting strategies using the CRISPR technology for in vitro applications, such as targeting different cell lines. One interesting project we are involved in is in vivo targeting to produce cancer mouse models. This strategy will bypass the generation of new lines along with their associated time and breeding costs.

The field of animal transgenesis is evolving in an unprecedented manner with more efficient and precise tools to make the technology more versatile and available to any application. TPF is regularly participating in annual meetings and courses in order to keep up with the latest advances in this technology. Together with other transgenic facilities within the UK, we are organising a series of technical workshops to build an active community of transgenic technologists.

Histology

Garry Ashton, Caron Abbey, Keren Dawson[1], Janice Kerrigan[2], Katherine Lally[1], Marta Madureira da Graca, Usman Mahmood, Emma Watson, Deepti Wilks (Haematological Malignancy Biobank)

[1] Joined in 2018 [2] Left in 2018

The Histology facility continues to underpin the research activities of a large number of both basic and translational research groups within the CRUK MI. It allows the adoption of tissue-based experimental approaches to all research programmes. The unit's remit is to offer a full range of both routine and advanced histological services for oncology research. As the range and complexity of the services offered continues to grow, the training and continued professional development of staff has ensured the unit continues to offer a comprehensive and flexible service at all times.

The core facility is now housed at Alderley Park with a small satellite lab offering routine histology services and immunohistochemistry also based within the Oglesby Cancer Research Building (formerly the MCRC Building). Two new scientific officers have also been recruited within the year.

Histology routinely processes, embeds and sections both human and mouse tissues in addition to organotypic assays, spheroids, agar plugs and cell pellets. Vibratome sections of 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 unit 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 have also been collected and processed. The samples are of the highest quality ensuring maximum value to any research program.

The high throughput routine immunohistochemistry service, troubleshooting and antibody validation services have once again seen exceptional demand. In addition the unit has incorporated sophisticated labelling techniques into routine practice. mRNA in situ hybridisation and multiplex immunohistochemistry are labelling techniques used by several research groups. Multiplexing using both mRNA in situ hybridisation and protein immunohistochemistry on single tissue sections is also now in routine use.

Laser capture microdissection followed by the downstream extraction of both RNA and DNA, giving sufficient quantity and quality for NGS from relatively small amounts of material, is now routine and has seen a large increase in demand. The evaluation of several commercial extraction kits has been undertaken whilst the development of laser capture microdissection together with immunohistochemistry is ongoing.

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

One interest of the Stem Cell Biology group is to study the development of the haematopoietic system and identify new regulators of this process. Histology has been instrumental in characterising some of these newly identified regulators by performing section and whole-mount staining of mouse embryos. The facility has also optimised the combination of in-situ hybridisation (ISH) staining and multiplexed IHC using fluorescent labelling for this project. In addition we have provided extensive support in the development of multiplexed IHC antibody panels (up to 5 fluorescent labels). These panels have been applied to a variety of tissue samples, including mouse prostate organoids and urogenital systems, and human patient tissue microarrays in order to characterise the expression of RUNX1 in the context of other markers. The unit continues to be used heavily by the CEP Preclinical and IHC Biomarkers teams. CDX models are phenotyped routinely on our automated IHC platforms ensuring consistency, reproducibility and standardisation.

Molecular Biology Core Facility

Wolfgang Breitwieser, Andzhela Abu Rashed, Chris Clark, Gillian Williams[1], Amy Priestman, Rachel Horner, and John Weightman

[1] Left in 2018

The Next Generation Sequencing (NGS) services offered by the Molecular Biology Core Facility (MBCF) provide a critical component to the Institute's scientific infrastructure. For a number of years the NGS service has been supported mainly by three platforms; Illumina's HiSeq2500, NextSeq500 and MiSeq. Methodologies include sequencing of whole genomes, whole exomes, transcriptomes, PCR amplicons, as well as interrogations of protein-DNA interactions (e.g. ChIP-Seq), and chromosomal architecture (4C-, HiC-Seq). The increasing demand for the technology is paralleled by a continuously growing range of methods and the service has been striving to develop, validate, and improve NGS workflows, including novel methods for ChIPSeq (NEBNext UltraII), analysis of immune repertoire (Immunoseq), and improved genome and transcriptome analysis of formalin fixed samples (SureselectXT, RNADirect).

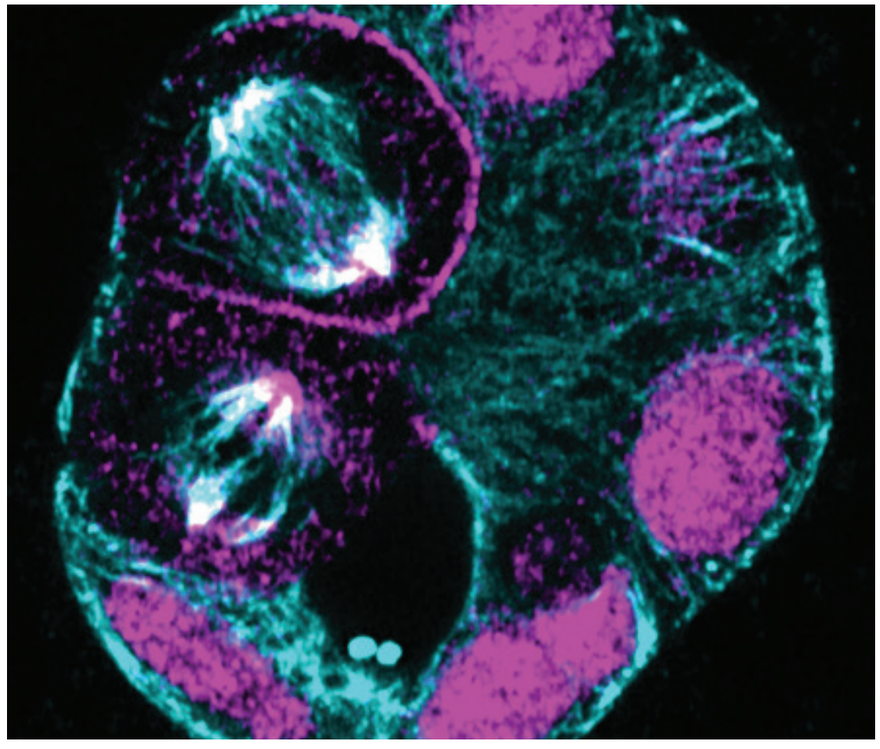
A rapidly developing area in the NGS field is single cell (sc) analysis. Supported by a set of state-of-the-art automation platforms (Echo, Mantis, Bravo), MBCF has developed workflows for high throughput methodologies for sc-RNA sequencing (Smartseq2, Celseq2). In addition we have installed a 10XGenomics Chromium platform for automated single cell encapsulation to support sc-RNA sequencing and genomic analysis including sc-CNV and sc-ATAC Seq. As a result, MBCF now has the capabilities to rapidly process hundreds to many thousands of single cells for transcriptome and genome interrogations. These types of single cell analyses are rapidly gaining traction as methods of choice in immunological and cancer biology investigations and consequently, over the last year we have processed an ever increasing number of single cell NGS projects from a diverse set of tissues, including blood, bone marrow, xenografts, and tumour biopsy material.

Another exciting development in the NGS field is presented by long read sequencing. MBCF has been an early adopter of this technology and we have validated a number of workflows using Oxford Nanopore's MinION platform for genome, transcriptome, and targeted sequencing. In combination with the high throughput capability and accuracy of Illumina's short read technology, we have been exploiting long read information using Nanopore sequencing for, e.g. the interrogation of chromosomal rearrangements, or improved accuracy in determining transcript splice variants.

A recent addition to MBCF's services has been the provision of bespoke chemical compound libraries for drug screening purposes. This service is supported by an automation set up for

Some proteins undergo major changes in localisation during the cell cycle. NuMA (magenta), contained in the cell nucleus until the start of cell division, will move to the mitotic spindle (cyan) poles. NuMA concentration increases to anchor microtubules for correct orientation of the mitotic spindle and separation of chromosomes. Imaged on the Zeiss AiryScan super-resolution microscope.

Image supplied by Andrew Porter (Cell Signalling)



high throughput compound dispensing (Access, Echo). Using these automation platforms we also support high throughput compound dispensing, and combinatorial dosing experiments. As more researchers recognise the benefits of automated compound dispensing, the service is experiencing an increasing number of complex projects. The recent installation of an instrument specific combination screening software thus allows us to program work flows for complex plate layouts.

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, Kevin Doyle[1], Jack Heal[2], Rishi Ramgolam[1], Neil Venables[2], ZhiCheng Wang

[1]Joined in 2018 [2]Left in 2018

The Scientific Computing core facility (SciCom) faced both challenges and opportunities in 2018. It started well with Rishi Ramgolam joining the team in January. He is a software developer with a focus on implementing bioinformatic analysis pipelines for clinical trials. Kevin Doyle started in November as Linux Systems administrator and will work on the infrastructure for running virtual servers. Sadly, the software architects Neil Venables and Jack Heal left SciCom in September 2018, but not before finishing their work on a new web framework called Octopus.

Octopus is an easy to use web frontend that contains several modules for the pre-processing of sequencing data. Currently, modules for pre-processing Illumina and Nanopore sequencing data and a 10x Genomics module are based on the Cell Ranger software for single cell DNA sequencing. Pre-processing includes steps for demultiplexing as well as automated quality and contamination checks. The modules were implemented by SciCom in close cooperation with the Molecular Biology Core Facility and the CEP NAB team. Great progress has been made regarding the implementation of the bioinformatic mutation calling pipelines for the TARGET trial. The focus in 2018 was on increasing the stability, the reproducibility and portability of the pipelines, using technologies like Linux containers. These containers allow the creation of defined software stacks, so that the pipelines can easily be ported on cloud or standby High-Performance Computing systems during a Disaster Recovery Process. The different versions of the pipelines for TARGET are automated, so that they can be integrated as a module into the Octopus framework. This will greatly simplify their usage and improve reproducibility by reducing the chance for human errors. New precision medicine solutions are being developed in cooperation with the TARGET team and digital ECMT that allow the loading of sequence data into cBioPortal, which enable the uploading of patient's tumour biopsy and circulating tumour DNA sequence data into cBioPortal and REACT (REal-time Analytics for Clinical Trials). The results of this work are also beneficial for other projects including those that can inform regarding the genomic status of a patient.

A novel platform for analysing, visualising and managing data from in vitro screening assay technologies based on the Genedata Screener® software has been implemented for DDU by SciCom. The software and its components were installed on our virtual server infrastructure in close cooperation with Genedata, Advanced Imaging and IT core facilities. The installation serves as a prototype to further data integration projects. A bioinformatic analysis pipeline for high content single cell analysis was successfully implemented with CEP and the University of Southern California's Convergent Science Initiative in Cancer. The standardised components of the compute intensive pipeline are created by Peter Kuhn's lab at USC and run on the SciCom virtualisation infrastructure.

In 2018 the re-installation of the SciCom High Performance Computing (HPC), virtualisation and storage infrastructure were fully completed. We used this opportunity to optimise hardware designs and software configurations as well as the introduction of new cluster utilities to improve the stability, manageability and efficiency of the systems. For instance, the introduction of the new workspace concept has led already to a more efficient use of cluster's storage resources, since unnecessary data is automatically cleaned up. The security and stability of the cluster could be further improved through a comprehensive Linux operating system and batch system upgrade on the Phoenix HPC cluster, which was carried out at the end of the year. Finally, additional 1.4 PByte storage and backup capacity for the SciCom research storage were purchased in December. This storage upgrade and the purchase of new hardware for running compute intensive virtual servers in 2019 allows the implementation of new integrated data analysis methods, fully exploiting the potential of the high-speed access to a shared central storage system

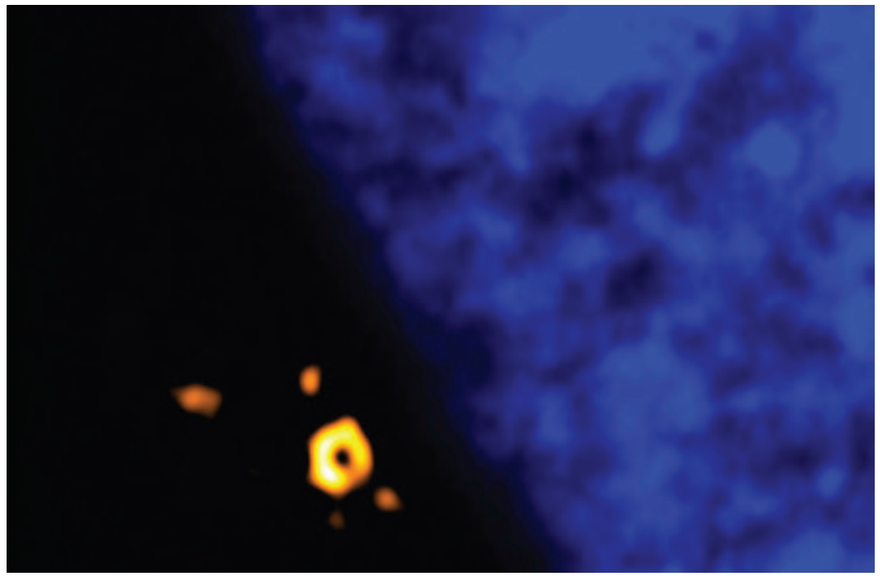
Computational Biology Support

Hui Sun Leong[2], Sudhakar Sahoo, Samuel Taylor, Pieta Schofield, Nitin Sharma[1]

[1]Joined in 2018, [2]Left in 2018

The Computational Biology Support Team provides expertise in the analysis of large biological data sets that originate from high throughput technologies such as Next Generation Sequencing, Mass Spectrometry, and Advanced Bio-imaging. Computational Biology Support is responsible for the initial pre-processing and analysis of data sets generated by the Institute's Core Facilities, and also for providing bio-statistical support to research groups. For this the Service draws on the most advanced methodologies for analysis of DNA-, RNA-, ChIP-seq, proteomics and bio-molecular imaging. The team utilise open-source as well as custom-built software tools running on the on-site high performance computing facility and we also contribute to the building of automated workflows in data processing. For example, we have also been involved in developing models and algorithms using R code to study the survival of patients across a number of cancer types. The team's other contributions include the development of gene signatures to predict outcomes of patients, e.g. in lung carcinoma. In addition, we help to interrogate publically available data resources, e.g. The Cancer Genome Atlas (TCGA) or the Database for Genotypes and Phenotypes (dbGaP). To aid this process we have drafted guidelines to help facilitate access for the Institute's investigators to open as well as restricted information, e.g. gene expression information, and de-identified clinical and demographic data.

In 2018 the CBS team contributed to a number of high profile publications. In one study we supported the bio-statistical analysis of histone methylation changes upon pharmacological intervention in AML cells (Maiques et al., Cell Reports 2018). Our contribution included evaluation of ChIPSeq data as well as Gene Set Enrichment Analysis (GSEA). In a separate investigation on tumours that lacked known driver mutations, we interrogated these tumours for the presence of chromosomal abnormalities and copy number gains using Whole Genome Sequencing, and using Whole Exome Sequencing for the identification of actionable mutations (Torres Ayuso et al., NPJ Genomic Medicine 2018). In a study of microRNA mediated tumour suppression, we undertook miRNA expression analysis of TCGA datasets from lung adenocarcinoma (LUAD) samples as well as tumour cells (Shi et al., Cell Death & Disease 2018). Current projects involve for example the analysis of p53 transcriptional network in non-small cell lung cancer, lncRNA-mediated transcriptional and post-transcriptional regulation of metastasis in lung cancer, and KRAS copy number association with worsened prognosis in pancreatic cancer.



Super-resolution microscopy probes the structure of cells in new ways. Here, Pericentrin (orange) forms an almost perfect ring around the centrosome, a complex structure that helps organise the microtubule cytoskeleton of the cell. The centrosome is in close proximity to the cell nucleus (blue), where the mottled pattern gives a hint at the structure of the chromosomes inside.

Image supplied by Andrew Porter (Cell Signalling)

CANCER RESEARCH UK MANCHESTER INSTITUTE

PUBLICATIONS AND ADMINISTRATION

Multiplexed immunofluorescent staining of an ex-vivo model of prostate development. Image scanned on the Leica Aperio Versa.

Image supplied by Renaud Mevel (Stem Cell Biology)

RESEARCH PUBLICATIONS

Cancer Inflammation and Immunity (page 14)
Santiago Zelenay

Refereed research publications
Böttcher JP, Bonavita E, Chakravarty P, Blees H, Cabeza-Cabrerizo M, Sammicheli S, Rogers NC, Sahai E, Zelenay S, Reis e Sousa C. (2018)
NK Cells Stimulate Recruitment of cDC1 into the Tumor Microenvironment Promoting Cancer Immune Control. ***Cell***, 172(5):1022-1037.

Other publications
Bonavita E, Pelly VS, Zelenay S. (2018)
Resolving the dark side of therapy-driven cancer cell death. ***Journal of Experimental Medicine***, 215(1):9-11.

Cell Signalling (page 20)
Angeliki Malliri

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Woroniuk A, Porter A, White G, Newman DT, Diamantopoulou Z, Waring T, Rooney C, Strathdee D, Marston DJ, Hahn KM, Sansom OJ, Zech T, Malliri A. (2018)
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Other publications
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Compartmentalisation of RAC1 signalling. ***Current Opinion in Cell Biology***, 54:50-56.

Clinical and Experimental Pharmacology (page 22)
Caroline Dive

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Torres-Ayuso P, Sahoo S, Ashton G, An E, Simms N, Galvin M, Leong HS, Frese KK, Simpson K, Cook N, Hughes A, Miller CJ, Marais R, Dive C, Krebs MG, Brognard J. (2018)
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Drug Discovery (page 26)
Caroline Springer/Donald Ogilvie

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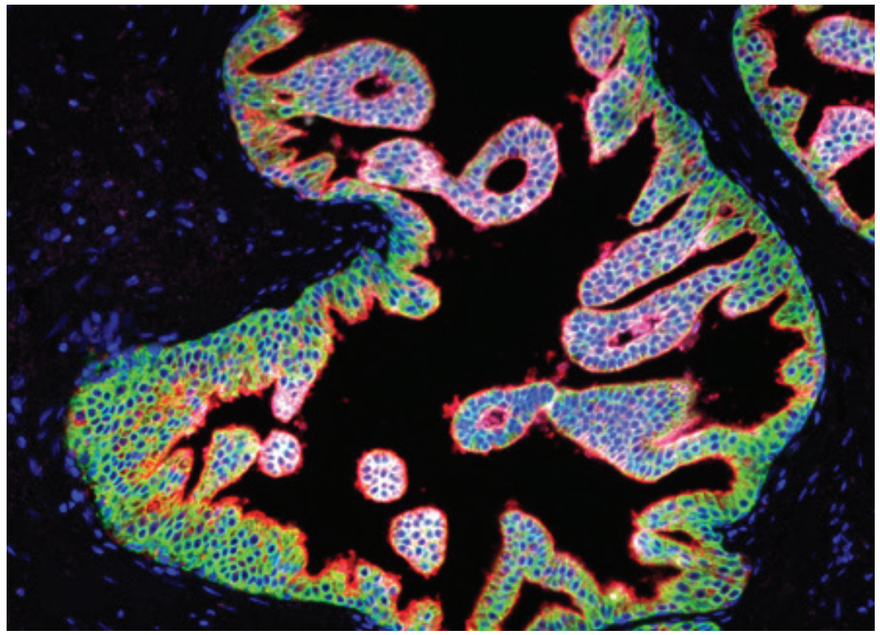
Image of focal expression of the progenitor marker LY6D (magenta) in the human prostate.

Image supplied by Ivana Steiner (Prostate Oncobiology)



Head and Neck Cancer Biology (page 28)
Robert Metcalf

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Leukaemia Biology (page 30)
Tim Somervaille

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Molecular Oncology (page 32)
Richard Marais

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Prostate Oncobiology (page 34)
Esther Baena

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RNA Biology (page 36)
Crispin Miller

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Skin Cancer and Ageing (page 38)
Amaya Virós

Refereed research publications
Trucco LD, Mundra PA, Hogan K, Garcia-Martinez P, Viros A, Mandal AK, Macagno N, Gaudy-Marqueste C, Allan D, Baenke F, Cook M, McManus C, Sanchez-Laorden B, Dhomen N, Marais R.
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Stem Cell Biology (page 40)
Georges Lacaud

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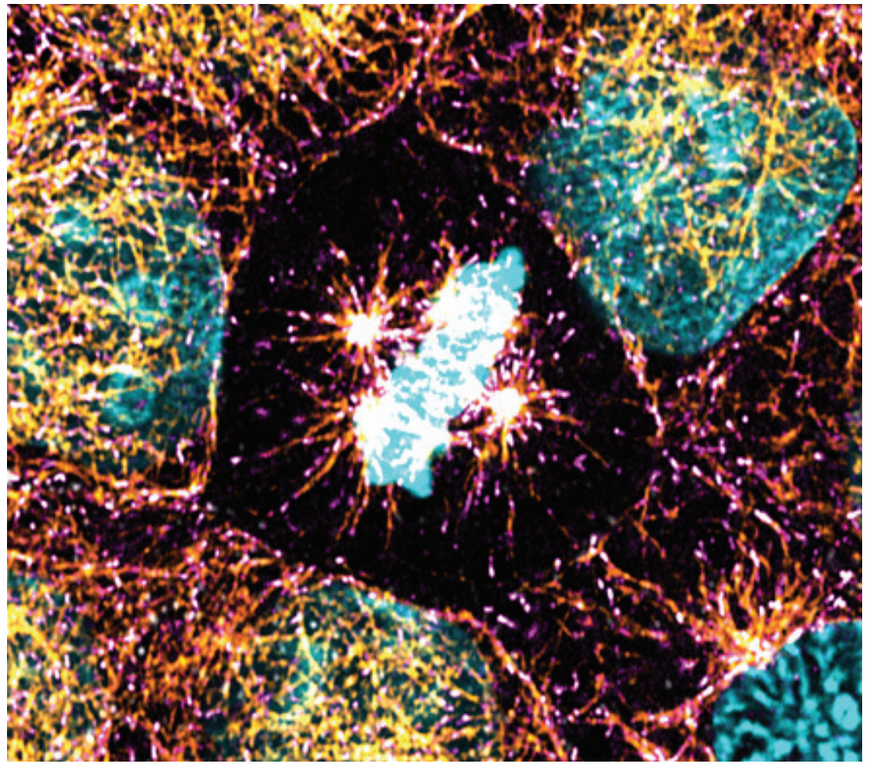
Martinez-Soria N, McKenzie L, Draper J, Ptasinska A, Issa H, Potluri S, Blair HJ, Pickin A, Isa A, Chin PS, Tirtakusuma R, Coleman D, Nakjang S, Assi S, Forster V, Reza M, Law E,

Microtubules (orange) give structure to cells and form the basis of the mitotic spindle in dividing cells (centre cell), which help segregate chromosomes (cyan) into two new daughter cells. Protein EB1 (magenta) is seen at the growing tips of the microtubules. In the bottom right, a dense patch of microtubules marks the centrosome location in a cell at an earlier phase of division.

Image supplied by Andrew Porter (Cell Signalling)



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Transcriptional Networks in Lung Cancer (page 44)

Michela Garofalo

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Tumour Supressors (page 48)

Patricia Muller

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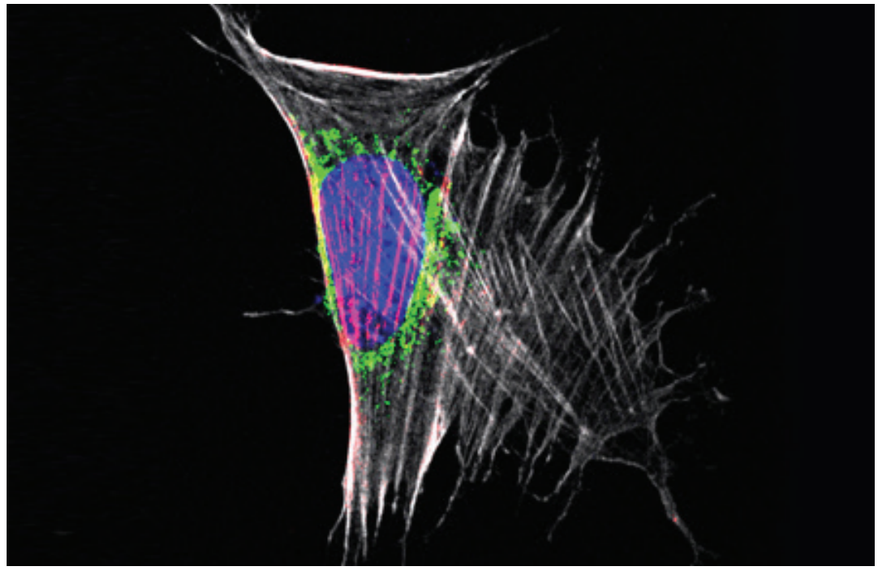
STEF-depleted cell showing nucleus (blue) with active Rac1 (green) targeted to the nuclear membrane where it has restored the cables of the actin cap (red). Other actin structures are shown in white.

Image supplied by Andrew Porter (Cell Signalling)



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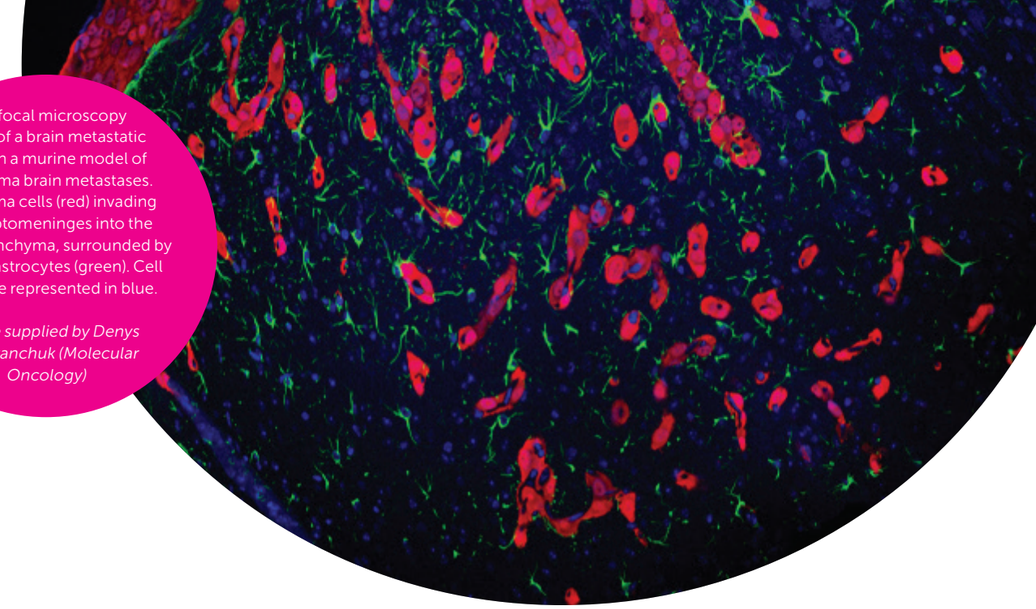
EXTERNAL SEMINAR SPEAKERS 2018



Confocal microscopy image of a brain metastatic lesion in a murine model of melanoma brain metastases. Melanoma cells (red) invading from leptomeninges into the brain parenchyma, surrounded by resident astrocytes (green). Cell nuclei are represented in blue.

Image supplied by Denys Holovanchuk (Molecular Oncology)

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. Postdoctoral researchers and other scientists at the Institute also give weekly seminars which are very well attended and help to integrate the entire cancer research efforts of the Institute.

Julia Newton-Bishop
University of Leeds

Holger Bastians
Goettingen Center for Molecular Biosciences (GZMB)

Dominique Bonnet
The Francis Crick Institute

Ingo Ringshausen
University of Cambridge

Stefan Fröhling
German Cancer Research Center

Balca Mardin
BioMed X Innovation Center

John Le Quesne
University of Leicester

Ravid Straussman
Weizmann Institute of Science

Dr Nicholas McGranahan
UCL

Andreas Trumpp
German Cancer Research Center (DKFZ)

Violeta Serra
Vall d'Hebron Institute of Oncology

Jakob Nilsson
The Novo Nordisk Foundation Center for Protein Research, University of Copenhagen

Ian Collins
Cancer Research UK Cancer Therapeutics Unit at The Institute of Cancer Research

Paul Huang
The Institute of Cancer Research

Stefano Piccolo
University of Padova

Eleonora Leucci
KU Leuven

Ken Lau
Vanderbilt University

Trudy G. Oliver
Huntsman Cancer Institute

Dieter Saur
Technical University of Munich (TUM)

Jonathan Brody
Thomas Jefferson University

Martine Jager
Leiden University

Antony M. Carr
Genone Damage and Stability Centre, University of Sussex

Marianna Kruithof-de Julio
University of Bern

Bertie Göttgens
University of Cambridge

Vladimir Kirkin
The Institute of Cancer Research

Serena Nik-Zainal
University of Cambridge

Maggie Cheang
The Institute of Cancer Research

Phil Jones
University of Cambridge - Wellcome Trust Sanger Institute

Katherina Stankova
Maastricht University

John Poirier
Memorial Sloan Kettering Cancer Centre

Catrin Pritchard
University of Leicester

Breast Cancer Now Seminars

Daniel Rea
Institute of Cancer and Genomic Sciences - University of Birmingham

Mohamed Bentires-Alj
University of Basel

Christina Scheel
German Research Center for Environmental Health - Institute of Stem Cell Research

Antonis Antoniou
University of Cambridge

POSTGRADUATE EDUCATION

Postgraduate Education Manager
Julie Edwards

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Angeliki Malliri

Postgraduate Director and Chair of the Education Committee
Tim Somervaille

The Cancer Research UK Manchester Institute offers a postgraduate degree (PhD) 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 2018, we welcomed nine graduate students and one clinical research fellow to our PhD programme, working in a variety of fields from translational oncogenomics, cell plasticity and epigenetics, molecular oncology, skin cancer and ageing, prostate oncobiology, through to cell biology. It was also particularly gratifying to see that, over the past twelve months, one of our students has published two first author papers in Clinical Cancer Research and British Journal of Pharmacology.

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. The speakers are internationally renowned scientists and we consider it essential that our students are exposed to outstanding research from leaders in different disciplines, which will give them a broad understanding of many aspects of cancer research and basic biology. In addition, we hold a series of weekly postdoctoral research seminars and attendance from PhD students is an integral part of the seminar programme. While students themselves are asked to give talks at key points during their PhD, they also have opportunities to present their work at lab meetings and during student forums within the Institute. STAy (short for Science TakeAway) is a group run by junior scientists in the CRUK Manchester Institute. Meetings are open to all early career scientists - PhD students, postdocs and scientific officers from the Institute and The University of Manchester Division of Cancer Sciences co-located with us. 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 2018 have included their annual Science Showdown, patient-focused engagement event "STAy Patient With Us" and various 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 2018, The Lizzy Hitchman student prize went to PhD Student Colin Hutton from the Systems Oncology group for his PhD work describing a novel approach in identifying two fibroblast populations in pancreatic tumours.

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

In 2018, the 12th IPSCC was organised by PhD students from The Francis Crick Institute, London and held in June. The Institute was represented by 12 of our PhD students in their 2nd and 3rd years, and the forum provided a unique opportunity for the students to present their work and network with some of Europe's best cancer research institutes. The students enjoyed a welcome talk from Professor Sir Paul

Nurse, and keynote lectures from Dr Patrick Vallance, Professor Fiona Watt, Professor Ester Hammond and Professor Steve Jackson.

The conference was attended by over 90 students in total with 18 talks and 74 posters scheduled over the two and a half days. It is mandatory for participating student delegates to submit a poster to showcase their research either through a poster or oral presentation. Posters are scored and ranked by the student delegates during the poster sessions, with the top three posters receiving prizes. CRUK Manchester Institute student Joe Maltas, from the Cell Signalling group, was awarded joint first poster prize for showcasing his work on "The nuclear roles of the Rac activator tiam1 in NSCLC".

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

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 currently based over two sites at Alderley Park, Cheshire and the Olgesby Cancer Research Building, Manchester (formerly the Manchester Cancer Research Centre Building).

Education Committee 2018

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 of Education Committee
Angeliki Malliri
Postgraduate Tutor
Richard Marais
Ex-Officio Member
Julie Edwards
Postgraduate Manager
Claus Jørgensen
Georges Lacaud
Jonathan Tugwood
Caroline Wilkinson
Wolfgang Breitwieser

Student Representatives

Denys Holovanchuk[2]
Jakub Chudziak
Callum Hall[1]

[1] Joined in 2018
[2] Left in 2018

OPERATIONS

Chief Operating Officer
Caroline Wilkinson

Chief Laboratory Officer
Stuart Pepper

Head of Finance
Mike Berne

Head of Human Resources
Rachel Powell

The Operations' team provides the necessary services that facilitate the running of the Institute. It is overseen by four operational managers. Caroline Wilkinson is the Chief Operating Officer with responsibility for scientific administration and communications. She also acts as the primary point of operational contact within the Institute for both The University of Manchester and Cancer Research UK. Rachel Powell is Head of the Human Resources team which this year welcomed Laura Bayliff; Stuart Pepper is the Chief Laboratory Officer overseeing all aspects of general laboratory management and also oversees IT and Health and Safety. The fourth operational manager is Mike Berne who started at the Institute in 2018 to head our Finance team following Margaret Lowe entering a phased retirement. Margaret has served the Institute with distinction for over 25 years and we wish her all the best for the future when she leaves us in early 2019. Mike brings a wealth of experience from the finance team at The University of Manchester's Faculty of Biology, Medicine and Health and has settled well into Institute life.

This year has seen some restructuring of the Operations' team with Logistics reporting to Colin Gleeson, and Neil Carne and Tony Woollam from the Estates team seconded to The University of Manchester and our Logistics team respectively. Belen Conti was promoted to the position of Executive Assistant to Stuart Pepper and Caroline Wilkinson, and Jayne Fowler was recruited as Executive Assistant to Caroline Springer (Director of the DDU). Delydd Jones joined the Institute to replace Belen in the Director's Office team. The year has been dominated by moving the Institute to Alderley Park and working with our operational counterparts at the site to ensure a smooth transition to our interim premises. The team has also been working hard on arrangements for the redevelopment of the Paterson Building site.

Institute Administration Team

Ruth Cox, Maria Belen Conti, Jayne Fowler[1], Delydd Jones[1]

[1] Joined in 2018

Ruth Cox is Executive Assistant to the Institute Director and manager of the Institute Admin Team. The team grew to four members this year; we welcomed Jayne Fowler as Executive Assistant to the new Director of the Drug Discovery Unit and Belen Conti was promoted to a new position as Executive Assistant to the Senior Management Team, while Delydd Jones joined us as Admin Services Coordinator. The extra support has been invaluable during this extremely busy year.

The team provides administrative support to the Director and the Institute Faculty in addition to assisting with the Institute's move to Alderley Park and the organisation of several events over the course of the year, including the Paterson Fire Anniversary Event and Institute Colloquium. Belen also manages the Institute's social media accounts.

Delydd has taken over from Belen in organising the external seminar series, which has continued to be a great success in 2018. The seminars serve to foster collaboration and encourage interaction with the wider scientific community. We ensure that staff at both Alderley Park and OCRB are able to participate in the visits and view the seminars using a

video-link between sites. We aim to provide a varied programme of national and international speakers. Details can be found at www.cruk.manchester.ac.uk/seminars.

Finance and Purchasing

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

[1]Joined in 2018 [2] Phased retirement began in 2018

It has very much been a year of transition for the Institute Finance Team with the appointment of Mike Berne as the new Chief Finance Officer, replacing Margaret Lowe, who began a phased retirement in July 2018. In addition to this, there was also the transition between working environments as the team relocated to Alderley Park alongside a shift in workloads as the team dealt with significant increases in daily activity due to the management of fire-related expenditure.
The Institute Finance Team supports the Director with the management of the Institute's £30m, which is devolved across the various Research Groups, Service Units and Operational Activities. This has been an area of particular complexity this year, given the expansive network as groups were originally split across a number of distant sites, before finally managing to relocate, in the most part, to Alderley Park.

The team continues to support the research groups by providing effective and efficient professional advice when costing new research proposals and contracts, while also providing guidance relating to any purchasing and logistical changes as a result of our new location.

While the fire created additional operations the Institute still receives funding from many different sources and we have the on-going responsibility to ensure these funds are used in the intended manner following both the financial regulations of the University as well as the Terms and Conditions of the funders.
We monitor all individual awards and provide the feedback required to the funders and the Principle Investigators.

Human Resources

Rachel Powell, Rachel Craven, Julie Jarratt, Laura Jones, Emma Lloyd, Laura Bayliff[1], David Stanier[3], Natalie Taylor[2]

[1] Joined in 2018 [2] Left in 2018
[3] Joint with administration

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

During 2018, we completed 126 recruitment rounds and successfully appointed 81 individuals to enhance the work of the Institute. This was compared to 105 recruitment rounds in 2017; an increase of 20% in one year. Also, in 2018 the department administered the successful promotion of 10 individuals. We have continued our commitment to joint partnership working with the Union, which has resulted in the revision of several HR policies and procedures and the renewal of the workforce agreement for scientists. There has also been a review of the salary scales for the CRUK scientific and non-scientific pay and grading framework.

The Institute is committed to working towards the Athena Swan accreditation and this will be a priority over the next 12 months. We have continued to provide support to our EU staff during the uncertain time as the UK prepares to leave the European Union. Therefore, we were pleased to announce to EU staff that The University of Manchester was part of a new pilot scheme from the Home Office which allows staff to apply to continue to live and work in the UK after 2021. In addition, we have reimbursed staff the fees for the EU Settlement Scheme.

Next year, the focus will be on the Athena Swan accreditation, recruitment of new research groups in line with the Institute's strategy and the implementation of a new candidate management system to support the application process and enhance the candidate experience.

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 almost every aspect of their work.

Once again, 2018 was another year of unprecedented change in IT. In readiness for the relocation and regrouping of the majority of CRUK MI staff at Alderley Park (AP), Q1 2018 was primarily spent rebuilding our physical IT infrastructure on this new site. To this end, we have now implemented a dedicated CRUK MI resilient wired and wireless network infrastructure across all CRUK MI research facilities at AP and re-established an enterprise-class file storage facility for our research data. This is based on a replicated design, hosted in two geographically separate datacentres, to provide a resilient, high availability, redundant, fit for purpose, storage facility.

Q2 2018 and onwards was largely spent supporting around 330 CRUK MI staff moving into their new offices and labs at AP, whilst rebuilding our services to operate across multiple sites, primarily Alderley Park and also the Oglesby Cancer Research Building. Facilitating service provision across multiple sites has required careful planning and more widespread adoption of new services to support remote working. For example, we have real-time multipoint network monitoring to rapidly identify the source of any outages. We also now make greater use of automated deployment tools to deploy new client computers. In addition, our adoption of 'self-service' application installation now enables research staff to resolve a significant number of IT Service Requests themselves.

The process of relocation and rebuilding has enabled us to work more closely with The University of Manchester IT Services and other CRUK MI service groups and take advantage of new synergies. In particular, we have formed closer working relationships with the Scientific Computing group and the Advanced Imaging group. This has enabled researchers to fast track implementation of new technologies to facilitate their research projects.

Alongside rebuilding our IT infrastructure and services afresh we are also now beginning to shape our return to our previous 'home' in Withington based on The Christie NHS Foundation Trust site, in a new custom built cancer research building to be shared by three partners; CRUK MI, The University of Manchester and The Christie.

Looking forward, the planning for our move back will gather pace as the year progresses. This will be an exciting time for all involved as we start to design a cancer research facility for the future.

Safety and Facilities Management

Colin Gleeson

Over the last year services related to a variety of functions across safety, logistics and laboratory support and these three elements have been organised to sit together under a single maintenance facility that is managed by Colin Gleeson. This puts safety at the heart of all these vital support functions for the Institute, and also promotes efficient coordination of activity between these teams. The relocation to Alderley Park placed many demands on these teams and it is a testament to the flexibility and hard work of everyone listed here that by the end of summer 2018 the Institute was running efficiently. This section details some of the work that has taken place to allow the relocation to be completed.

As we look ahead to the next couple of years there will be an important role to play in contributing to the new building, to ensure that the critical infrastructure necessary to run a research building can operate efficiently.

Health and Safety

Colin Gleeson

The relocation of CRUK MI to Alderley Park was completed in 2018. This demanded a wide range of activities, including laboratory re-design projects for some areas to guarantee the space was appropriate for our research needs; operational meetings with our landlords to ensure the services they provide would be sufficient for our needs; and major equipment rebuild and relocation to maintain a

comprehensive range of research facilities available for our research. All of these activities required a considerable input from a health and safety point of view over a long and busy timeline.

Concomitantly routine health and safety activities continued, which included inspections of facilities; reviewing risk assessments, including numerous new genetic modification assessments; a review of our current assessments for transgenics, creating new DSEAR assessments for our laboratories; arranging statutory inspections of equipment; investigating incidents and non-compliance issues and the development of working relationships and liaising with health and safety colleagues at the AP site and University main campus. We also greatly improved our first-aider provision with ten people gaining the full 3-day first-aid at work qualification and another 6 gaining the 1-day emergency first-aid qualification.

Further, in the latter half of the year, some final close-down work for the old Paterson Building was completed. This involved the partial surrender of our authorisation for radioactive work within the Paterson Building, whilst at the same time retaining a small laboratory for radioactive work within The Christie NHS Foundation Trust site. We also embarked on the development of an application for radioactive work at the Alderley Park site. In 2019, we look forward to contributing to the preparatory and planning work for the new building on the old site.

Laboratory Services

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

[1]Left in 2018

During 2018, Lab Services continued to support the various research buildings with a main base of operations at the Oglesby Cancer Research Building containing the glass washers and autoclaves. From this base they supply clean, sterile glass and plastics for the research groups based at OCRB and provide sterile plastics to the groups at Alderley Park.

At AP the department continue to collaborate with the onsite support, facilities and catering teams, and at OCRB deliver the service directly to the lab groups. In partnership with the porters and Logistics team, they continue to deliver items from OCRB to AP via the daily shuttle van service.

In 2018 the team relocated the media service and now manufacture liquid media and agar plates from the OCRB and deliver to the research groups across OCRB and AP.

The Lab Services department also continues to support the research groups in other ways:

- maintenance and servicing of the photographic dark rooms at each site;
- providing a drop in monthly pipette clinic at both sites;
- organising the delivery of clean general and tissue culture lab coats.

Additionally, in consultation with the Health and Safety Manager, Mark Craven has maintained appropriate first aid supplies across the sites. He also supports the Chief Laboratory Officer and coordinates servicing of shared equipment such as microbiological safety cabinets and lab water systems. Alongside the Facilities Manager, he reports and resolves lab-based faults at AP. Mark has coordinated the removal of CRUK MI laboratory waste ensuring compliance with local rules.

Logistics

Andrew Lloyd, Michael Alcock, Edward Fitzroy, Sedia Fofana, Stephen Keane, Jonathan Lloyd, Robin Sherratt, William Glover, Nigel Fletcher, Tony Woollam

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

It has been a challenging year for the Logistics team. With the additional support from Nigel Fletcher (BRU) and Tony Woollam (Estates), we have managed to support both Olgesby Cancer Research Building and Alderley Park sites with our usual day to day service, and supported sample movement by introducing a transport service.

At the start of the year the team worked extremely hard behind the scenes preparing for the relocation to AP. The team was tasked with setting up the office spaces, which involved large numbers of furniture deliveries and many office rearrangements. In April the team successfully relocated all the Operations teams from OCRB to AP.

We now have a fully operational Logistic team at AP and they work closely with the various onsite Logistics and waste teams.

The team has continued to deliver an efficient and effective service providing support for the research carried out at AP and OCRB. This includes the receipting, checking, booking in and distribution of goods ordered by staff. We also facilitate the delivery of dry ice, liquid nitrogen and gas cylinders.

Researchers can order central stores stock items via the intranet, which can be collected personally or distributed by the Logistics team. Included in this system are the enzymes and media stored in the Institute freezers at the OCRB (Sigma, Life tech, Promega, New England Bio labs, and Qiagen). A list of stocked items can be found on the intranet. We continued to make savings by buying in bulk from our suppliers.

Over the past year the team has offered a sample transfer service from OCRB to AP, with daily transfers in the morning and afternoon; a daily afternoon service from AP to OCRB; and depending on demand, a service to other University of Manchester buildings.

Electronics

Yunis Al-hassan, Steve Powell, Tony Woollam

As part of the Institute's electrical and fire safety strategies, this year the Electronics team have PAT tested well over a thousand pieces of electrical equipment across multiple sites. The work included PAT testing our newly acquired equipment. Additionally, the Institute electrical engineer has repaired numerous items of scientific research equipment. In some cases, repairs are carried out at electronic component level. This repair facility provides a significant economic benefit to the Institute, in that unnecessary expenditure on replacement equipment can be avoided. The Institute electrical engineer also tracks Institute equipment that are under warranty, service contract or in-house repair. Again, this provides a significant economic benefit to the Institute.

Scientific Operations and General Administration

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

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

Scientific administration is overseen by Caroline Wilkinson who is the Chief Operating Officer for the Institute and acts as the main point of contact for both The University of Manchester and Cancer Research UK. The team moved to Alderley Park in early 2018 and have helped with arrangements for operations at the Institute's new location. The team is also responsible for communications and in 2018 launched a new external website for the Institute, which was produced by web developer Tom Bolton with content overseen by Gill Campbell. Gill is the Institute's Grants Advisor who helps our scientists apply for external funding to extend the breadth of research that we conduct. She works closely with the Institute's Grants Committee, chaired by Iain Hagan, who undertake a review of all prospective applications and also help prepare applicants for interviews associated with fellowships or awards. Tom has adapted the online PhD recruitment portal that he produced in 2016 to create an online application system for staff recruitment, which will be launched in 2019.

Julie Edwards is the Postgraduate Education Manager and has helped our students with the transition to Alderley Park and in particular with arranging extensions for those whose PhDs were interrupted by the fire at the Paterson Building in 2017. Early on in 2018, we held our PhD student recruitment day at Alderley Park for the first time, which was organised by Julie and ably supported by David and Gill. David Stanier is also responsible for general administration and oversees the Institute's transport arrangements between Alderley Park and the Oglesby Cancer Research Building. This latter site is home to two of our research teams who have remained close to the Christie Hospital site for operational reasons. David is also the Institute's Information Governance Co-ordinator supporting Caroline Wilkinson as the Institute's Information Governance Guardian. These responsibilities are required by The University of Manchester and interact with the University's Information Governance Office

to help ensure that we operate to the appropriate legislative requirements.

Caroline Wilkinson oversees the Research Integrity Committee, which reviews all manuscripts before final submission for publication to ensure that our research is conducted in accordance with the highest standards of integrity. This year, Simon Poucher has taken on a role with the committee in order to check that any in vivo research is reported in accordance with the NC3R's ARRIVE guidelines.

Steve Morgan continues in his role at the Oglesby Cancer Research Building where he works closely with staff from the University's Faculty of Biology, Medicine and Health to ensure that reception runs smoothly and to operate the Institute's switchboard.

Animal Welfare

Simon Poucher, Regulatory Liaison and Training Officer, **Janet Watso**n, Animal Welfare and Ethical Review Body (AWERB) Chair, **Caroline Wilkinson**, Establishment 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 (ASPA) 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, the facilities for the care and use of mice, and 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 2018, our AWERB met formally on seven occasions with two additional meetings involving all licensees at the Institute. These two meetings were also attended by our Home Office Inspector. In the year, six applications to the Home Office for new Project Licences and 11 applications for amendments to existing Project Licences were reviewed by the AWERB. Significant input is provided to the applicants by the Home Office Liaison Coordinator (HOLC), Named Veterinary Surgeon (NVS) and Named Animal Care and Welfare Officer (NACWO) at the draft stage but review by the wider AWERB membership contributes to further improvements. Licences have incorporated a number of techniques new to CRUK MI this year (including a model of ocular melanoma, orthotopic brain tumours, and bladder catheterisation) and the AWERB has required evidence of how licensees can develop expertise. The NVS provides invaluable advice on all matters, including this year improved surgical practice, scoring of skin ulceration effects and recommendations for best anaesthetic equipment and stereotaxic rigs. A total of 27,018 mice were used at the Institute in regulated procedures under the Act in 2018.

Licensees are required by law to report any unforeseen adverse effects on animals or breaches of the controls and limits in their licence. Six incidents were self-reported to the Home Office in the year, including unexpected reactions to treatments or unexpected tumour growth patterns; all of which were satisfactorily resolved with the inspector. The AWERB reviews these reports and uses these to share learning across licensees.

By the start of 2018, experimental work was fully established at Alderley Park under a mutual agreement with AstraZeneca to share their facility and CRUK MI subsequently exited operations at the University's Stopford building. The CRUK MI AWERB has continued to interact with other establishments through the NW AWERB Hub; the Establishment Licence Holder (ELH) and HOLC attend regular meetings with the Home Office Animals in Science Regulation Unit (ASRU) and the ELH sits on the national ELH forum as well as helping to train new Establishment Licence Holders. Starting this year, the AWERBs of CRUK MI, AstraZeneca and Agenda Life Sciences, all based on the Alderley Park site, have held joint meetings to share ideas, presentations and training opportunities - in October, the three held a joint 3Rs' poster event.

Fulfilling our commitment under the Concordat on Openness on Animals Research, CRUK MI staff have talked about our research with mice to the public at a 'Pint of Science' evening in a Manchester pub and at an event at Manchester's Science and Industry Museum, as well as Institute Open Days; a workshop run by the Understanding Animal Research organisation in May helped our volunteers to communicate comfortably with members of the public at these events.

Cancer Research UK Commercial Partnerships

Martyn Bottomley

Cancer Research UK Commercial Partnerships (CP) Team (formerly Cancer Research Technology (CRT)) is a specialist oncology-focused development and commercialisation team which is part of 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.

From April 2018, the CP Team has undergone a reorganisation from a "cradle to grave" model into functionally distinct sub-teams in order to provide greater strength, depth and accountability in our core activities supporting translation and commercialisation, as well as providing clearer and more streamlined interfaces with other teams across Research and Innovation with whom we collaborate to achieve our joint goals of progressing CRUK science.

The new structure comprises four core teams: Opportunity Sourcing & Translation (OST); Business Development & Transactions; Partnerships & Strategic Alliances; and Business Operations. Notably, our interface with the academic community through OST will now have a geographical focus, with four regions each having an expanded team of Translation Managers and Executives under a Regional Translational Lead and OST Associate Director. We hope this will build deeper and more strategic relationships with our funded centres, institutes and universities, as well as improving internal information flow and collaboration.

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 Translation Lead is based within Manchester and is currently hot-desking at the Oglesby Cancer Research 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) based at Alderley Park to facilitate the development of drug therapies to satisfy the unmet clinical needs of cancer patients. Martyn continues to be involved with the management of collaborations with Pharmaceutical partners such as 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. During 2018, Martyn also completed the license of the DNMT1 drug discovery program from the Manchester DDU to GSK.

Commercial Partnerships is also currently actively managing a broad portfolio of development programmes and exciting licensing opportunities originating from the Cancer Research UK Manchester Institute that continue to attract commercial partners. We look forward to building on our successes and continuing to work closely with the Cancer Research UK funded researchers in Manchester under the new CP structure to advance discoveries to beat cancer in the years ahead.

Staff from CRUK Corporate Partner, Flybe visit the MBCF labs in the Oglesby Cancer Research Building.

CANCER RESEARCH UK'S RESEARCH ENGAGEMENT

Research Engagement Manager

Tim Hudson

Cancer Researcher UK's Research Engagement Team brings CRUK-funded research to life for its supporters, the public and its staff, working regionally with researchers to develop face-to-face engagement opportunities. The team creates compelling engagement content for local supporters and the public to drive interaction with life-saving research happening near them.

Almost 7,000 people interacted with the work of the Manchester Institute during 2018, at events in our own labs or externally at fundraising events and science festivals.

During 16 visits to Institute labs at Alderley Park and the Oglesby Cancer Research Building, 160 donors, fundraisers, volunteers, corporate partners and CRUK staff had the opportunity to gain a close-up view of the research their support helps to fund.

A highlight came in the autumn when we welcomed both the Warburton family and the company's Charity Champions to Alderley Park, celebrating a four-year partnership which has resulted in £1.5million towards research funding. The Charity Champions enjoyed a talk by Claus Jørgensen before meeting Lizzy Hogg in the labs and learning about CyTOF with Steve Bagley. The Warburton family enjoyed speaking with Caroline Dive, whose lab they have allocated fundraising to for the coming year, and were treated to a special visit to the CEP group's Good Clinical Practice lab.

Our scientists also took their work out to the community at 25 external events.

In the spring, Institute scientists teamed-up with Research Nurses from The Christie NHS Foundation Trust, other University of Manchester research groups and others representing the CRUK Manchester Centre to deliver engagement

Images top left to right.
CRUK's Events Interns meet MI PhDs in the Oglesby Cancer Research Building to get hands-on with our research.

The University of Manchester's Science Spectacular, part of the Manchester Science Festival.

Images bottom.
Institute scientists meet the public at the Science & Industry Museum's Platform for Investigation.

activities to over 400 members of the public at the Science and Industry Museum's *Pi: Platform for Investigation*. Demonstrations included DNA mutation and flow cytometry games, clinical trials activities and a special animal welfare station, hosted by the BRU team and others.

Further collaboration took place during the Manchester Science Festival in September, when MI staff teamed up with researchers from the Division of Cancer Sciences, the Biomedical Research Council and The Christie NHS Foundation Trust at The University of Manchester's *Science Spectacular*, to engage over 200 members of the public with a range of research themes – prevention, early detection, treatments and targeted therapies.

The 13th Relay for Life Stockport took place in the height of the summer, featuring the team of researchers from the Manchester Institute captained by Steve Lyons. As well as organising a host of pre-event fundraising and taking part in the 24-hour walk event, the team hosted a research stall, talking to participants about their work and getting hands-on with some strawberry DNA extraction.

Our researchers engaged with national and international audiences too; PhD student, Denys Holovanchuk delivered a talk to school students in Portugal, whilst Adele Green featured in a video exhibit for the Science Museum's acclaimed exhibition, *The Sun: Living With Our Star.*

Artistic collaborations resulted in works exhibited in prestigious settings. Manchester Metropolitan University student, Alice Thickett visited Steve Lyons and Steve Bagley in the Oglesby Cancer

Artist Alice Thickett with the piece she created following her work with our researchers.

Research Building and developed a piece titled *Cancer Research: Written in the Stars*. The textile print features cell imagery from the labs, provided by Division of Cancer Sciences PhD student Daiana Drehmer, and was exhibited as part of the Pint of Science and Manchester Science Festivals this year.

On a larger scale, Institute scientists and staff featured on one of 80 giant bee sculptures which went on display in Manchester city centre, as part of *Bee In The City*. Scientists were invited to add their signatures to artist Kelly Stanford's STEM Bee. The 12 cancer research autographs joined signatures from over 80 physicists, biologists, chemists, mathematicians and engineers in the city. After the trail ended the bees were auctioned to raise funds for The Lord Mayor of Manchester's charity – We Love MCR, to improve the lives and life chances of Manchester people, with Kelly's STEM Bee selling for an incredible £20,000.

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.

Images left to right.
Relay for Life Stockport's Researcher Team at this year's event.

Artist Kelly Stanford's STEM Bee, displayed outside Manchester's Oxford Road Rail Station as part of Bee in the City.

Alicia Marie-Conway was one of the MI scientists who joined with partners from The Christie and The University of Manchester to sign the STEM Bee.

Members of the Warburton family visit the CEP labs with Caroline Dive

ACKNOWLEDGEMENT FOR FUNDING FOR THE CANCER RESEARCH UK MANCHESTER INSTITUTE

The total funding of the CRUK Manchester Institute for 2018 was £30m. The major source of this funding was awarded by Cancer Research UK via a core grant of £13.3m plus additional strategic funding of £5.9m. 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.

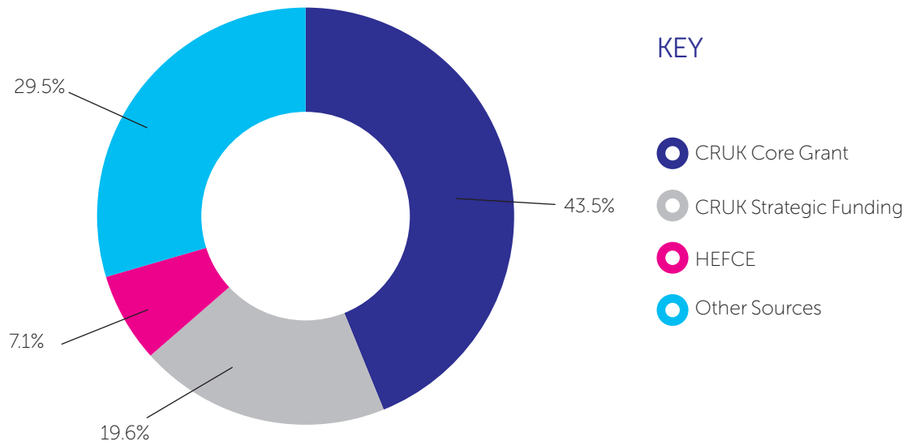
The balance of the Institute's funding is received from a number of additional sources. The research carried out through these additional projects enhances and supports the research undertaken by the core funding.

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
- David & Ruth Lewis Trust
- Euclises Pharmaceuticals Inc
- European Commission
- European Organisation for Cancer Research and Treatment of Cancer
- European Research Council
- Fondation ARC pour la Recherche sur le Cancer
- GlaxoSmithKline
- Harry J Lloyd Charitable Trust
- John Swallow Fellowship
- Kay Kendall Leukaemia Fund
- Leo Pharma Foundation
- Menarini Biomarkers Singapore
- Merck
- Moulton Charitable Trust
- National Institute of Health Research
- Ono Pharmaceuticals
- 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 2018



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.

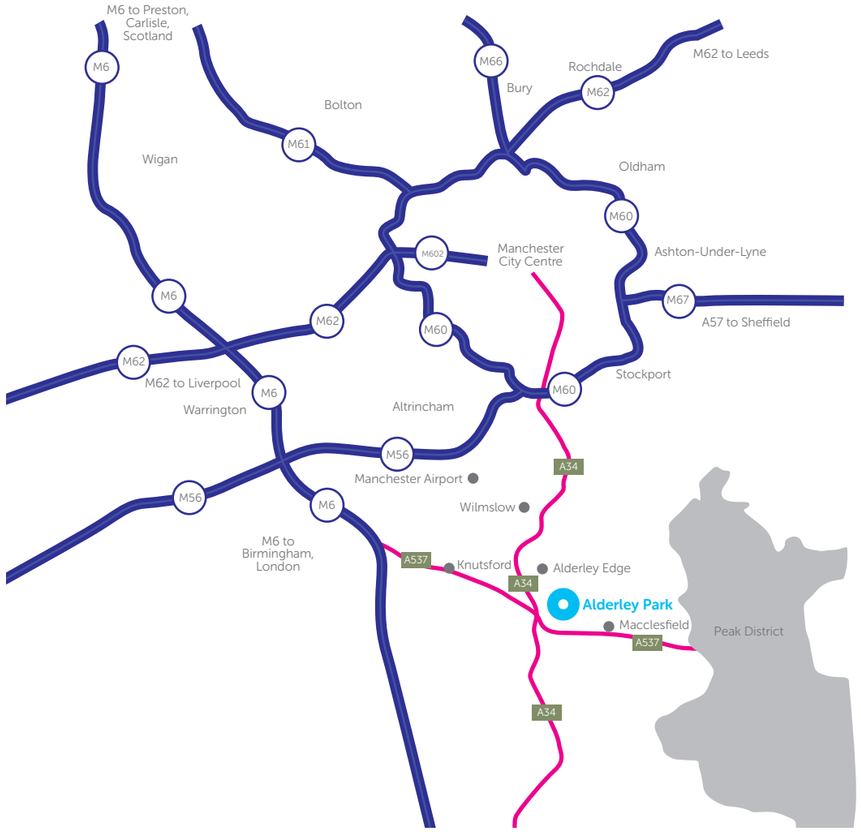
Opportunities exist at a number of levels in the Institute. We have a well-established programme of degrees by research which is described in the section on Postgraduate Education. We encourage applications from suitably qualified graduates to apply to join either the PhD or MD programmes. Graduates 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 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.

CONTACT DETAILS



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