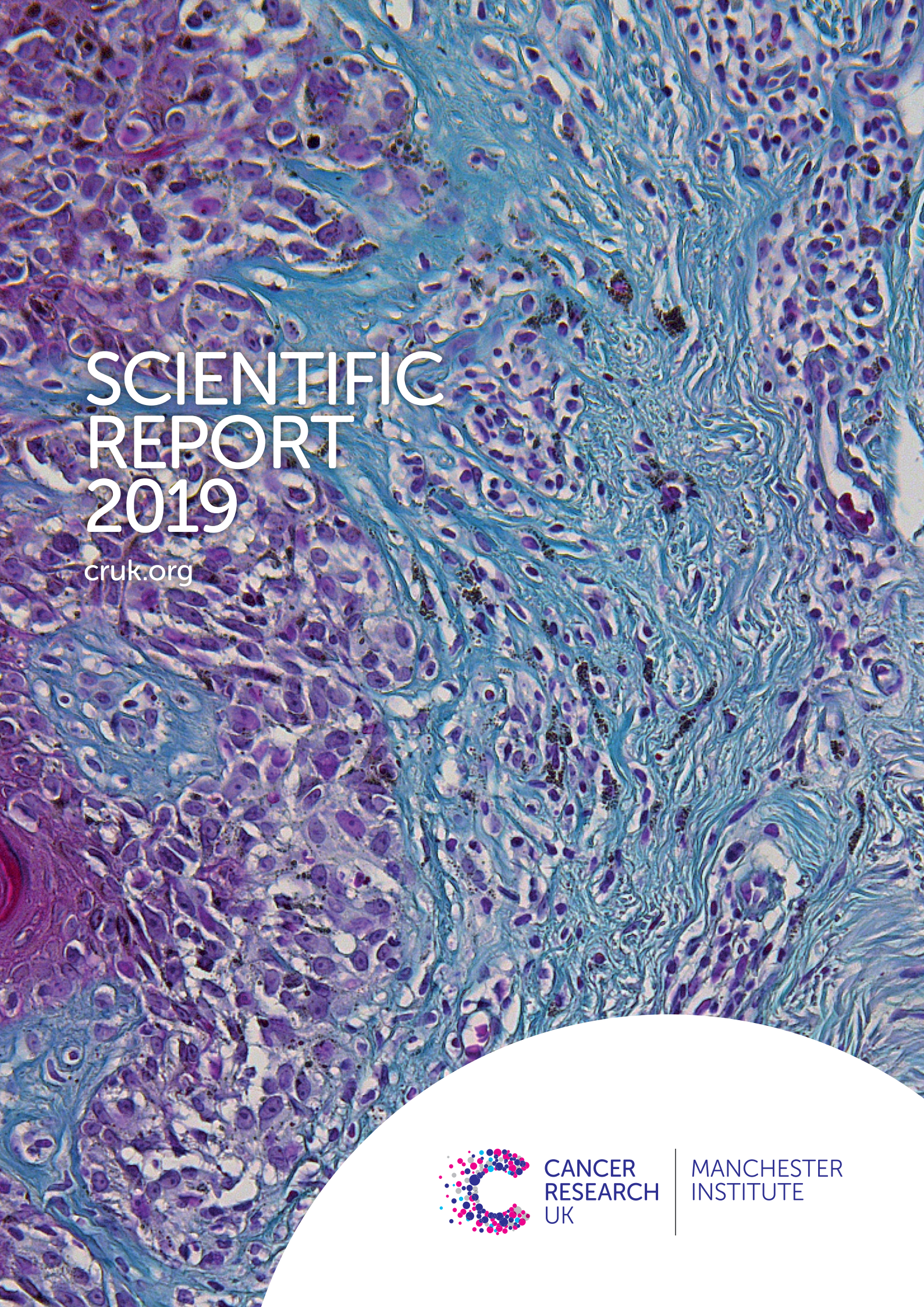


SCIENTIFIC REPORT 2019

cruk.org

CANCER RESEARCH UK

MANCHESTER INSTITUTE

COVER IMAGE

Primary cutaneous melanoma (nests of purple cells) invading the dermis. The specimen is stained with Masson's trichrome stain. Cyan blue reveals collagen fibre architecture.

Image supplied by Amaya Viros (Skin Cancer and Ageing)

SCIENTIFIC REPORT 2019

MANCHESTER INSTITUTE

MANCHESTER 1824
The University of Manchester

CANCER RESEARCH UK | MANCHESTER INSTITUTE

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

During 2019 we continued our recovery from the 2017 Paterson Building fire by consolidating our interim base at Alderley Park. The Paterson Building has been demolished and good progress is being made on the plans for its replacement.

The project received a significant boost in the summer through a £25m award from the Research England UK Research Partnership Investment Fund and towards the end of 2019, the Rewrite Cancer campaign was launched by the project's partners to raise the final £20m required for the build. As 2020 proceeds, we look forward to finalising the design and watching the new facility start to emerge and take shape.

Despite the disruption of the move, we have celebrated many scientific highlights that illustrate the depth and breadth of research at the Institute. It was a particularly successful year for the Institute's Deputy Director Caroline Dive and her Clinical and Experimental Pharmacology group who continue to be at the forefront of the development of biomarkers to aid clinical decision making. In April, they received an "Outstanding" rating at their quinquenniual review, which took place over two days at Alderley Park. Later in the year, they were part of the successful renewal of the CRUK Lung Cancer Centre of Excellence, and towards the end of the year, we renamed the group as the Cancer Research UK Manchester Institute Cancer Biomarker Centre (CBC) to recognise the scope and extent of their exciting scientific activities. We also welcomed Dr Alistair Kerr and Dr Carlos Garcia Lopez to the CBC as the new Head of the Bioinformatics and Biostatistics team, and as an Institute Fellow respectively.

One of the CBC's key studies was published in *Nature Medicine* and it describes the prediction of non-small cell lung cancer (NSCLC) relapse through pulmonary venous circulating tumour cells. These cells are isolated from stage I-IIIa NSCLC patients during surgery, and through their enumeration, risk of recurrence can be predicted. Prof Dive's team is partnering with Lloyds Pharmacies in Greater Manchester where phlebotomists take blood samples from patients following lung cancer surgery with the aim of using these "liquid biopsy" approaches to detect relapse at an early stage. The TARGET trial has been another exciting development for the CBC in recent years, together with clinical colleagues at the Christie Hospital. This ctDNA-driven selection phase I clinical trial is being run across Experimental Cancer Medicine Centres in the North and was also published in a *Nature Medicine* publication, and the trial will now be expanded to assess patient survival. It is very pleasing to see how well the CBC has recovered from the fire.

The Institute's Drug Discovery Unit (DDU) has also made an excellent recovery, since establishing their facilities at Alderley Park in early 2018. Their programme of greater integration with the Institute's scientists is developing well in addition to fostering exciting new collaborations with the wider CRUK community including a joint position with Stephen Taylor at The University of Manchester, and a second joint position with Richard Bayliss at The University of Leeds. They published two studies in 2019 that are the first to describe orally available drug-like compounds against lysyl oxidase (LOX). This enzyme forms cross-links between collagens and elastin in the extracellular matrix and plays a critical role in regulating tumour growth and metastasis. Their work has added to the growing interest in LOX as a potential therapeutic target, and the lead inhibitors developed by the DDU display promising effects in delaying tumour growth in a mouse model of breast cancer.

The Cell Signalling group uncovered a new regulator of mitotic spindle orientation through the interaction of the calcium/calmodulin-dependent serine protein kinase (CASK) with the tumour suppressor Dlg1. Former Clinical Fellow, Dan Wiseman, now Oglesby Senior Leukaemia Research Fellow in the Division of Cancer Sciences at The University of Manchester, together with colleagues in the Leukaemia Biology group, uncovered an intriguing relationship between splicing and epigenetic regulation underpinning a new molecular subtype of Acute Myeloid Leukaemia. Alongside, the Leukaemia Biology group continue to develop their programme on the potential AML target Lsd1 and have identified mTORC1 inhibition as a synergistic therapeutic approach.

I was delighted that the DETECTION clinical trial was funded by CRUK and that the CACTUS trial is now up and running. Both applications for these melanoma trials have been driven by Rebecca Lee, a former clinical fellow in the Molecular Oncology group, and rely on the ctDNA expertise developed in the CBC. Also, many of our Group Leaders contributed to the successful application for an Academic Researcher Clinical Training Innovation in Cancer programme (ARCTIC) as part of the CRUK Manchester Centre's Clinical Academic Training (CAT) initiative and we look forward to welcoming some of the trainees to CRUK MI in due course. Our scientists also played a major role in the success of several other CRUK Manchester Centre initiatives such as the ACED early detection alliance and the radiation research network RADNET, while the Belfast-Manchester Movember Centre of Excellence for Prostate Cancer, which involves three of our research groups, was renewed in 2019. Moreover, together with partners in Italy and Spain, the CBC's digital Experimental Cancer Medicine Team were awarded a CRUK Accelerator award to produce digital tools for clinical teams across the CRUK Experimental Cancer Medicine Centres' network providing real-time access to patient data to facilitate faster clinical decision-making. Georges Lacaud, who leads the Stem Cell Biology group, received funding from the charity Bloodwise (now Blood Cancer UK) to investigate MOZ histone acetyl transferase activity in leukaemia.

A number of our staff and students received awards and prizes during the year. Caroline Dive was one of The University of Manchester's Researcher of the Year, and she received the 2019 Heine H. Hansen Lectureship Award for Small Cell Lung Cancer in recognition of her pioneering discovery research in this field. The Deputy Group Leader of the CBC, Ged Brady, was awarded a Professorship by The University of Manchester in recognition of his contributions to nucleic acid biomarker research. Manchester Cancer Research Centre Director and CRUK MI Senior Group Leader Rob Bristow was elected Fellow of the Academy of Medical Sciences for advancing medical science through his outstanding contributions towards understanding the genomics of prostate cancer progression and treatment response.

Our early career researchers' efforts were recognised at several meetings during the summer. Postdoctoral Research Fellow, Lucas Trucco, from the Molecular Oncology team received the AACR Scholar-in-Training Award to attend the AACR Annual Meeting, while Brian Lee from Systems Oncology was awarded a poster prize at the BACR Tumour Microenvironment meeting. Several of our PhD students attended the International PhD Student Cancer Conference in Amsterdam, with two of our students, Max Schenk and Chris Bromley, winning prizes for their presentations. Sakis Paliouras received a University of Manchester Doctoral Academy Graduate Society conference award and a BACR-CRUK student travel award to attend the AACR annual meeting. In July, the MCRC and Experimental Cancer Medicine Centre organised an exciting meeting in Manchester "Phase 1: Where Science Becomes Medicine" at which Julie Stevenson and Paul O'Regan from the digital Experimental Cancer Medicine Team were jointly awarded prizes for their posters on eTARGET - a digital solution for the Manchester Molecular Tumour Board. PhD students Denys Holovanchuk, Amy McCarthy and Jakub Chudziak all received awards to attend conferences, Amin Ali received The University of Manchester's Presidential Award and Joe Maltas was awarded The Humane Research Trust Margaret Pritchard Memorial Prize. Postdoctoral Research Fellow Victoria Pelly received a CRUK Research Travel Award, which facilitated a visit to the group of Ton Schumacher at the NKI. We have continued to celebrate our achievements in pursuing developments in the principles of the 3Rs – Replacement, Reduction and Refinement – during in vivo procedures and to promote sharing and dissemination of ideas and best practice, we held a joint 3Rs' Poster event with AstraZeneca and Agenda Life Sciences. I am delighted that CRUK MI picked up awards in all three categories at this event, through the work of Lisa Doar, Chris Below, Callum Hall and Yannick von Grabowiecki.

Junior Group Leader Santiago Zelenay received the inaugural BIAL Prize in Biomedicine, along with his former mentor at The Francis Crick Institute, Caetano Reis e Sousa. This new award recognises significant achievements in biomedicine published over the past decade. During his time at the Crick, Santiago discovered a mechanism used by cancers to escape detection by the immune system, which has the potential to be exploited therapeutically. This work has formed the basis of several clinical trials exploring the combination of anti-inflammatory drugs with immunotherapy in a range of cancers and is underpinning the

research programme of Santiago's Cancer Inflammation and Immunity group.

Each year the Institute awards its own prize to recognise the most outstanding achievements by a young scientist. Named after a former Institute Director, the 2019 Dexter award was presented jointly to Mark Williams and Alice Lallo. During his time as a Clinical Research Fellow in the Leukaemia Biology group, Mark uncovered the molecular mechanism underpinning up-regulation of the drug efflux pump ABCB1, which is a key driver of chemoresistance in acute myeloid leukaemia. His work, published in the Journal of Clinical Investigations, suggested a potential therapeutic approach to overcome this form of resistance and enhance the activity of ABCB1 inhibitors. Mark is set to pursue a career as a clinician scientist in the field of stem-cell transplantation, and in August 2020 we look forward to welcoming him back to CRUK MI, through a University of Manchester Presidential Fellowship.

Alice Lallo's work in the CBC focused on characterising DNA damage response deficiencies (DDR) in small cell lung cancer using cell lines and circulating tumour cell derived explant (CDX) models. Her research has supported translation of the combination of two DDR inhibitors to the clinic and resulted in a first author publication in *Clinical Cancer Research*. A critical part of her study involved the use of ex vivo CDX cultures to screen for drug efficacy as well as investigating molecular mechanisms of drug response. In 2019, Alice published her second first author paper in the *British Journal of Pharmacology*, in which she described the development and characterisation of her CDX ex vivo culture system.

Our core facilities continue to evolve and support our research through the provision of cutting-edge technological platforms. The Mass Spectrometry facility was severely affected by the Paterson Building fire but after a sustained effort to replace equipment and optimise the environment in which to house it, the service returned to being fully operational and I congratulate Duncan Smith and Yvonne Connelly for all their sterling work during this challenging time. An evolving theme has been increased collaboration between the facilities to great effect together with better integration with our Computational Biology and Scientific Computing activities. During the year, plans developed to hold a core facilities retreat to provide a platform for enhanced discussion across the CRUK Institutes. I look forward to seeing the outcomes from this initiative in the coming years.

Our Science Take Away (STAy) group continues to drive a programme of events for our cohort of early career researchers and to organise opportunities to engage with the public. In 2019, they invited and hosted several of our external seminar speakers, organised media training and grant writing workshops and put together a highly successful World Cancer Day "Behind the Labels" public engagement evening - a large event incorporating clinician, researcher, and patient talks, lab tours and table-top activities. The group put forward their views as part of Cancer Research UK's consultation process with researchers over the PlanS coalition on open access publishing. Together with Steve Bagley (Head of Visualisation, Irradiation and Analysis) and Gill Campbell (Grants Advisor), the group submitted a successful application to the Royal Society Summer Science Exhibition 2020. Unfortunately, this event was postponed due to the SARS-CoV2 pandemic, but we look forward to them displaying their exhibit on the theme of the Institute's work on the tumour microenvironment at some point in the future.

It is a great privilege to engage with CRUK's supporters, facilitated through the excellent work of Tim Hudson, CRUK's Research Engagement Manager based in Manchester. During 2019, we welcomed supporters to our Laboratories at both Alderley Park and the Oglesby Cancer Research Building while also attending events such as CRUK's Relay for Life Stockport. We hosted a visit by three members of the Science Museum's exhibition curation team to help them prepare for the CRUK-sponsored cancer exhibition, which is due to take place in 2021.

At the time of writing, we are in the midst of the SARS-CoV2 pandemic. Like many other research establishments, we halted our laboratory work during the lockdown and sought other ways to continue our research efforts by working from home. I was very proud that so many of our researchers volunteered to assist the national effort on COVID-19 testing. Pleasingly, we have reopened our laboratories, albeit with the restrictions imposed by reduced occupancy and social distancing, but I am again proud that our staff are back at work and pursuing their projects with so much renewed enthusiasm.

RESEARCH HIGHLIGHTS

In this section we highlight some research publications from 2019 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.

Chemi F, Rothwell DG, McGranahan N, Gulati S, Abbosh C, Pearce SP, Zhou C, Wilson GA, Jamal-Hanjani M, Birkbak N, Pierce J, Kim CS, Ferdous S, Burt DJ, Slane-Tan D, Gomes F, Moore D, Shah R, Al Bakir M, Hiley C, Veeriah S, Summers Y, Crosbie P, Ward S, Mesquita B, Dynowski M, Biswas D, Tugwood J, Blackhall F, Miller C, Hackshaw A, Brady G, Swanton C, Dive C; TRACERx Consortium.
Pulmonary venous circulating tumor cell dissemination before tumor resection and disease relapse.
Nature Medicine 2019; 25(10):1534-1539.

In early stage non-small cell lung cancer (NSCLC), tumour recurrence post-surgery occurs in approximately 50% of cases and most commonly at distant sites. In this study, members of the Cancer Biomarker Centre addressed whether pulmonary vein circulating tumour cells (PV-CTCs) detected in early stage NSCLC at surgical resection with curative intent represent lethal subclone(s) responsible for subsequent disease relapse. They enriched and enumerated blood samples from 100 patients enrolled into the TRACERx study using CellSearch platform. PV-CTCs were detected in 48% of patients but was not significantly associated with clinicopathologic factors such as age, sex, histologic subtype, pathologic stage, smoking status, and treatment received. An exploratory analysis showed that PV-CTC-high status (≥7 PV-CTCs per 7.5 ml blood) was significantly associated with lung cancer relapse and remained an independent predictor in multivariate analysis when adjusting for tumour stage. The team molecularly profiled PV-CTCs to establish genetic links between PV-CTCs, the primary tumour and the relapse tumour. In a case study, genomic profiling of single PV-CTCs collected at surgery revealed higher mutation overlap with metastasis detected 10 months later (91%) than with the primary tumour (79%), suggesting that early-disseminating PV-CTCs were responsible for disease relapse. The evolutionary origin of the PV-CTCs and metastasis was confirmed by phylogenetic analysis that revealed both the PV-CTCs and metastasis were part of the same specific branch, which was distinct from all other subclones of the primary tumour. Finally, they examined the mutations shared by PV-CTCs and the metastatic biopsy yet absent from the primary tumour. In this patient, the PV-CTC and metastatic-associated mutations included a putative inactivating driver mutation in the tumour-suppressor gene LZTS1, which has been shown to inhibit tumour migration and whose lower expression has been linked to poor overall survival in NSCLC. In summary, they showed the potential utility of PV-CTCs as early predictors of NSCLC recurrence after surgery.

Rothwell DG, Ayub M, Cook N, Thistlethwaite F, Carter L, Dean E, Smith N, Villa S, Dransfield J, Clipson A, White D, Nessa K, Ferdous S, Howell M, Gupta A, Kilerci B, Mohan S, Frese K, Gulati S, Miller C, Jordan A, Eaton H, Hickson N, O'Brien C, Graham D, Kelly C, Aruketty S, Metcalf R, Chiramel J, Tinsley N, Vickers AJ, Kurup R, Frost H, Stevenson J, Southam S, Landers D, Wallace A, Marais R, Hughes AM, Brady G, Dive C, Krebs MG.
Utility of ctDNA to support patient selection for early phase clinical trials: the TARGET study.
Nature Medicine 2019; 25(5):738-743.

Researchers in the Cancer Biomarker Centre have analysed the genetic mutations in a blood sample of cancer patients, which could help match those patients with no other treatment options to clinical trials with experimental medicines. Together with colleagues at The

Christie NHS Foundation Trust, AstraZeneca and the NIHR Manchester Biomedical Research Centre (BRC), in this promising study they demonstrated that a blood test can be carried out and analysed in a timeframe that can help clinicians select a matched, targeted treatment. Currently, enrolment to trials depends on a patient's type of cancer or genetic data obtained from an invasive tumour biopsy, which is often months or years old and may not represent a patient's current disease due to their tumours' evolutionary changes over time.

The team from CBC showed that a small volume of blood can contain up-to-date genetic information about a patient's cancer to inform treatment choices. In this feasibility study of the first 100 patients, 11 were enrolled onto an available and molecularly matched clinical trial.

In the first of the two-part trial, called TARGET (Tumour chARacterisation to Guide Experimental Targeted therapy), the researchers were able to collect, process and analyse blood samples from 100 patients in the Manchester area. The second part of TARGET, which is already underway, will show how often the blood test is successful at matching patients to early phase clinical trials and the impact this has on their overall survival. There is also an option of referring patients to other clinical trial sites within the Experimental Cancer Medicine Centre (ECMC) network, if suitable matched trials are available in other parts of the country.

Mohan S, Ayub M, Rothwell DG, Gulati S, Kilerci B, Hollebecque A, Sun Leong H, Smith NK, Sahoo S, Descamps T, Zhou C, Hubner RA, McNamara MG, Lamarca A, Valle JW, Dive C, Brady G.
Analysis of circulating cell-free DNA identifies KRAS copy number gain and mutation as a novel prognostic marker in pancreatic cancer.
Scientific Reports 2019; 9(1):11610.

Using serial biopsy of pancreatic ductal adenocarcinoma (PDAC) to chart tumour evolution presents a significant challenge. The Cancer Biomarker Centre examined the utility of circulating free DNA (cfDNA) as a minimally invasive approach across a cohort of 55 treatment-naïve patients with PDAC; 31 with metastatic and 24 with locally advanced disease. Somatic mutations in cfDNA were detected using next generation sequencing in 15/24 (62.5%) and 27/31 (87%) of patients with locally advanced and metastatic disease respectively. Copy number changes were detected in cfDNA of 10 patients of whom seven exhibited gain of chromosome 12p harbouring *KRAS* as well as a canonical *KRAS* codon 12 mutation. In multivariable Cox Regression analysis, they show for the first time that patients with *KRAS* copy number gain and *KRAS* mutation have significantly worse outcomes, suggesting that this may be linked to PDAC progression. The simple cfDNA assay the team describe will enable determination of the presence of *KRAS* copy number gain and *KRAS* mutations in larger studies and clinical trials.

Mohan S, Foy V, Ayub M, Leong HS, Schofield P, Sahoo S, Descamps T, Kilerci B, Smith NK, Carter M, Priest L, Zhou C, Carr TH, Miller C, Faivre-Finn C, Blackhall F, Rothwell DG, Dive C, Brady G.
Profiling of circulating free DNA using targeted and genome wide sequencing in patients with small cell lung cancer.
Journal of Thoracic Oncology [Epub 16 October 2019]

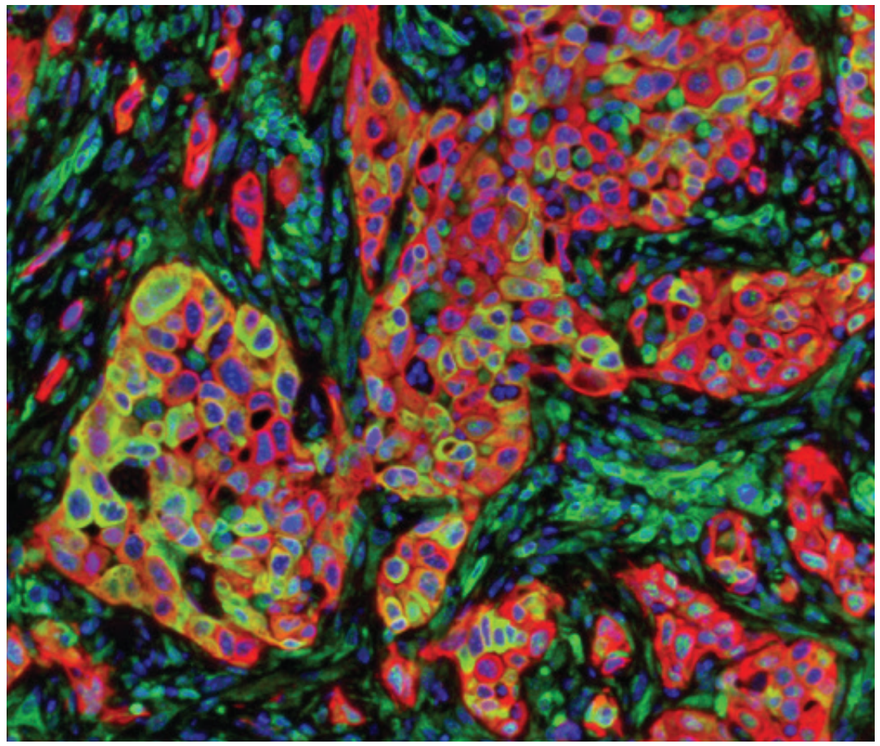
Tissue biopsies and especially serial biopsies pose a significant challenge in SCLC as they are typically of poor quality and/or unavailable for research. Surgical resections of the tumour are rare, yet by necessity most genomic studies so far have been performed on resected tumours and hence, under representative of the majority of the patients. Several targeted therapies have now entered clinical trials and analysis of circulating free DNA (cfDNA) is a minimally invasive approach for disease monitoring and stratification. To this end, members of the Cancer Biomarker Centre developed a novel cfDNA workflow for disease monitoring using genome-wide copy number aberration (CNA) analysis as well as targeted mutation analysis of 110 SCLC associated genes. This workflow was applied to pre-treatment cfDNA from 69 patients with extensive-stage (ES) and limited-stage (LS) SCLC to establish sensitivity and feasibility of the approach. Their data show tumour-related changes (CNAs and/or somatic mutations) could be detected in cfDNA from 94% LS and 100% of ES patients. Among the 110 genes analysed, a total of 272 somatic mutations were identified, including driver alterations in 69% of samples of which 60% were potentially targetable. In multivariate analysis, both metrics from CNA analysis as well as the number of mutations were significantly associated with the stage of disease. Further, in a subset of six patients the team explored the utility of cfDNA analysis for disease monitoring using longitudinal samples. Four patients had baseline CNAs that were undetectable on completion of treatment, a fifth patient had 10-fold reduction of CNA and no notable CNA change in a sixth patient. At relapse, CNAs were detectable in four of the five patients and the sixth patient with undetectable CNA relapsed with only brain metastasis and stable lung disease. The liquid biopsy approaches reported here have been shown to provide effective baseline analysis and longitudinal monitoring of both LS and ES disease and are now being incorporated into extended SCLC studies and trials.

Porter AP, White GRM, Mack NA, Malliri A.
The interaction between CASK and the tumour suppressor Dlg1 regulates mitotic spindle orientation in mammalian epithelia.
Journal of Cell Science 2019; 132(14) jcs230086.

Epithelial cells normally align their divisions with the plane of the epithelium, ensuring that daughter cells are produced side-by-side. In 3D this leads to single layers of epithelial cells surrounding a hollow lumen or acini, such as those found in the kidney or breast. Spindle misorientation can disrupt the integrity of the epithelial architecture, potentially promoting tumourigenesis. The Cell Signalling group uncovered a new regulator of mitotic spindle orientation, calcium/calmodulin-dependent serine protein kinase (CASK). CASK binds to and recruits the tumour suppressor Dlg1 to the lateral cell membrane where Dlg1 in turn recruits core components of the spindle orientation complex. Cells depleted for CASK show reduced Dlg1 recruitment, misoriented cell divisions, and form 3D structures containing multiple lumens. They showed the significance of the CASK-Dlg1 interaction in two ways. First, direct disruption of the CASK-Dlg1 interaction by inducibly expressing an interfering peptide phenocopies the effect of depleting CASK. Second, depletion of Dlg1 results in spindle misorientation, which can only be rescued by expression of wild-type Dlg1 and not by Dlg1 lacking the CASK interaction domain. Together, these results reveal the critical importance of the CASK-Dlg1 interaction for spindle orientation, which has important implications for the correct formation of epithelia.

Leung L, Niculescu-Duvaz D, Smithen D, Lopes F, Callens C, McLeary R, Saturno G, Davies L, Aljarah M, Brown M, Johnson L, Zambon A, Chambers T, Ménard D, Bayliss N, Knight R, Fish L, Lawrence R, Challinor M, Tang H, Marais R, Springer C.
Anti-metastatic inhibitors of lysyl oxidase (LOX): design and structure-activity relationships.
Journal of Medicinal Chemistry 2019; 62(12):5863-5884.

Lysyl oxidase (LOX) is a secreted copper amine oxidase that serves as the master crosslinker of



Multiplex IHC images of murine prostate (pten-/-/ p53-/-) tumours stained for STING (green), CK8 (Red), and DAPI (Blue) following treatment with radiotherapy. Radiotherapy leads to an increase in STING staining. IHC staining was performed using the Bond (Rx) staining platform (CRUK MI Core facility) and imaged using Olympus VS120 microscope (Advanced imaging).

Image supplied by Debayan Mukherjee (Targeted Therapy - Division of Cancer Sciences, The University of Manchester)

collagens and elastin in the extracellular matrix. LOX and its isoforms are critical mediators of tumour growth and metastatic spread, and have been firmly established as therapeutic targets in oncology and fibrosis. However, the only effective LOX inhibitor available until recently is β-aminopropionitrile, a non drug-like molecule that is unsuitable for clinical development. The Drug Discovery Unit describe the discovery of a novel series of aminomethylenethiophene (AMT) inhibitors in the first detailed study dedicated to inhibitors of this LOX isoform. Significantly, they detailed structure-activity relationships around the AMT pharmacophore leading to great improvement in potency over the HTS hit. This is followed by further structural modifications to overcome in vivo stability issues while retaining LOX inhibition potency, leading to the orally available LOX inhibitor CCT365623. They demonstrated that CCT365623 also inhibits LOXL2 at a similar level and has an excellent selectivity profile against four other non-LOX amine oxidases. Above all, this inhibitor is effective in suppressing breast cancer metastasis to the lungs in their transgenic mouse model and thus demonstrating its promise as a drug candidate.

Smithen DA, Leung LMH, Challinor M, Lawrence R, Tang H, Niculescu-Duvaz D, Pearce SP, Mcleary R, Lopes F, Aljarah M, Brown M, Johnson L, Thomson G, Marais R, Springer C.
2-Aminomethylene-5-sulfonylthiazole inhibitors of lysyl oxidase (LOX) and LOXL2 show significant efficacy in delaying tumor growth.
Journal of Medicinal Chemistry [Epub 4 September 2019]

The lysyl oxidase (LOX) family of extracellular proteins play a vital role in catalysing the formation of crosslinks in fibrillar elastin and collagens leading to extracellular matrix (ECM) stabilisation. These enzymes are known to promote tumour progression and metastatic disease, and have thus become an attractive therapeutic target for many types of invasive cancers. Following on from the Drug Discovery Unit's recently published work on the discovery of aminomethylenethiophenes (AMTs) as potent, orally bioavailable LOX/LOXL2 inhibitors, this report details the development of aminomethylenethiazoles (AMTz) as potent inhibitors of three LOX isoforms, as well as a subseries of LOXL2-selective inhibitors. Major findings include the discovery that a thiazole core leads to improved potency towards LOXL2 via an irreversible mode of inhibition. Structure-activity relationship studies enabled the development of a predictive LOXL2 3DQSAR model, and further selectivity and ADME assessment resulted in the identification of a lead compound (21b) that demonstrates excellent selectivity over common amine oxidases and hERG, as well as improved Caco-2 permeability and pharmacokinetic properties in two rodent species. This inhibitor was also evaluated in our transgenic LOX-driven breast cancer model where it displays excellent anti-tumour efficacy, with significantly reduced tumour growth, thus showing promise as a drug candidate for further study.

Deb G, Wingelhofer B, Amaral FMR, Maiques-Diaz A, Chadwick JA, Spencer GJ, Williams EL, Leong HS, Maes T, Somervaille TCP.
Pre-clinical activity of combined LSD1 and mTORC1 inhibition in MLL-translocated acute myeloid leukaemia.
Leukemia [Epub 28 November 2019]

Acute myeloid leukaemia (AML) is a heterogeneous group of proliferative malignancies characterised by an accumulation of poorly differentiated myeloid blasts in bone marrow and blood. The histone demethylase lysine-specific demethylase 1 (LSD1 or KDM1A) has emerged as a candidate therapeutic target in AML and 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, in particular those with AML associated with translocations targeting the Mixed Lineage Leukaemia gene. However, as for all agents in AML, it is unlikely that LSD1 inhibitors will be highly effective as single agents. Through a genome-wide CRISPR-Cas9 dropout screen, the Leukaemia Biology group identified genes and cellular pathways that collaborate with LSD1 to maintain the leukaemic phenotype, and which could be targeted by combination therapies. They identified multiple components of the amino acid sensing arm of mTORC1 signalling as cellular sensitisers to LSD1 inhibition. Knockdown of mTORC1 components or mTORC1 pharmacologic inhibition in combination with LSD1 inhibition synergistically induced the expression of a set of transcription factor genes associated with terminal monocytic lineage differentiation, resulting in enhanced differentiation in both cell line and primary cell settings in vitro and in vivo. Altogether, the data suggested that dual mTORC1 and LSD1 inhibition represent a candidate combination approach for enhanced differentiation in *MLL*-translocated AML, which could be evaluated in early phase clinical trials.

Williams MS, Amaral FM, Simeoni F, Somervaille TC.
A stress-responsive enhancer induces dynamic drug resistance in acute myeloid leukemia.
Journal of Clinical Investigation [Epub 26 November 2019]

The drug efflux pump ABCB1 is a key driver of chemoresistance, and high expression predicts for treatment failure in acute myeloid leukaemia (AML). In this study, the Leukaemia Biology group identified and functionally validated the network of enhancers that controls expression of *ABCB1*. They show that exposure of leukaemia cells to daunorubicin activated an integrated stress response-like transcriptional program to induce *ABCB1* through remodelling and activation of an ATF4-bound, stress-responsive enhancer. Protracted stress primed enhancers for rapid increases in activity following re-exposure of cells to daunorubicin, providing an epigenetic memory of prior drug treatment. In primary human AML, exposure of fresh blast cells to daunorubicin activated the stress-responsive enhancer and led to dose-dependent induction of *ABCB1*. Dynamic induction of *ABCB1* by diverse stressors, including chemotherapy, facilitated escape of leukaemia cells from targeted third-generation ABCB1 inhibition, providing an explanation for the failure of ABCB1 inhibitors in clinical trials. Stress-induced up regulation of *ABCB1* was mitigated by combined use of pharmacologic inhibitors U0126 and ISRIB, which inhibit stress signalling and have potential for use as adjuvants to enhance the activity of ABCB1 inhibitors.

Yoshimi A, Lin KT, Wiseman DH, Rahman MA, Pastore A, Wang B, Lee SC, Micol JB, Zhang XJ, de Botton S, Penard-Lacronique V, Stein EM, Cho H, Miles RE, Inoue D, Albrecht TR, Somervaille TCP, Batta K, Amaral F, Simeoni F, Wilks DP, Cargo C, Intlekofer AM, Levine RL, Dvinge H, Bradley RK, Wagner EJ, Krainer AR, Abdel-Wahab O.
Coordinated alterations in RNA splicing and epigenetic regulation drive leukaemogenesis.
Nature 2019; 574(7777):273-277.

Dan Wiseman, formerly of the Leukaemia Biology group, and colleagues had previously identified in their Christie NHS Foundation Trust patient cohort an unappreciated frequent association between gain-of-function mutations in the metabolic gene IDH2 and the spliceosome component SRSF2 in acute myeloid leukaemia (AML). In collaboration with Memorial Sloan Kettering Cancer Center they confirmed this across several large publically available sequencing datasets, characterising a new AML molecular subtype affecting 5-10% of patients with distinctive clinical and DNA methylation features. Through in vivo modelling studies they demonstrated a functional collaboration between these mutations, which promoted acute leukaemogenesis when present together as compared with either alone. Comprehensive RNA sequencing of patient samples, including a large cohort from the MCRC Biobank, identified substantially more aberrant splicing in double mutant cases than SRSF2 single-mutants. Amongst the genes most differentially spliced in this context was INTS3, which encodes a component of the Integrator complex with multiple roles in transcriptional regulation. A double intron retention event resulted in increased nonsense-mediated decay, and was associated with broader integrator dysfunction. Mechanistically they showed that INTS3 missplicing was concordant with increased stalling of RNA polymerase II, and dependent on mutant SRSF2 binding to cis elements in INTS3 mRNA. They further showed that INTS3 depletion phenocopied SRSF2/IDH2 mutations in promoting hallmarks of leukaemogenesis in myeloid differentiation cell model systems. Overall, this study identified a pathogenic crosstalk between altered epigenetic state and aberrant splicing in a distinct subset of AML. This may have translational importance, given that targeted therapies inhibiting both mutations are in clinical trials.

CANCER RESEARCH UK MANCHESTER INSTITUTE

RESEARCH GROUPS

Organotypic brain slice (grey scale) invasion by patient-derived melanoma cells tagged with GFP (green). Illustrating melanoma cell capacity to invade into the brain tissue ex vivo.

Image supplied by Denys Holovanchuk (Molecular Oncology)

www.cruk.manchester.ac.uk/Our-Research/Cancer-Biomarker-Centre

Cancer Research UK Manchester Institute Cancer Biomarker Centre

Group Leader
Caroline Dive

Deputy Group Leader
Ged Brady

Staff Scientists
Alastair Kerr[1]
Elaine Kilgour
Dominic Rothwell
Jonathan Tugwood

Associate Scientists
Kris Frese
Kathryn Simpson

Research Associate
Carlos Lopez-Garcia[1]

QA Manager
Tony Price

Postdoctoral Fellows
Katarzyna Bloch
Francesca Chemi
Adam Fletcher[2]
Ellyn Hughes[1]
Sumitra Mohan
Maria Peiris-Pages
Ruth Stoney[2]
Stuart Williamson[2]

Bioinformaticians
Tine Descamps
Saba Ferdous
Cheryl Jones
Bedirhan Kilerci[1]
Katarzyna Murat[1]
Bethanie Neale[2]
Simon Pearce
Matthew Roberts
William Rowe[2]
Cong Zhou

Clinical Informaticians
Jenny Bradford[2]
Fouziah Butt
Katherine Dempsey[2]
Paul Fitzpatrick

2019 was a busy year for the team. In February our new Good Clinical Practice (GCP) facilities at Alderley Park were inspected by the MHRA. In April, we had a successful CRUK quinquenial review that resulted in a new name, the CRUK Manchester Institute Cancer Biomarker Centre (CBC). We played a significant role in the renewal and expansion of the CRUK Lung Cancer Centre of Excellence and our digital Experimental Cancer Medicines Team obtained a CRUK Accelerator Award for Digital Clinical Trials. Our overall goals remain a) to discover, develop, validate and implement biomarkers that inform personalised cancer medicine; and b) to characterise and exploit our panel of circulating tumour cell (CTC) derived models of small cell lung cancer (SCLC) to discover new targets and test new therapies. We developed our immune-oncology biomarker activities and recruited Dr Alastair Kerr to lead the new Bioinformatics and Biostatistics (BBS) team. CBC's highlighted study published in Nature Medicine reported utility of ctDNA to inform selection of a patient's Phase I clinical trial and non-small cell lung cancer (NSCLC) relapse risk prediction by pulmonary vein CTC number at surgery. CBC contributed to Manchester's success with the International Alliance for Cancer Early Detection (ACED) and is supporting Cancer Research UK RadNet Manchester with biomarker sciences.

The biomarker portfolio

CBC supported 19 clinical trials in 2019. The pioneering CACTUS melanoma trial led by Professors Paul Lorigan and Richard Marais employs a CBC validated ctDNA assay primary endpoint, reporting in real-time to instruct treatment switch from targeted- to immunotherapy. CBC biomarker development also supports upcoming trials examining proton beam therapy in the newly established Proton Beam Therapy Centre at The Christie NHS Foundation Trust (CHFT). A CBC 'first' this year involved transfer of our GCP ELISA for plasmaTie2 (pTIE2) to The CHFT Diagnostic Biochemistry Laboratory for further validation towards routine NHS use. pTIE2 dynamics inform management of anti-angiogenic therapies, a hypothesis examined within the CRUK funded VALTIVE1 trial in ovarian cancer; a culmination of >10 years biomarker research with Professor Gordon Jayson with biomarker data modelling by the BBS team.

Understanding SCLC biology and the search for new therapy targets

Despite multiple clinical trials, SCLC standard of care remained unchanged for >3 decades until the recent introduction of immunotherapy, which benefits a minority of patients. Although phenotypic heterogeneity was reported 30 years ago, the World Health Organisation recognises a single morphological classification of SCLC. Molecular classification based on neuroendocrine transcription factors (NE TFs, developed in 2019) may support future precision medicine. The Preclinical Pharmacology (PP) team applied RNAseq to our 47 patient CTC derived explant models (CDX) and hierarchically clustered the data; principal component analysis revealed at least 4 subgroups, including those expressing previously identified transcriptional drivers ASCL1, NEUROD1, and POU2F3 and a new subgroup expressing ATOH1 (Figure 1). Roles of ATOH1 in tumour maintenance and/or progression and druggable downstream targets are under investigation.

Hannah Frost[1]
Andrew Hughes
Laura Hutchinson[2]
Donal Landers
Leanne Ogden
Paul O'Regan
Jenny Royle
Laura Stephenson
Julie Stevenson
Siobhan Southam[2]
Jason Swift
Jenny Ward

Clinical Fellows
Alicia Conway
Victoria Foy
Melissa Frizziero[1]
Matthew Howell

Graduate Students
Alessia Catozzi
Jakub Chudziak
Sam Humphrey
Alice Lallo
Julia Ogden[1]
Sarah Pearsall
Max Schenk

Scientific Officers
Eleanor Anderson[2]
Sophie Atkinson[1]
Mahmood Ayub[2]
Samantha Barlow[2]
Dominic Birch
Kieran Bradbury
Nicholas Brittain[2]
Laura Booth[2]
Stewart Brown
Debbie Burt
Holly Butterworth
Rebecca Carroll[1]
Mathew Carter
Alex Clipson
James Copley[1]

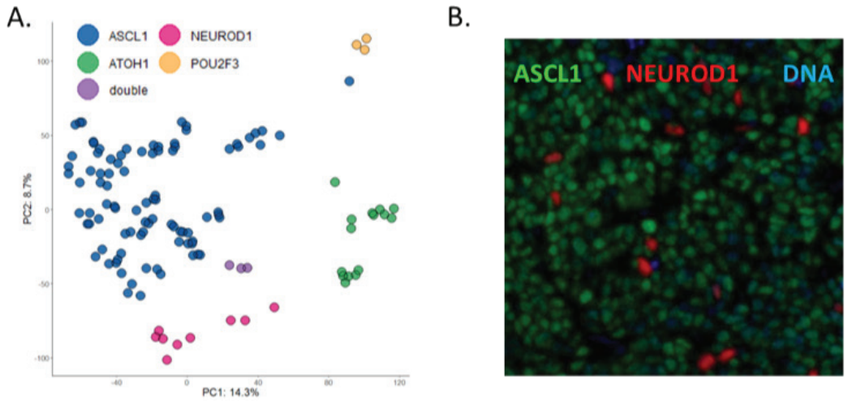


Figure 1. SCLC subtype based on NE TFs: A) PCA plot of CDX model RNAseq data. B) Multiplex immunofluorescence showing co-expression of ASCL1 and NEUROD1 in a CDX model.

CDX models generated from the same patient before treatment and at disease progression are facilitating discovery of acquired drug chemoresistance mechanisms, including a nitric-oxide activated pathway and acquired resistance by serial chemotherapy treatment of mice bearing CDX augments our chemoresistance mechanism(s) search (Figure 2).

Vasculogenic mimicry in SCLC

Angiogenesis is a well characterised mechanism exploited by solid tumours to overcome limited blood supply and enable tumour growth. Vasculogenic mimicry (VM) is an alternative, tumour autonomous mechanism of *de novo* vessel genesis. The PP team previously reported VM in SCLC biopsies associated with poor prognosis. VM is present in most CDX models and in two SCLC genetically engineered mouse models (GEMMs) (Figure 3).

SCLC *ex vivo* cultures often contain morphologically and phenotypically distinct cell populations; neuroendocrine (NE) and non-neuroendocrine (NNE) cells grow in suspension and as adherent monolayers respectively. Only the minority NNE cells form characteristic VM networks when grown on matrigel (Figure 4 shown overleaf). In both GEMM and CDX models, NNE cells remodel collagen in the matrigel to form VM structures, indicating a role

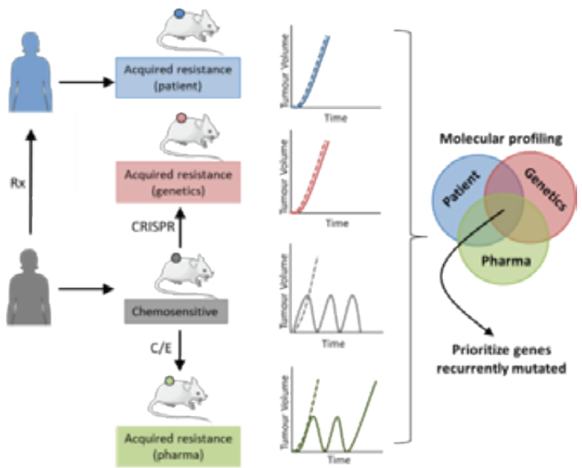


Figure 2. Comparison of acquired resistance developed in the patient and in the lab will reveal molecular mechanisms of drug resistance.

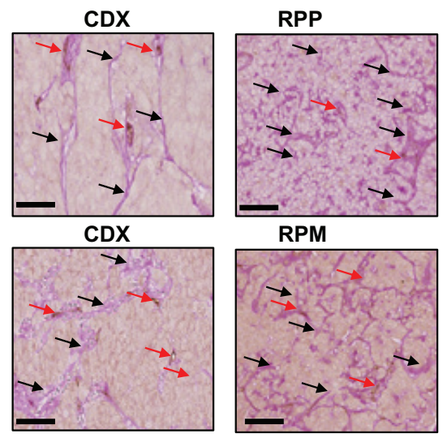


Figure 3. VM vessels in CDX and GEMMs. PAS+/CD31- VM vessels (pink/black arrows), CD31+ endothelial vessels (brown/red arrows). RPP and RPM GEMMs are Rb/p53/p130 triple KO and Rb/p53 double KO with overexpression of c-Myc respectively. Black scale bar, 50 µm.

Joanne Felce
Alice Fickling[2]
Adam Freestone[1]
Lynsey Franklin
Melanie Galvin
Laura Glass[1]
Molly Glenister-Doyle[1]
Weronika Golinska[2]
Eleanor Gregory
Hannah Gregson[2]
Grace Hampson
Thomas Helps[2]
Rebekah Higgins[2]
Sarah Hilton[2]
Michael Hoffs
Elizabeth Hunter[1]
Aileen Jardine
Hana Jelassi
Noel Kelso
Luke MacGregor[1]
Simrah Mohammad
Derrick Morgan
Karen Morris
Kamrun Nessa[2]
Anthony Oojageer
Nicola Overton
Safwan Patel
Rosalind Peason[1]
Jackie Pierce
Sabah Rashid[1]
Alan Redfern
Mitchell Revill
Caroline Roberts
Jordan Roebuck
Karishma Satia
Daniel Slane-Tan
Karen Smith[1]
Nigel Smith
George Thompson[2]
Isabelle Thompson[1]
Simon Topham
Erich van Hechnova[2]
Amelie Viratham[2]
Hannah Walsh
Daniel White

Junior Translational Scientist
Simon Hood[1]

Laboratory Manager
Matthew Lancashire

Laboratory Support Technician
Andrew Stevens

BRC Project Manager
Jonathan Wake

Programme Manager
Ekram Aidaros-Talbot

Administrative Coordinator
Suzanne Bickley

[1] Joined in 2019
[2] Left in 2019

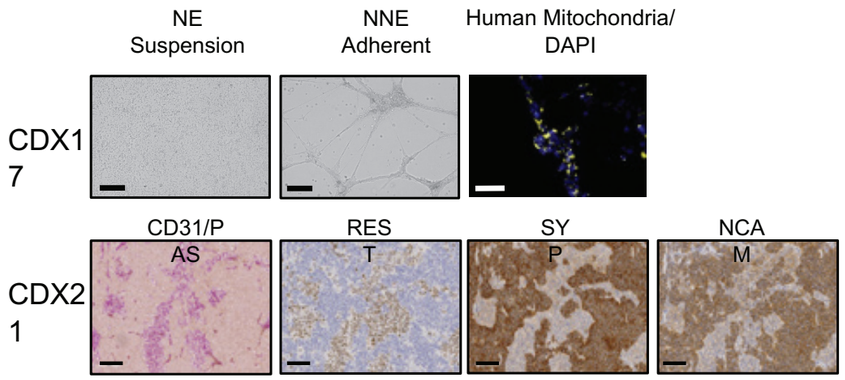


Figure 4. NNE cell undergo VM. Top row: VM networks in matrigel. Bottom row: IHC showing VM rich regions (PAS+/ CD31-, pink) co-localise with the NNE marker REST but not NE markers SYP and NCAM. Black scale bars, top row (bright field), 500 µm, black scale bars, (IHC) and white scale bar (IF, 100 µm).

in sensing and adapting to the tumour microenvironment. A deeper interrogation of cell intrinsic and environmental regulators of VM is underway.

Liquid biopsies for SCLC

The Nucleic Acids Biomarkers (NAB) team developed a molecular profiling and disease monitoring liquid biopsy workflow to support targeted treatment of SCLC. We examined genome-wide copy number aberrations (CNA) and targeted mutation analysis of 110 SCLC associated genes, including those within DNA damage repair pathways. We applied this workflow to pre-treatment cfDNA from patients with extensive-stage (ES) or limited-stage (LS) SCLC to establish sensitivity. Tumour-related changes (CNAs and/or somatic mutations) were detected in >93% patients with potentially targetable mutations in >50% cases (Mohan et al, JTO, 2019). In multivariate analysis, CNA analysis and mutation number significantly associated with disease stage. We explored utility of cfDNA analysis for disease monitoring; CNA profiles showed dynamic changes through therapy to disease relapse. We are currently extending longitudinal analysis to 130 patients to establish if our approach can become a routine patient-monitoring tool in the clinic.

Non-small cell lung cancer (NSCLC) CTCs at surgery to predict disease relapse and explore tumour evolution

Within the TRACERx consortium trial (TRAcking non-small cell lung Cancer Evolution through therapy [Rx]), we addressed whether CTCs in the pulmonary vein (PV-CTCs) of early stage NSCLC patients are responsible for metastatic dissemination and disease relapse. We enumerated PV-CTCs from 100 patients. PV-CTC number was associated with lung cancer specific relapse and remained an independent predictor of relapse in multivariate analysis adjusted for tumour stage. We performed molecular profiling of PV-CTCs to query genetic links between PV-CTCs, the resected tumour and subsequent metastasis. In a case study, there was a higher mutation overlap between PV-CTCs and metastasis detected 10 months later (91%) than between metastasis and primary tumour (79%), suggesting that early disseminating PV-CTCs were responsible for disease relapse (Chemi et al, Nat Med, 2019). We also examined genome-wide CNA from 70 single PV-CTCs compared to matched tumours. Two distinct populations of PV-CTCs were identified; 'Type A' showed clear CNA changes in common with the tumour, 'Type B' did not match the tumour. We now need to elucidate the origin of 'Type B' cells and ask whether and how they participate in early metastatic dissemination.

ctDNA to assist selection of Phase I clinical trials

TARGET Trial (Tumour chARacterisation to Guide Experimental Targeted therapy) recruitment with monthly Molecular Tumour Board meetings is ongoing with integration of clinical data, tumour mutation profiling and contemporaneous ctDNA data utilising our visualisation tool, eTARGET.

Feasibility stage A (100 patients) was published (Rothwell et al, Nat Med, 2019). Stage B is ongoing with >420 patients' ctDNA analysed. We are adding tumour mutation burden in ctDNA and immune biomarkers in biopsies to assist selection to immunotherapy trials (Figure 5).

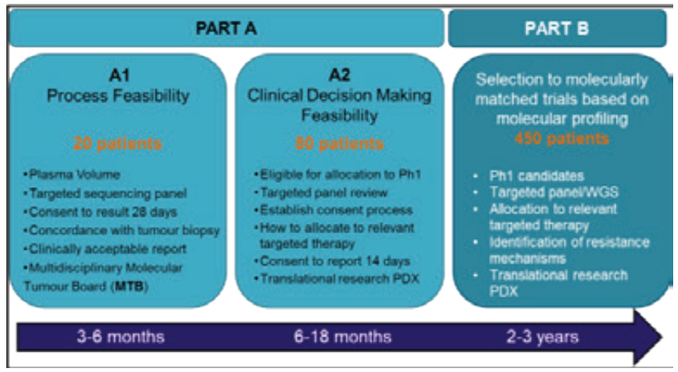


Figure 5:
Schematic of TARGET study.

ctDNA informing treatment switching

The CACTUS Trial (CirculAting Tumour DNA gUided therapy Switch) for advanced cutaneous melanoma phase II patients opened in May 2019. With PIs Professors Richard Marais and Paul Lorigan and Dr Rebecca Lee, the NAB team developed a primary endpoint, GCP validated, droplet digital PCR (ddPCR) ctDNA assay measuring BRAF and NRAS mutations (variant allele frequencies) to instruct treatment switch from targeted- to immunotherapy. The expertise with real-time reporting, developed during CACTUS, is being applied to DETECTION (Circulating tumour DNA guidEd Therapy for stage IIB/C BRAF or NRAS mutant- positive mElanoma after surgiCal resecTION), a CRUK funded trial of stage IIB/C melanoma patients that will involve ddPCR ctDNA analysis to detect early relapse/micro-metastatic disease and select patients for targeted therapy.

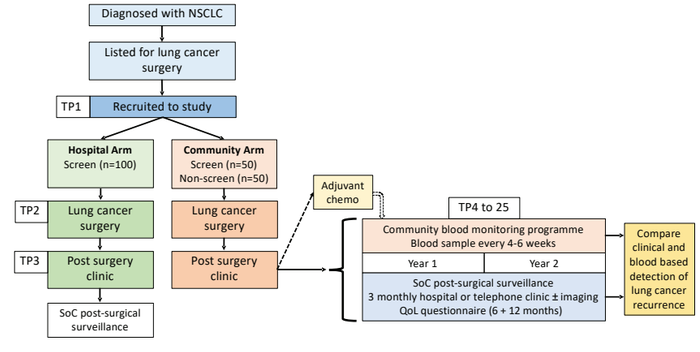


Figure 6:
The COMPASS MRD study.

ctDNA for the early detection of cancers

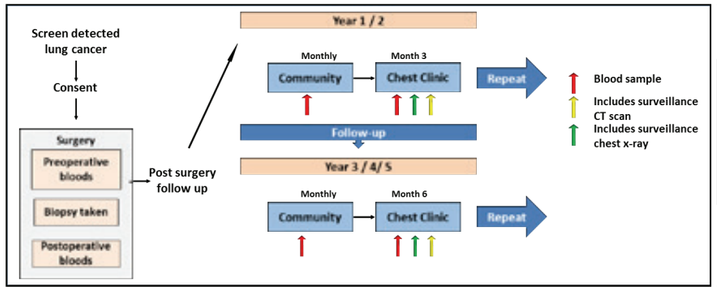
The NAB team continued to develop ctDNA methylation assays and molecular barcoding approaches to increase assay sensitivity. Assay evaluation is ongoing using our biobank of plasma samples from the Manchester Community Lung Health Study pilot with Dr Philip Crosbie (Manchester University NHS Foundation Trust, MFT) where results of low dose CT scans and cancer diagnoses are known. We are also developing nanoparticle-based approaches to enrich for ctDNA in collaboration with Professor Kostas Kostarelos (The National Graphene Centre).

Minimal residual disease (MRD) monitoring in NSCLC

In collaboration with Dr Phil Crosbie and his clinical team at MFT, set up of the COMPASS study was completed and will be open to recruitment in January 2020. After surgery, patients with community screen-detected NSCLC give monthly blood samples in their community Lloyds Pharmacy for collection and transport to CBC laboratories in vans with the CRUK logo (Figure 6) to support development of a liquid biopsy that detects relapsing disease earlier. Resected tumour tissue and nasal epithelium (as a surrogate tissue) are collected at surgery to seek early prediction of relapse. Nasal samples will be analysed in collaboration with Professor Avrum Spira, Boston University Medical Centre by our CRUK Lung Cancer Centre of Excellence (LCCE)-funded travelling postdoc, Dr Kate Bloch in the Spira laboratory. Tumour and blood sample analysis will be performed in collaboration with Professor Charlie Swanton's team at University College London within the LCCE. With Dr Santiago Zelenay we will assess the prognostic significance of his COX2 inflammatory signatures in early stage tumours.

Biomarkers to inform immunotherapy trials

The Cells and Proteins (CAP) team expanded their core immune biomarker 'toolkit'. New assays include T-cell receptor sequencing (TCRseq) with



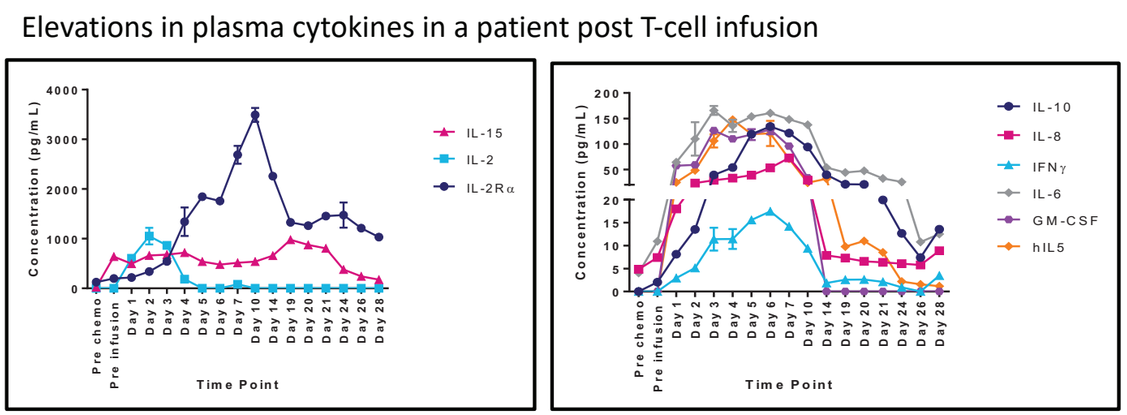


Figure 7:
Plasma cytokine dynamics levels from a patient after T-cell infusion therapy.

the NAB team, multiplex IF assays to landscape the tumour microenvironment with the Tissue Biomarker team, and assays for PDL1 in CTCs. In partnership with ThermoFisher, we installed the IonTorrent platform for immune biomarker assays including TCRseq for analysis of clonality, diversity and convergence. With Professor Fiona Blackhall, we are assessing CTCs, ctDNA and TCRseq in the first SCLC patients receiving immunotherapy at the CHFT. Tissue and circulating immune assays are being implemented in melanoma, renal cancer, cancer of unknown primary and extra pulmonary-neuro endocrine tumour patient studies.

Ex vivo co-cultures of matched peripheral blood lymphocytes and CTCs from SCLC patients are under development as a potential platform to predict immunotherapy responses and explore resistance mechanisms. Within iMATCH (Innovate Manchester Advanced Therapies Centre Hub), we validated a multiplex ELISA assay to monitor cytokine storms in patients receiving advanced T-cell therapies with Professor Fiona Thistlethwaite (CHFT, Figure 7), and with Dr Phil Monaghan (CHFT Diagnostic Biochemistry Laboratory) we will transfer and further develop this assay for routine clinical use.

With the digital ECMT, we are evaluating feasibility of home blood sampling kits to assess cytokines as an early warning of adverse events and cytokine release syndrome in patients receiving immunotherapy and advanced T-cell therapies.

Tissue biomarkers for preclinical research and clinical trials

The Tissue Biomarker (TB) team expanded their portfolio of chromogenic assays (>20 antibodies) enabling more detailed interrogation of SCLC subtypes based on NE TFs, MYC family proteins (Figure 8), VM and acquired chemoresistance in CDX models. The TB team is developing tissue biomarkers applicable to patient biopsies within clinical trials of immunotherapies linking with the CAP team. The TB team will merge with the CHFT Academic Pathology Unit in 2020, bringing a wealth of pathology expertise to assist with CBC goals.

Bioinformatics and biostatistics

The Bioinformatics and Biostatistics (BBS) team is involved in multiple collaborations and runs a weekly statistics clinic to assist medical students and clinicians (15 projects in 2019). In an ongoing collaboration, the angiogenesis biomarker plasma Tie2 was examined in three clinical trials culminating this year in CRUK funding to translate it from GCP to routine NHS use. BBS supports experimental design, for example: the NOTION study with digital ECMT to assess feasibility of at home blood sampling to monitor patients treated with immunotherapy; TRACERx NEO within the LCCE to continue exploration of NSCLC evolution; and the EPIC study with Phase I Trialists at CHFT to access impact of patients' physiological status on clinical outcome. The BBS team implemented best practice pipelines including quality control metrics and is working with CRUK MI Scientific Computing to enable use across the Institute. The bespoke pipeline for the TARGET trial was upgraded to include the new MuTec2 mutation caller and new pipelines are in development, e.g. for the NAB team's new T7 workflow helping shape experimental decisions and codifying robust analysis.

Digital clinical trials

The digital Experimental Cancer Medicine team (www.digitalecmt.org) made significant progress in development and release of several digital healthcare systems supporting experimental cancer medicine clinical trials: (i) eTARGET, the tool used by the monthly MCRC Molecular Tumour Board to identify cancer-driving genetic alterations and then identify the best treatment/clinical trial; and (ii) REACT, enabling intuitive and iterative visualisations of patient data derived from clinical trials for investigators to better understand the safety and efficacy profile of a drug. REACT is being used by a number of clinical trial sponsors including the CRUK Centre for Drug Development and Pharma companies conducting clinical trials at MCRC.

'Technology' clinical trials

The dECMT also established several 'technology' clinical trials (software, algorithms, medical devices), which evaluate not only performance characteristics of technology but also how they enable changes in the patient care pathway inside and outside of hospitals. These included clinical trials of a system that captures patient-reported outcomes electronically and remotely; a trial measuring renal function in a patient's home based on a pin-prick of blood; and a clinical trial in which patients post a dry bloodspot which is reconstituted by the CAP team to measure immune function of patients on immunotherapy combinations.

Publications listed on page 64

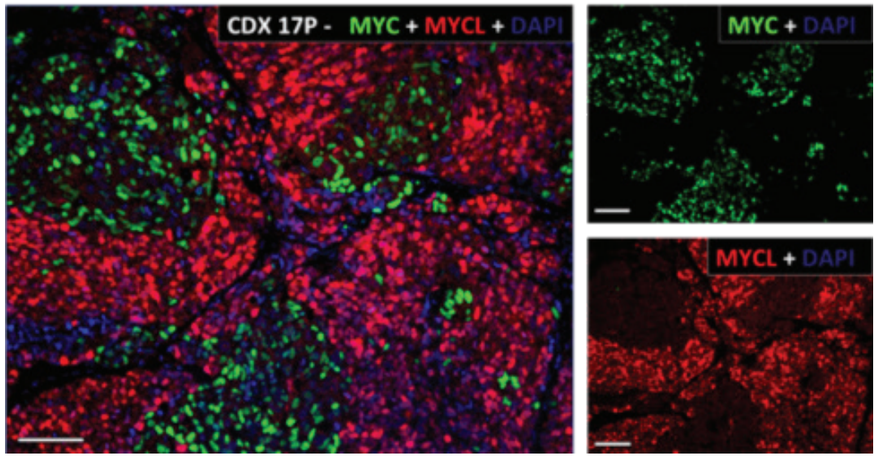


Figure 8:
MYC family proteins in a SCLC CDX.
MYC (green), MYCL (red) and DAPI (blue), white scale bars, 100 µm.

CANCER INFLAMMATION AND IMMUNITY

Group Leader
Santiago Zelenay

Postdoctoral Fellows
Eduardo Bonavita
Agrin Moeini
Victoria Pelly

Scientific Officer
Shih-Chieh Chiang

Graduate Students
Charlotte Bell
Christian Bromley
Eimear Flanagan

Basic research in immunology has led to the development of practice-changing treatments such as those based on the use of immune checkpoint inhibitor blocking antibodies. These therapies have led to unprecedented responses in patients with late-stage cancers. While they continue to be tested across multiple malignancies and in different clinical settings, it has become evident that for nearly all cancer types only a very reduced fraction of patients experience profound and long-lasting responses. Much more often than not patients derived transient or no benefit at all.

The Cancer Inflammation and Immunity group studies the principles that determine outcome from immunotherapy with a focus on the underlying mechanisms that favour immune escape and treatment failure. Combining basic and translational research, our work has uncovered broadly conserved pathways associated with pro-tumourigenic inflammation and immune evasion. Monitoring the activity of these inflammatory pathways in pre-treatment patient biopsies shows promise as an indicator of treatment outcome. Moreover, their therapeutical manipulation represents a promising approach to augment the efficacy of immunotherapy and other cancer treatments that rely on the anti-cancer function of the immune system.

Already recognised as pillars of oncology treatment, immune checkpoint drugs have transformed the landscape of cancer management. In cutaneous melanoma, the deadliest form of skin cancer, these treatment modalities have become the standard of care for patients with advanced disease, and specific combinations of checkpoint inhibitor drugs result in a five-year survival rate for more than half of patients. However, the fraction of complete and durable responses is much lower for the overwhelming majority of tumour types. In this context, the greatest challenges in the immunotherapy field are the lack of effective alternative treatments for non-responders and of suitable biomarkers to predict who those patients might be. This is particularly relevant given immunotherapy is very costly and has side effects, often leading to the development of serious toxicities even in patients with tumours that do not respond. Under the conviction that solutions to these problems require further basic understanding of the cellular and molecular players involved in anti-cancer immune responses, we continue our search for instructive pathways that influence the inflammatory profile and immunity at the tumour site.

In this context, a qualitative step forward during last year was the successful incorporation into our set of research tools of cutting-edge methodologies that allow a comprehensive profiling of the tumour immune microenvironment at the single cell level or enable multiplexing with spatial resolution. Collectively, the use of these technologies has provided further support to our general working paradigm that the inflammatory response at the tumour site is a vital determinant of tumour fate and response to immunotherapy. Our data suggests that certain immune cells, even when classically present in very low frequency within tumours, can have a major effect in the overall activation and polarisation status of multiple populations, including those that are much more prevalent intratumourally. Likewise, we have often encountered situations in our mouse models in which, at particular points in time, the cellular immune composition of tumours can be remarkably similar and yet the molecular landscape is radically different. Of note, the latter more robustly anticipate the fate of those tumours exposing the limitation of approaches that merely aim at monitoring the relative abundance of immune cells without establishing their activation status through molecular profiling.

These analyses combined with that of patient datasets provided further evidence to our theory that there is no such thing as 'inflamed' or 'non-inflamed' tumours but rather different types of inflammatory 'flavours'. In this regard, we have identified a limited set of inflammatory factors that can be used to define type of inflammatory landscape. A group of these factors, routinely co-expressed across all cancer types, promote immune evasion, therapy resistance and progressive growth. Others by contrast, instigate a cancer-restraining response and mediate spontaneous and therapy-induced immune-dependent control of tumours. Of special interest, translating our results to human cancer we have found evidence that the inflammatory status of baseline samples monitored by transcriptional profiling of a selected number of mediators has profound prognostic utility. Likewise, these inflammatory signatures predict outcome from immunotherapy in many tumour types and compare favourably when benchmarked against current established biomarkers of response to immune checkpoint inhibitors. One of these gene signatures and the scoring method to establish it from transcriptional data have been patented by Cancer Research UK Technologies Ltd. A remarkable feature is that its composition in terms of the individual gene elements was defined through the characterisation of our genetically-engineered transplantable mouse models. Yet, the gene signature predicts responses in human cancers highlighting the conserved features and parallelisms of mouse and humans.

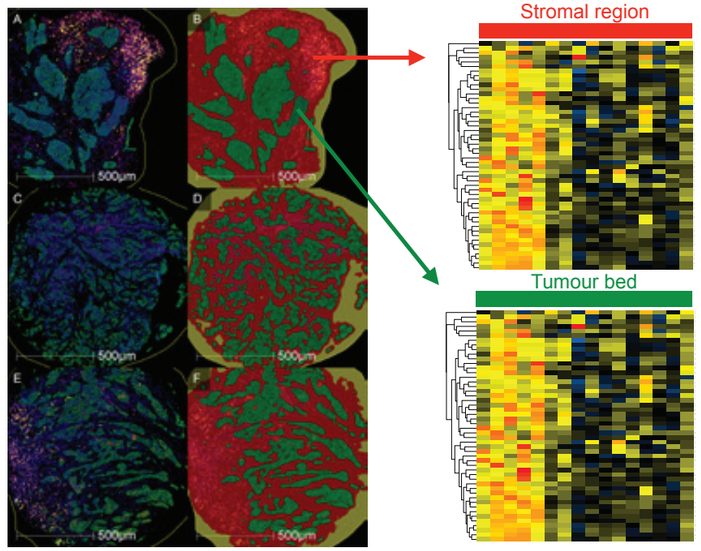


Figure 1
Classifying tumour and stromal regions in the triple-negative breast cancer microenvironment. Nuclear (blue), pan-cytokeratin (green), CD45 (red) and CD3 (yellow) staining of a tissue microarray containing cores from pre-treatment, needle biopsies from patients with triple-negative breast cancer. (A), (C) and (E) show the merged multiplex immunofluorescence image of three patient cores, and (B), (D) and (F) highlight algorithmically-defined regions of tumour bed and stroma. Using the Nanostring GeoMx platform we obtained transcriptomic profiles from these different tumour microenvironment compartments. Bioinformatic methods were then used to integrate data from digital pathology analysis, and from spatially-distinct transcriptomic profiling.

Encouraged by these exciting findings, we are refining our inflammatory signature using machine learning approaches. This bioinformatic analysis has, in turn uncovered that the approach is particularly prognostic for certain tumour types such as lung, breast or kidney cancer. Therefore, we are now analysing in further detail these three tumour types expanding our research portfolio considerably. Notably, we have very recently built and analysed a tissue microarray of baseline biopsies of patients newly diagnosed with triple-negative breast cancer. Profiting from a pump priming grant from the Manchester Biomedical Research Centre, we have subjected tissue microarray to a pioneering methodology to obtain both quantitative protein and gene expression levels and spatial context on a single tissue section (Figure 1). The data has provided new insights into the inflammatory and immune tissue biology of the tumour microenvironment in a cancer type of dismal prognosis in which an effective treatment remains an urgent unmet medical need.

In parallel, we continue our efforts to identify therapeutical targets to use in combination with immunotherapies based on immune checkpoint inhibitors. Aligned with our postulate that certain inflammatory responses prevent effective immunity at the tumour bed, we combined immune-based therapy and standard cancer treatments with specific anti-inflammatory drugs. Using mouse models and various types of drugs administered in a clinically relevant manner, we have made significant progress in teasing apart the underlying mechanistic basis for one of our most promising combinations.

This fundamental analysis in mouse models in conjunction with the mining of large publicly available datasets of cancer patients has provided the basis for the design of two clinical trials. Both led by Dr Anne Armstrong, Consultant Medical Oncologist from The Christie NHS Foundation Trust specialising in breast cancer, the trials will test the combination of non-steroidal anti-inflammatory drugs with immune-checkpoint inhibitors. The first one, which will imminently start recruiting patients with newly diagnosed triple-negative breast cancer, will test in a neoadjuvant setting the combination of high-dose aspirin, a widely-used anti-inflammatory drug, with a checkpoint inhibitor avelumab. The primary objective of this trial is to determine whether co-administering aspirin with immune checkpoint inhibitors can switch the inflammatory profile of tumours towards one that is more favourable to T cell-mediated tumour control. In the second one, for which we have recently secured funding from the J P Moulton Charitable Foundation, we will investigate whether addition of anti-inflammatory drugs to standard of care immune-checkpoint blockade enhances the efficacy of this treatment. This will be an exciting basket trial that will focus on three tumour types, triple-negative breast cancer, non-small cell lung cancer and clear cell renal cancer. Crucially, these three tumour types are, according to our bioinformatic analysis, particularly promising for manipulation of the inflammatory response. This trial also constitutes an ideal setting to test prospectively the utility of our inflammatory signature and to start developing it as a biomarker with diagnostic utility.

CELL DIVISION

Group Leader
Iain Hagan

Associate Scientist
Agnes Grallert

Postdoctoral Fellows
Daniela Eckert
Zoe Edwards
Lenka Halova
Cynthia Li[1]
Wei Ma
Pawan Singh
Neslihan Zohrap[1]

Scientific Officers
Keren Dawson[1]
Eleanor Wendy Trotter

Graduate Students
Zoe Lee
Millie Jones

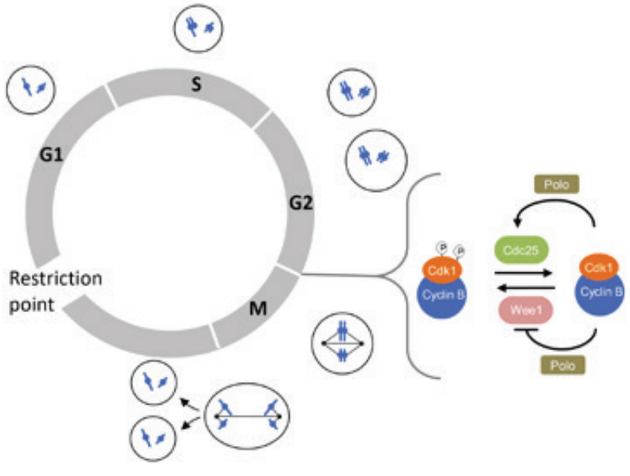
[1] Joined in 2019

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 key to understanding the biology of cancer. 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 study the targets of two of these therapeutically important checkpoint pathways: the commitment to and 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) (Figure 1). Growth, developmental and environmental cues determine 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.

Figure 1



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 inhibit Cdk1-Cyclin B during interphase by phosphorylating the catalytic Cdk1 subunit. Removal of this phosphate by Cdc25 phosphatases then promotes mitotic entry. A trigger level of 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 switch from one state (interphase) into another (division) (Figure 1). Mitotic commitment is blocked when DNA replication is incomplete, or DNA has been damaged by checkpoint pathways that boost Wee1 activity and inhibit Cdc25.

We study three core aspects of cell cycle control: the biology of the Wee1 family of kinases that restrain entrance into division in response to stress; the role played by the centrosome in determining when cells commit to genome segregation; and 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.

Tumour cells accumulate mutations to avoid the normal restraints upon passage through the restriction point. This adaptation often generates abnormally high Cdk-Cyclin activities that perturb the control of DNA replication. Consequently, tumour cells invariably display

Figure 2

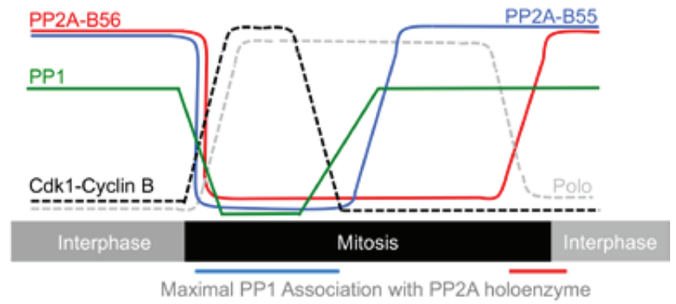


Figure 1:
Feedback control by Polo kinase in Cdk1-Cyclin B activation at the G2/M transition
Cdk1-Cyclin B activity is held in check in interphase as a consequence of phosphorylation of Cdk1 by Wee1. Cdc25 removes the inhibitory phosphate to trigger mitosis. This trigger level of Cdk1-Cyclin B then activates 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.

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.

"oncogene induced replicative stress (OIRS)" in which parts of the genome are under-replicated while others are amplified. OIRS increases reliance on the DNA integrity checkpoint controls. The application of further damage, through local radiation or systemic application of DNA damaging chemotherapy, can therefore overwhelm these checkpoint controls in tumour cells to induce a catastrophic division while neighbouring, normal cells simply delay their division. DNA damaging chemotherapeutic agents and irradiation are therefore among the most successful therapies in the clinic. This success has prompted intense interest in the prospect of compromising checkpoint controls in tumour types that are currently radio and chemo resistant. A lead agent in this quest is the Wee1 inhibitor AZD1775.

While AZD1775 is rapidly progressed through to clinical trials, we still know remarkably little about the basic biology of the three Wee1 family kinases in the cancer cell cycle. A full understanding of the biology of Wee1 family kinases will be key to identifying the optimal context for Wee1 inhibition in the clinic. We are therefore addressing when, where and how, these kinases are used in human cancer cells.

The initial appearance of active Cdk1-Cyclin B on human centrosomes, before propagation 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 have shown that release of Cdk1-Cyclin B or Polo kinase activity at the SPB will drive cells into division. In contrast, release of either kinase activity at any other location around the cell has 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.

Cdk1-Cyclin B activation promotes the activities of an array of mitotic protein kinases. The ensuing wave of mitotic phosphorylation drives chromosome condensation and the formation and function of the division apparatus. Once each phase of mitosis is complete, most phosphorylation events must be reversed to support transition to the next phase of division, otherwise cells remain trapped in mitosis. As a protracted mitotic arrest can trigger death, there is great interest in targeting the mitotic exit phosphatases to invoke such a mitotic arrest. The OIRS induced chromosome aberrations of tumours perturb chromosome segregation in mitosis to delay mitotic progression. This delay makes tumours more vulnerable to further delays arising from mitotic phosphatase inhibition than normal tissue.

PP1 and the protein phosphatase 2A isoforms PP2A-B55 and PP2A-B56 play key roles in driving mitotic exit. PP1 acts as a monomer, whereas PP2A enzymes are hetero-trimers comprising 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, 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 re-activates 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.

Publications listed on page 65

CELL PLASTICITY & EPIGENETICS

Institute Fellow
Maximiliano Portal

Postdoctoral Fellow
Yelizaveta Shlyakhtina

Graduate Student
Katherine L Moran

During neoplastic development, cancer cells are constantly exposed to changing conditions within their niche of origin and further within the ecosystems encountered whilst invading novel tissues. This fast-paced environment drives a micro-evolutionary process that gives rise to distinct sub-clones within a tumour, ultimately leading to increased heterogeneity along the course of the disease. Interestingly, this variability can either arise as a result of somatic mutations acquired during tumour development or may be established due to the ability of a single genotype to produce many discrete, sometimes dramatically different, phenotypes. In this context, and in contrast to genetic heterogeneity, non-genetic variability may provide a dynamic source of diversity supporting the rapid adaptation of cancer cells during numerous environmental perturbations. In our lab, we study the generation and inheritance of non-genetically encoded molecular traits with the aim to unravel their role in the cellular response to diverse cues, such as oncogene transformation and the challenge with therapeutic agents.

It is becoming increasingly apparent that individual cells within a clonal population show significant heterogeneity, particularly in their response to stimuli. Strikingly, this heterogeneity is present despite there being no genetic variability in the population, leading us to the hypothesis that the observed phenotypic differences instead rely on non-genetic information. Interestingly, the idea of non-genetic information and its transfer is not new. Inheritance of acquired characteristics has been a theory put forward by many scientists, including Darwin and Lamarck, long before the field of epigenetics was established. However, the identity of non-genetic information carriers and potential mechanisms of information transfer remain unclear. Under these premises, recent technical and conceptual developments from our lab lead us to postulate that non-genetic information plays a key role in maintaining phenotypic diversity. Indeed, we have recently shown that individual cells obtained from a clonal population (genetically identical cells) of RAS(G12V)-transformed cells (HA1ER-cells) inherently display phenotypic variability at various levels, including the ability to grow as 3D spheres and their capacity to develop resistance to cytotoxic agents. Interestingly, further sub-cloning of the original HA1ER cell line gives rise to populations of cells that only partially recapitulate the phenotypic heterogeneity of the parental one. In this light, the observed phenomenon may (i) either account for temporal dynamic fluctuations in gene expression patterns that may cause lineage switching – the ability of a cell to change its molecular properties and convert to a distinct phenotypic state; or (ii) represent the establishment of stable epigenetic states that can be propagated over time. To explore which of the above possibilities could explain the observed phenomenon, we developed an experimental/bioinformatics pipeline that enables the reconstruction of cellular lineage trajectories whilst concomitantly providing the individual transcriptome of each cell in a single step. By taking advantage of our technical development we were able to show that RAS-transformed cells harbour up to 10 alternative phenotypic metastable states that

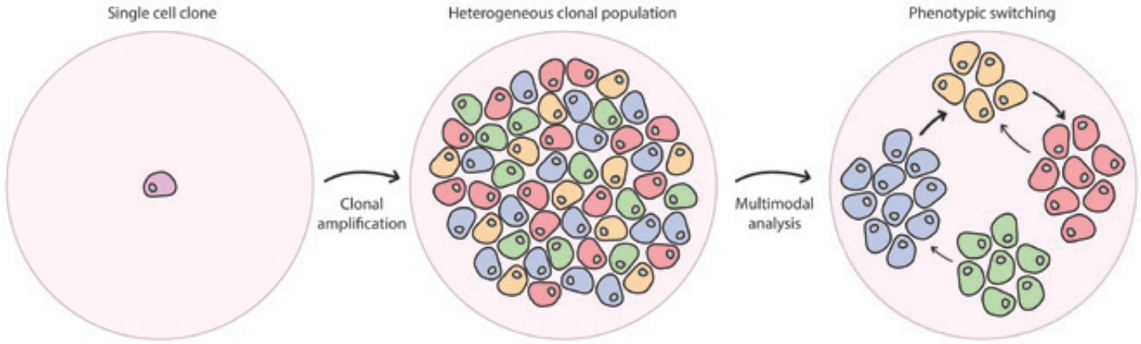


Figure 1:
Scheme illustrating the phenotypic diversity found in clonal cell populations.
We have shown that populations generated from the clonal amplification of a single cell exhibit significant phenotypic diversity, despite all cells being genetically identical. Multimodal analysis of these cells reveals that there are multiple phenotypic metastable states present in these populations and that cells can move between states over time, thus highlighting the dynamic nature of this system.

remarkably predict which cells in the clonal population will grow steadily in 3D cultures and which will fail to do so. Importantly, we have shown that cells in a clonal population have the capability to "migrate" from different phenotypic states, in some cases following constrained paths, suggesting that phenotypic diversification might be under strict molecular control. Notably, though we have shown the existence of predetermined phenotypic paths, we observed that cells are constantly switching from phenotypic states thus giving the population as a whole an enormous evolutionary potential. Indeed, further analysis of individual HA1ER clones by mass spectrometry show striking differences between clones at the protein level thus supporting our early observations and suggesting that the observed differences at the transcriptome level are actually translated into discernible phenotypes. These striking results suggest that, in clonal populations, phenotypic diversity is encoded at different levels other than the "genetic" one and highlights the relevance of non-genetic mechanisms of adaptation to environmental cues.

Following our observations, the main goal of our lab in the upcoming years is to unravel the key molecular players and mechanisms that orchestrate the establishment, maintenance and temporal propagation of stable phenotypic states within isogenic populations and investigate their potential role in the emergence of resistance to therapeutic agents. In that regard, we have already gathered preliminary data that suggest, in a fractional killing setting induced by the cytotoxic agent TRAIL, the apoptotic response is clone/cluster dependent indicating that the information obtained from our single cell assays can be used as proxy to predict phenotypic variations.

Ultimately, our work will develop our understanding of cellular heterogeneity and plasticity, and expand on the concept of non-genetic information. Importantly, it has the potential to reveal the molecular mechanisms underlying these phenomena which remain largely undiscovered. A greater understanding of cellular heterogeneity and plasticity could also revolutionise how we think about evolution and cancer evolution in particular, allowing the ideas of Lamarck and Darwin to be finally brought together into a single unified theory.

CELL SIGNALLING

Group Leader
Angeliki Malliri

Postdoctoral Fellows
Pauline Jeannot[2]
William McDaid[1]
Matteo Menotti[2]
Aishwarya Payapilly[2]

Scientific Officers
Gavin White
Andrew Porter

Graduate Students
Ryan Guilbert
Joe Maltas
Hannah Reed
Joshua Searle[1]

[1] Joined in 2019
[2] Left in 2019

The small GTPase RAC regulates many normal cellular processes, but it can also enhance the formation and progression of cancers via mutation, overexpression or altered regulation. The aim of the Cell Signalling laboratory is therefore to find RAC-dependent targets, which are specific to tumour cells, and hence provide new therapeutic avenues without disrupting the necessary physiological roles of RAC in healthy cells.

RAC is a cellular switch. When bound to GTP, RAC binds target proteins, stimulating a range of cellular processes including adhesion, migration, division and transcription. Guanine Nucleotide Exchange Factors (GEFs) facilitate the switch to a GTP-bound form from an inactive GDP-bound form. Mice lacking the RAC GEF TIAM1 were highly resistant to skin and intestinal tumour formation induced by activation of RAS, a common oncogenic driver. However, the few tumours that did form were more aggressive. This highlights two distinct roles for RAC signalling: stimulating tumour formation, and suppressing malignant progression. Our work has demonstrated how the balance between these two roles is determined by the action of different GEFs acting at different cellular locations.

The outcome of RAC activation depends on sub-cellular localisation

Much early work on TIAM1 focused on its role in strengthening cell-cell junctions, associated with anti-migratory effects. TIAM1 present at cell-cell junctions is degraded following phosphorylation by the oncoprotein SRC or treatment with hepatocyte growth factor, facilitating dispersal of epithelial cells.

The precise localisation of TIAM1 at cell junctions is important. We showed that a TIAM1 interactor, β2-Syntrophin, recruits TIAM1 to adherens junctions, establishing a gradient of RAC activity which diminishes apically. This gradient is important for tight junction formation. Artificially activating RAC at tight junctions led to weaker cell-cell adhesions and disruption of normal epithelial architecture.

Away from cell adhesions, we have uncovered further locations for TIAM1 and other GEFs driving RAC activity – at the centrosome, in the nucleus, and at the nuclear membrane – that stimulate radically different processes. Understanding which are pro- and anti-tumourigenic will help determine how aggressive or responsive to treatment particular tumours are.

TIAM1 localises to centrosomes during mitosis, helping to build the mitotic spindle required for chromosome segregation. TIAM1 balances the outward force of the motor protein Eg5, whose effect is to increase centrosome separation. For this function, TIAM1 needs to be phosphorylated by CDK1 leading to activation of RAC/PAK signalling. Eg5 is a potential therapeutic target as its inhibition leads to monopolar spindles and mitotic arrest; our findings suggest that loss of TIAM1 could cause resistance to Eg5 antagonists. Our recent unpublished work has uncovered a new role for TIAM1, regulating the duplication of centrioles, structures at the core of each centrosome. Cells normally duplicate centrioles only once per cell cycle. Centriole overduplication is common in many cancers, however, promoting aneuploidy. We observe centriole overduplication, lagging chromosomes and aneuploidy in normally chromosomally stable cells following TIAM1 depletion, which may explain why loss of TIAM1 expression promotes malignant progression.

We are also continuing to study the role of nuclear TIAM1, which we first described in colorectal cancer cells. Nuclear TIAM1 inhibits migration of colorectal cancer cells by preventing the transcriptional co-activator TAZ from binding with its transcription factor partner TEAD. Interestingly, patients with high nuclear TIAM1 showed significantly better survival than those with low nuclear TIAM1 and TIAM1 levels were significantly decreased in more advanced tumours.

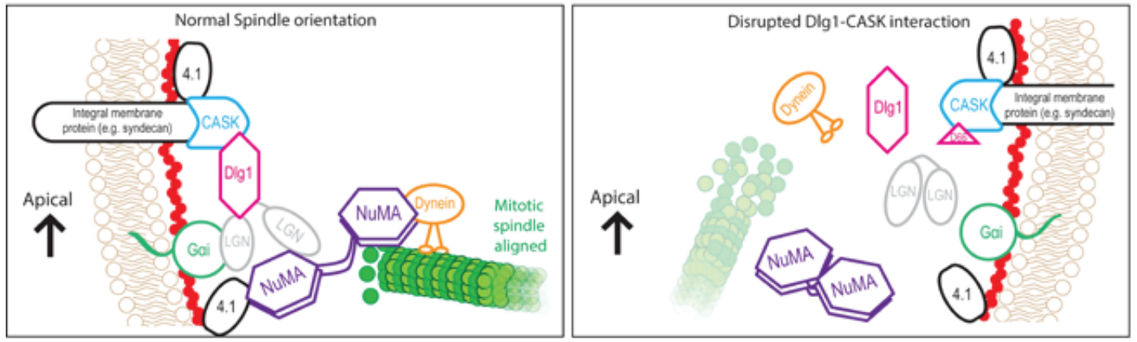


Figure 1.
Model describing the role of CASK in spindle orientation.
CASK localises to the cell cortex, where it recruits Dlg1, which in turn localises the core spindle orientation components of LGN and NuMA to the lateral cell membrane. There they bind astral microtubules, correctly orienting cell division. Disruption of the interaction between CASK and Dlg1 causes mislocalisation of this complex, leading to misorientation of the mitotic spindle. Reproduced with permission from Porter et al, Journal Cell Science, 2019.

Our recent data also demonstrated a role for perinuclear RAC in the regulation of a subset of the actin cytoskeleton known as the perinuclear actin cap – thick actin bundles that run over the nucleus, constraining its height, guiding nuclear orientation and facilitating cell migration. The RAC GEF STEF/TIAM2 localises to the outer nuclear membrane and is required for maintenance of the actin cap by activating RAC. Depletion of STEF led to reduction in myosin-generated tension at the nuclear envelope, decreased nuclear stiffness, and ultimately reduced TAZ-regulated genes, due to changes in mechanotransduction. Targeting an activated mutant of RAC specifically to the perinuclear region restored the actin cap in STEF-deleted cells.

We are continuing to study the role of both nuclear and perinuclear RAC signalling in migration of non-small cell lung cancer (NSCLC) cells. Lung cancer is the third most common cancer in the UK and with over 47,000 new cases per year is the most common cause of cancer death. Finding new treatments for late stage cancers (where the 5 year survival rate is just 3%), and preventing early stage cancers from spreading, are therefore high priorities. Moreover, TIAM1 and STEF/TIAM2 both contain a RAS-binding domain and are considered effectors of RAS. KRAS, one of the RAS family proteins, is frequently mutated in NSCLC. We are currently looking at the role of TIAM1 and STEF/TIAM2 in KRAS-induced lung tumourigenesis.

Different RAC GEFs can activate RAC with different consequences

RAC GEFs are often multi-domain proteins with many binding partners. We recently showed that GEFs themselves can determine the outcome of RAC activation. TIAM1 and P-REX1 have diametrically opposite effects on cell migration through RAC: TIAM1 promotes cell-cell adhesions to oppose cell migration while P-REX1 actively promotes migration. P-REX1 binds to Flightless-1, which is involved in remodelling the actin cytoskeleton to promote migration downstream of RAC. Over-expression of specific GEFs, which occurs commonly in many cancers, can therefore drive different oncogenic signalling pathways.

Role of spindle orientation in normal and abnormal epithelial cell divisions

In epithelial cells, the angle of cell division is controlled so that newly generated daughter cells sit side-by-side in the epithelium and in 3D produce a sheet of cells surrounding a single hollow centre. The angle of cell division depends on the orientation of the mitotic spindle. In turn, this is controlled by membrane-localised pools of NuMA and LGN, which bind astral microtubules emanating from the poles of the mitotic spindle. We recently established that CASK, a novel TIAM1 interactor, is a regulator of mitotic spindle orientation (Porter et al. J Cell Sci 2019). We found that CASK directly interacts with and localises Dlg1, a tumour suppressor, which in turn recruits LGN and NuMA. Expressing an interfering peptide and blocking the endogenous interaction between CASK and Dlg1, mislocalised NuMA and led to misoriented cell divisions. Moreover, depletion of Dlg1 led to misoriented cell division, and could only be rescued by Dlg1 which was capable of binding CASK. Further, depletion of CASK in 3D culture led to the formation of multilumen structures, a hallmark of misoriented cell divisions, which were reminiscent of early stages of epithelial cancers, such as ductal carcinoma in situ (DCIS), an early form of breast cancer. This suggests that mutation or deletion of CASK may promote neoplastic transformation. Accordingly reduced CASK expression correlates with worse prognosis in a number of cancer types, including breast cancer.

Publications listed on page 65

DRUG DISCOVERY

Director
Caroline Springer

Head of Medicinal Chemistry
Dan Niculescu-Duvaz
Allan Jordan[2]

Head of Biochemistry
Graeme Thomson

Head of Cellular Pharmacology
Graeme Walker

Head of Informatics & Modelling
Rae Lawrence

Chemists
Mohammed Aljarah
Michael Brown
Vikki Clayton[2]
Mark Dodsworth[2]
Efthymios Gavriil[1]
Paula Jackson[1]
Laura Johnson[2]
Chris Kershaw
Leo Leung
Rebecca Newton
Ali Raoof
Kate Smith[2]
Deborah Smithen

Bioscientists
Alex Baker[2]
Habiba Begum[2]
Elizabeth Blaikley
Aimee Blair[2]
Mairi Challinor
Andrew Clifton
Emily Crowley
Zoi Diamantopoulou[2]
Joanna Grabarek
Louise Griffiths[2]
Nicola Hamilton
Ben Hodgson
Paul Kelly
Filipa Lopes
Gareth Prosser[1]
Rebecca Holland[1]
Robert McLeary
Alice Pilborough
Alex Stowell

Informatics & Modelling
Matthew Roberts[1]

Graduate Students
Elizabeth Hogg[3]
James Stratford
Mihaela Ficu[1]
Megan Mylrea[1]

Undergraduate Students
Hannah Fortune[2]
Alex Anderton[1]

EA to Caroline Springer
Jayne Fowler

[1]Joined in 2019
[2]Left in 2019
[3]Joint with Systems Oncology

The last year has been a time of consolidation for a new Drug Discovery Unit. Many new academic collaborations have been fostered with our colleagues in the CRUK Manchester Institute and The University of Manchester, resulting in validated new cancer drug target projects.

Clinically, our pan-RAF programme completed its Phase I clinical trial at The Christie and Royal Marsden NHS Foundation Trusts. This orally bioavailable, well-tolerated panRAF/SRC inhibitor was developed in collaboration with Professor Richard Marais and is designed to treat mutant BRAF and mutant RAS melanoma. This project has been licenced to Basilea Pharmaceutica who are leading on future trials. Our RET pre-clinical candidate development programme has also been licenced and we anticipate that our RET inhibitor will enter first-in-human trials next year.

The main cause of death in cancer is due to metastasis and we are assessing our lysyl oxidase (LOX) inhibitors in cancer models of metastases. LOX is an enzyme that regulates cross-linking of structural proteins in the extracellular matrix and plays a critical role in metastasis as well as in the 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. We have discovered LOX inhibitors with good pharmacokinetic properties and have progressed our LOX drug discovery programme to late lead optimisation. Our inhibitors show therapeutic activity in many different primary tumour models as well as anti-metastatic efficacy in preclinical models. 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 are currently selecting the best drug candidates to progress to toxicology studies 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 the bulk of tumour cells, but CSCs escape treatment resulting in tumour regrowth and treatment failure. Thus, there is an urgent need for new discoveries to target the CSCs within tumours, for use in combination with the standard of care drugs. We are pursuing a lead optimisation drug discovery programme to target CSCs. We have discovered potent, 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 also collaborating with Professor Marais and The University of Manchester based breast cancer expert Dr Robert Clarke, in elucidating the biology of our target.

Our gene-directed enzyme prodrug therapy (GDEPT) programme uses novel newly engineered vaccinia viral vectors, in collaboration with Professor Richard Marais, 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. We have now shown highly selective tumour targeting with our viral vectors from a single systemic administration, which accompanies long-term tumour xenograft growth reductions in preclinical models.

Our partnership with IDEAYA Bioscience on our Poly(ADP-ribose) glycohydrolase (PARG) program continues to progress. In this collaborative effort IDEAYA have taken on responsibility for the further chemical optimisation and development of these series, looking to improve DMPK properties in order to facilitate demonstration of in vivo activity.

Biological studies to improve disease linkage are advancing at the DDU and through a collaboration with Professor Stephen Taylor at The University of Manchester.

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 work to ensure that our DDU projects are integrated with Professor Dive's biomarker discovery programme, with a number of joint appointments, so that all nominated targets have selection and predictive biomarkers. We also delighted to work closely with the excellent committed clinicians in the Christie NHS Foundation Trust.

Publications listed on page 65

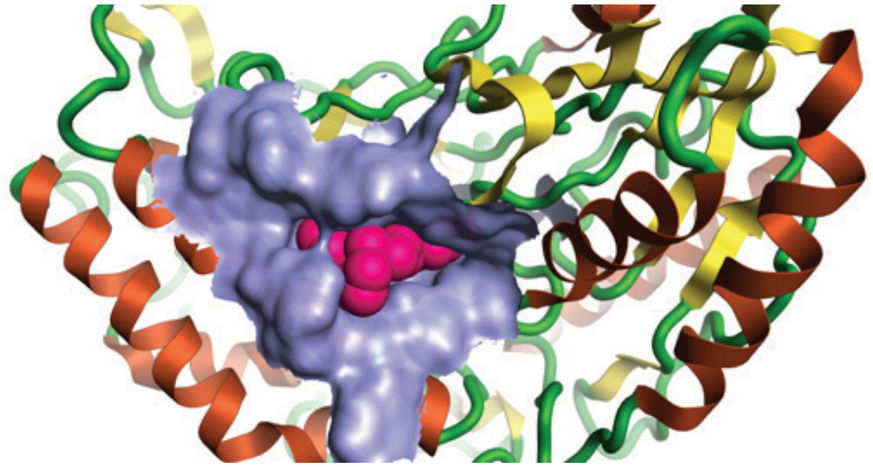


Figure 1.
A potent and selective CRUK MI designed ligand bound to a target involved in CSC growth.

HEAD AND NECK CANCER BIOLOGY

Institute Fellow
Robert Metcalf

Genetic alterations in the genomic profiles of head and neck tumour samples can be targeted for drug therapies. The focus of the Head and Neck Cancer Biology group is on salivary gland cancers as there is a significant unmet need in this patient group. 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 focuses on adenoid cystic carcinoma (ACC), in collaboration with Professor Caroline Dive and the Cancer Biomaker Centre. ACC is a salivary gland cancer that metastasises in > 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 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 ACC.

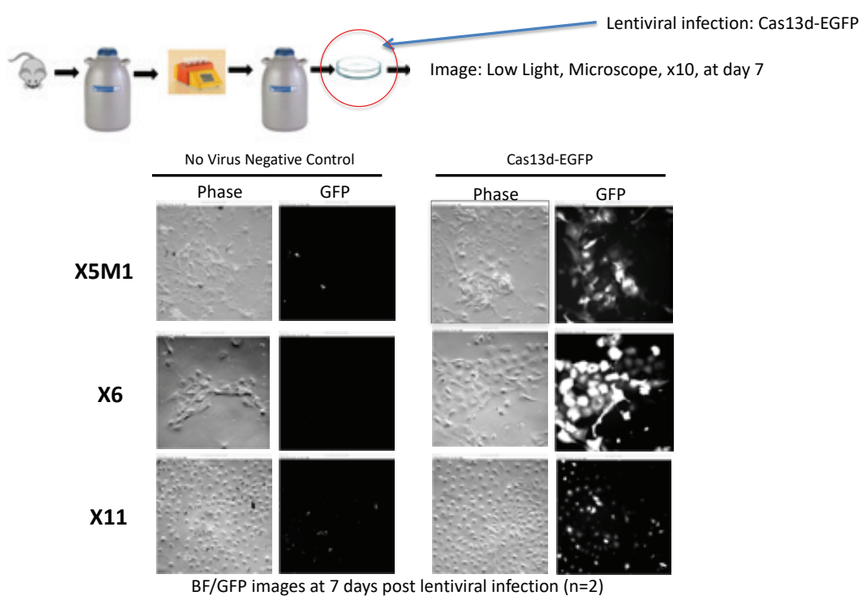


Figure 1.
Proof of principle lentiviral infection of ACC patient derived xenograft models (X5M1, X6 and X11) with Cas13-EGFP expressing vector following short term ex vivo culture confirmed by GFP expression on fluorescent microscopy images.

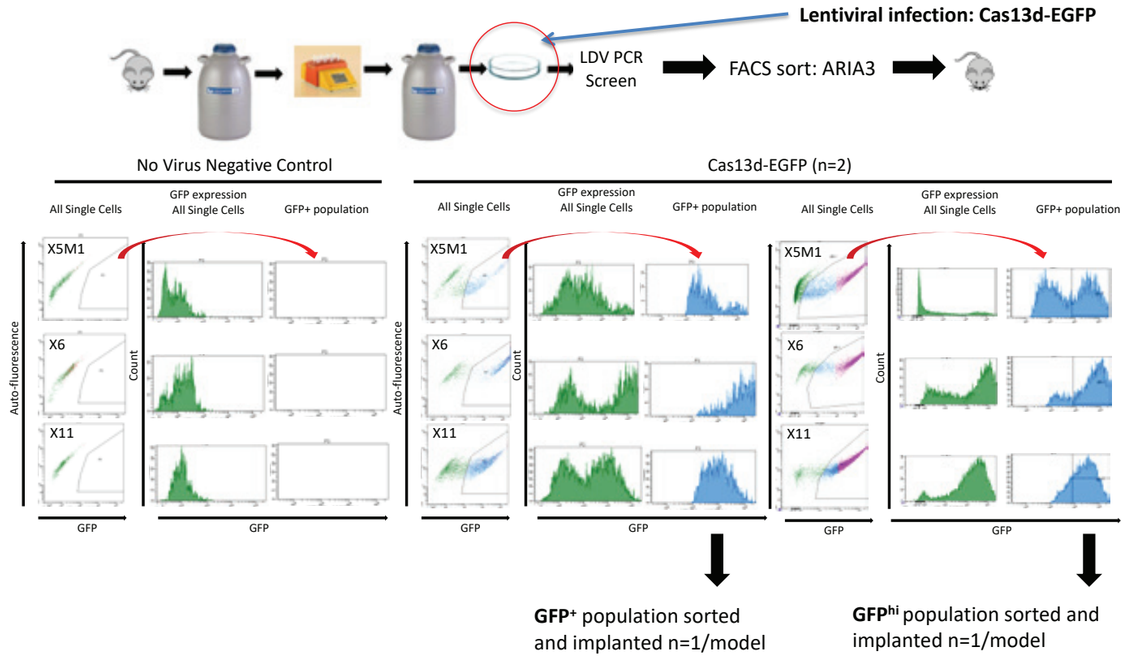


Figure 2.
Flow cytometry and FACS sorting of GFP positive ACC PDX cells following short term ex vivo culture and lentiviral infection with Cas13-EGFP expressing vector enables re-implantation of Cas13 modified cells in vivo.

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 rapidly 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, this research could have immediate global impact in the ACC 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.

Publications listed on page 66

LEUKAEMIA BIOLOGY

Group Leader
Tim Somervaille

Postdoctoral Fellows
Alba Maiques-Diaz[2]
Isabel Romero-Camarero
Bettina Wingelhofer
Hannah Seberg[1]

Clinical Research Fellows
Mark Williams
John Chadwick

Bioinformatician
Fabio Amaral

Senior Scientific Officer
Gary Spencer

Scientific Officer
Priyanka Bhattacharya[2]

Graduate Students
Fabrizio Simeoni
Francesco Camera
Bradley Revell[1]
Naseer Basma[1]

[1] Joined in 2019
[2] Left in 2019

The goal of the team is to identify new disease mechanisms in acute myeloid leukaemia (AML) and candidate therapeutic targets for development through to the clinic, aiming for patient benefit. This year we published two studies along these lines. In the first, we reported our discovery and functional evaluation of the enhancer landscape controlling expression of ABCB1, the most important chemotherapy resistance gene in AML. In the second, we reported our discovery by CRISPR screening that dual treatment of AML cells with inhibitors of the histone demethylase LSD1 and inhibitors of mTORC1 leads to synergistic induction of a terminal differentiation programme, and loss of leukaemia cell proliferative potential.

Resistance of leukaemia cells, including leukaemia stem cells (LSC) with disease reconstituting activity, to the chemotherapy drugs used in standard induction and consolidation regimens is the most common cause of treatment failure in acute myeloid leukaemia (AML). While a number of resistance mechanisms have been proposed over the years, the most significant and experimentally well established is high expression of the ABCB1 drug efflux pump (also known as MDR1 or P-glycoprotein). This cell membrane pump actively exports anthracyclines and other chemotherapy drugs from the interior of cells and its level of expression predicts for treatment failure in AML. More generally, ABCB1 is also highly expressed in many poor risk malignancies (e.g. ovarian cancer) as well as in normal gut, liver, kidney and the blood-brain barrier. Inhibitors of ABCB1 have been tested in clinical trials in AML but with limited success. In view of its significant role in the disease, the rationale for targeting ABCB1 remains a strong one. Furthermore, given the abundance of preclinical evidence supporting a role for ABCB1 in drug resistance, the failure in clinical trials of inhibitors of ABCB1 has not been adequately explained.

A prerequisite for the design of new therapeutic strategies is a greater understanding of the cancer-specific regulation of ABCB1 and its role in drug resistance. Specifically, until now it has been unclear how ABCB1 expression is established and maintained in human AML. Whether expression is constitutive or dynamic is of critical relevance to the clinical application of ABCB1 inhibitors: previous trials have assumed constant expression. Advances in enhancer biology have established that these distal regulatory elements govern cell-type specific gene expression and frequently respond to environmental conditions and homeostatic perturbations. Critically, the enhancer landscape of ABCB1 has yet to be defined.

In work led by Mark Williams, a clinical research fellow in the group funded jointly by a Kay Kendall Junior Research Fellowship and Cancer Research UK, we have recently identified and functionally validated the network of enhancers that controls expression of *ABCB1*. We show that both brief and prolonged exposure of myeloid leukaemia cells to daunorubicin activates an integrated stress response (ISR)-like transcriptional program to induce *ABCB1*. Using approaches such as chromatin immunoprecipitation with next generation sequencing, 4C-sequencing and CRISPR inhibition we were able to demonstrate that the mechanism of *ABCB1* up-regulation involved remodelling and activation of an ATF4-bound, stress-responsive enhancer in intron 4 of *ABCB1*. Protracted cellular stress primed this stress responsive enhancer for rapid increases in activity following re-exposure of cells to daunorubicin, providing an epigenetic memory of prior drug treatment.

We extended our studies from cell lines into primary patient AML cells, making use of our excellent Haematological Malignancy Biobank, which has been generated and curated by Deepti Wilks over the last decade. Remarkably, we also found that in primary human AML, exposure of fresh blast cells to daunorubicin activates the stress-responsive enhancer leading to dose-dependent induction of ABCB1. Furthermore, this dynamic induction of *ABCB1* was observed in response to diverse stressors, including chemotherapy. Such chemotherapy-induced up regulation of *ABCB1* facilitates escape of leukaemia cells from targeted third-generation ABCB1 inhibition, providing a novel explanation for the failure of ABCB1 inhibitors in clinical trials: chemotherapy drugs acutely induce their own cellular resistance mechanism.

In experiments aiming to identify a pharmacologic method of inhibiting this process, we discovered that stress-induced up-regulation of *ABCB1* could be mitigated by combined use of pharmacologic inhibitors U0126 and ISRIB. Combinatorial use of these drugs, or their analogues, hold promise for testing in early phase clinical trials as adjuvants to enhance the activity of ABCB1 inhibitors.

In a second study led jointly by Bettina Wingelhofer and Gauri Deb, respectively current and former postdocs in the group, we extended our long-standing interest in the histone demethylase Lysine Specific Demethylase 1 (LSD1) as a candidate therapeutic target in AML. Pre-clinical studies have demonstrated that LSD1 contributes to the differentiation block that is the cardinal feature of AML. We have previously reported that LSD1 knockdown or LSD1 pharmacologic inhibition promotes differentiation of, in particular, AML cells with chromosomal translocations targeting *MLL*. Development of more potent and specific tranylcypromine-derivative inhibitors such as iadademstat (ORY-1001, from Oryzon Genomics) has facilitated early phase clinical trials, which are ongoing. In patients with AML iadademstat is well tolerated and induces molecular and morphological differentiation of blast cells in leukaemias driven by *MLL* gene rearrangements.

While these early clinical trial results are encouraging, all effective treatments in AML are currently delivered in combination regimens. Identification of genes and cellular pathways whose loss of function collaborates or synergises with pharmacologic inhibition of LSD1 to promote differentiation represents an attractive strategy for uncovering novel drug combinations for testing in early phase trials. This research question led us to make use of CRISPR technology. Using a genome wide loss-of-function CRISPR-Cas9 screening approach we identified multiple components of the amino acid sensing arm of mTORC1 signalling - RRAGA, MLST8, WDR24 and LAMTOR2 - as cellular sensitisers to LSD1 inhibition. This was confirmed by knockdown of certain mTORC1 components as well as mTORC1 pharmacologic inhibition. In particular, we found that dual treatment of AML cells with an LSD1 inhibitor and the mTORC1 inhibitor rapamycin potently enhanced differentiation in both cell line and primary AML cell settings, in vitro and in vivo, and substantially reduced the frequency of clonogenic primary human AML cells in a modelled minimal residual disease setting. We observed synergistic up-regulation of a set of transcription factor genes associated with terminal monocytic lineage differentiation. Taken together our data indicate that dual mTORC1 and LSD1 inhibition is an attractive candidate combination approach for enhanced differentiation in MLL-translocated AML for evaluation in early phase clinical trials.

Publications listed on page 66

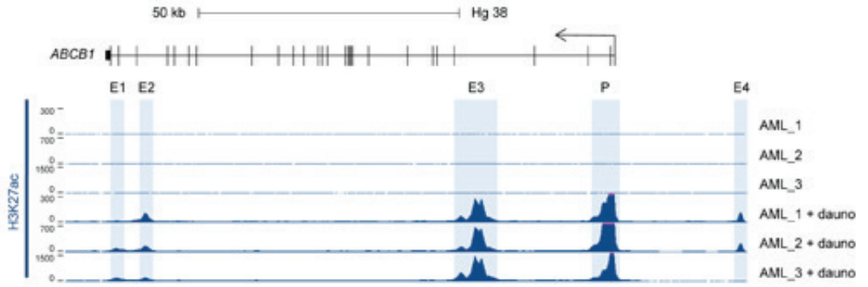


Figure 1:
ABCB1 enhancer landscape
ChIPseq tracks for H3K27Ac surrounding ABCB1 (chr7:87,495,508-87,626,404; hg38) in human K562 AML cells. Putative enhancers (E1-4) are highlighted in blue. P indicates the promoter. To generate resistant K562 AML cells, drug sensitive lines 1-3 were exposed to progressively escalating concentrations of daunorubicin over a three-month period.

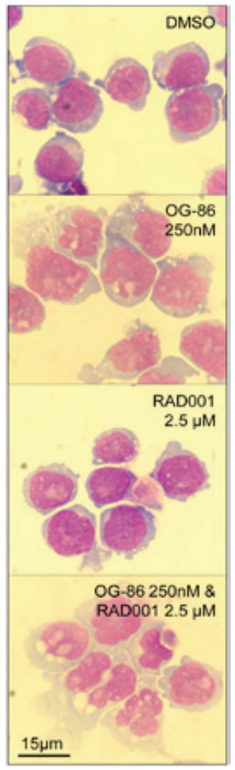


Figure 2:
Combined pharmacologic inhibition of LSD1 and mTORC1 in AML cells
Indicative cytospin images show THP1 AML cell morphology following 120hrs of treatment respectively with DMSO vehicle, LSD1 inhibitor OG-86, mTORC1 inhibitor RAD001 or the combination.

MOLECULAR ONCOLOGY

Group Leader
Richard Marais

Associate Scientists
Nathalie Dhomen
Valeria Pavet

Senior Clinical Scientist
Martin Cook

Senior Research Scientist
Adele Green

Postdoctoral Fellows
Franziska Baenke[2]
Candelaria Bracalente
Daphné Brisard[1]
Alessio Cannistraci
Elena Galvani
Pauline Hascoët
Sjors Kas
Patricia Pacios-Centeno[1]
HaoRan Tang[2]
Lucas Trucco
Joshua Tweedy[2]

Bioinformaticians
Piyushkumar Mundra

Clinical Fellows
Pablo Garcia Martinez
Zena Salih
Sara Valpione

Scientific Officers
Christopher Chester
Darryl Coles
Megan Grant
Clare McManus[2]
Jessica Walker[1]

Translational Research Project Manager
Jackie Mitchell

Graduate Students
Luke Chisholm
Denys Holovanchuk
Matthew Wilson

[1] Joined in 2019
[2] Left in 2019

As members of the CRUK MI community, our aim is to expand our knowledge of the basic biology underlying tumorigenesis in melanoma, breast and prostate cancer and translate this to patient benefit. Working in a multidisciplinary team composed of cell and molecular biologists as well as clinicians ensures that our discoveries are exploited in the fields of early detection of disease, personalised medicine and the development of novel therapeutic approaches.

Immunotherapy has changed the way melanoma is treated and in particular, immune checkpoint inhibitors (ICI) have made a major contribution to increased survival rate for patients with advanced stage disease. However, many metastatic melanoma patients still face a significant mortality risk, in part due to our limited insight into who will respond to therapy and how to unleash the potential of ICI in combination with other treatments for the benefit of all patients. Designing more effective clinical trials begins with increased understanding of the biological impact of therapies on both the cancer cells and the tumour microenvironment (TME). To that end, we examined how ICI treatment affected T cell behaviour in responding and non-responding melanomas.

T cells are components of the immune system that protect us from pathogens. ICIs re-programme T cells, boosting their ability to kill cancer cells. T cells recognise cancer cells through specific proteins on their surface called T cell receptors (TCR), which are generated by somatic recombination of the *TCR* locus to create the antigen recognition repertoire variability needed for effective immune function. Since each TCR is specific to an individual T cell clone, we studied how immunotherapy affected the evolution of the TCR repertoire by sequencing *TCRs* in the T cells in the blood of patients receiving ICI. In parallel, we analysed the *TCR* repertoire in the DNA released into the blood by T cells that have died in the course of fighting against cancer cells (Figure 1). Our data suggest that there is a dynamic awakening of the immune system under the selective pressure of immunotherapy, which was revealed by clonal expansion and contraction of specific T cells within three weeks of the first cycle of ICI treatment. Critically, we identified a signature within these early peripheral T cell responses that could anticipate which patients would respond to treatment. Moreover, expansion of a specific subset of immune-effector peripheral T cells occurred only in the patients that responded to therapy, suggesting that these cells could be responsible for mediating the anti-tumour effects. Since these events are prognostic for response, occur within 3 weeks of starting immunotherapy and can be measured via minimally invasive liquid biopsies, they may be able to provide an early response assessment for melanoma patients, and therefore have the potential to allow personalised delivery of immunotherapy care.

Patient-derived materials have helped us to understand better the dynamic interactions between tumours and the immune system. However, our ability to detect subtle changes that could impact therapy response is hampered by the diversity and plasticity of the immune system in the human population, and its shaping by diverse variables including genetic background, lifestyle and prior lines of treatment. Conversely, tractable and easy to manipulate models, such as transplantable mouse tumours, xenografts and organoids, lack normal tumour heterogeneity, a co-evolved inflammatory environment and the TME. We previously reported that our $BRAF^{V600E}$/UVR mouse melanoma model recapitulates the cardinal genomic features of human melanoma, including a UVR-induced mutational signature, high C-to-T load and recurrent mutations in the same top 10 genes as occur in human cutaneous melanoma. Our mice have a fully native immune system and the tumours develop a normal TME, but can be controlled for genetic and environmental variables that cannot be controlled when working with human-derived samples. We used our model to evaluate the biological features of durable response to ICI targeting the PD-1 checkpoint and identified a

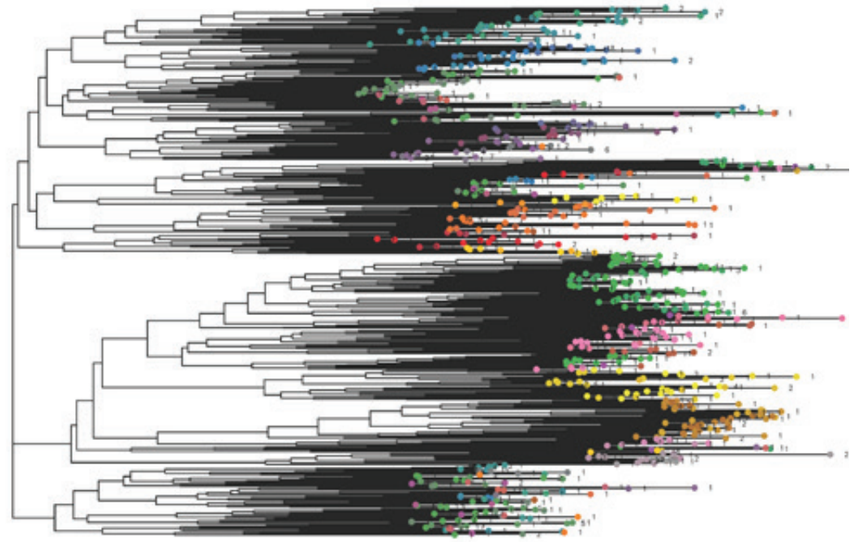


Figure 1:
Example of phylogenetic tree of the T cell receptor.
Phylogenetic tree of the T cell receptor in-frame rearrangements obtained from ctDNA of one patient treated with immunotherapy for metastatic melanoma. Each leaf represents a distinct sequence, and colours indicate the different V, D, and J gene from individual rearrangements (the number next to each leaf refers to the sequence count in the pool). The distance scale is proportional to the number of different nucleotides between the sequences.

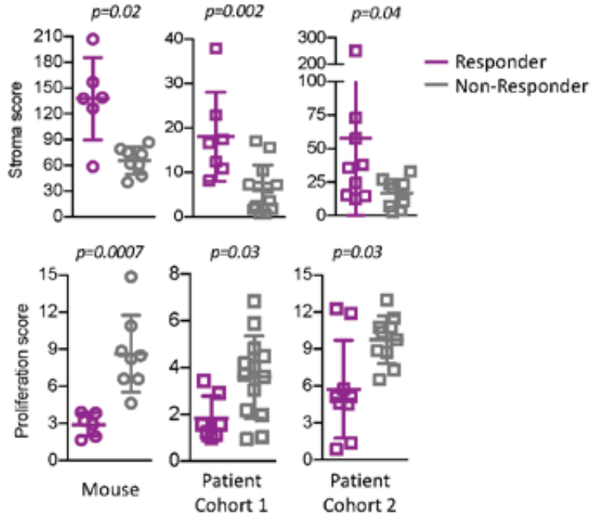


Figure 2:
Stroma remodelling and reduced cell division define durable response to therapy with anti-PD-1 in melanoma. (Top) Stroma score defining the level of expression of the 10-gene signature identified in our mouse model (left) and validated in two independent human cohorts (middle, right). (Bottom) Proliferation score defining the expression of the 7-gene panel identified in our mouse model (left) and validated in two independent human cohorts (middle, right).

set of 10 genes associated with stroma remodelling that were consistently upregulated in tumours responding to therapy. Additionally, a 7-gene proliferation signature was downregulated in responding tumours. We validated that our gene signatures could identify which patients would respond within 3-4 weeks of starting treatment with ICI in two independent patient cohorts (Figure 2). This study shows that our preclinical model can reveal insights into human disease that are difficult to identify in the human population itself.

In the past year, we have expanded the scope of our studies by generating a new mouse model for uveal melanoma, a rare melanoma subtype that arises in the melanocytes of the eye. Despite developing from the same cell of origin (melanocytes), uveal and skin melanoma are driven by quite different genetic events, and we recapitulate these in our mouse models. Oncogene specificity is also observed in different types of skin cancer, as different oncogenes promote the transformation of melanocytes into melanoma and keratinocytes into squamous and basal cell carcinomas. Indeed, oncogene specificity is observed in many tissues, and together with teams from the US and The Netherlands, we hypothesised that understanding the biological circuits that promote or prevent malignant transformation of particular cell types by different oncogene drivers will increase our knowledge of the malignant process and support the development of new therapeutic approaches. We are delighted that this ambitious hypothesis and research plan was selected to be one of the CRUK Grand Challenges in 2019 (www.specificancer.org).

Where possible, we translate our findings into patient benefit, and so we are excited to have secured funding for DETECTION, a clinical trial to assess if longitudinal monitoring of tumour DNA in the blood (ctDNA) can reveal minimal residual disease after curative intent surgery in stage IIB/C melanoma patients (CRUK Experimental Medicine Award programme). DETECTION will also determine if ctDNA-guided earlier treatment improves patient outcomes. Alongside CAcTUS (**C**ircul**A**ting **T**umour DNA g**U**ided therapy **S**witch), our ongoing trial (opened in April 2019) to investigate if ctDNA can guide the switch between targeted and immune therapy in advanced cutaneous melanoma patients, DETECTION builds on our discoveries that ctDNA can be used to monitor melanoma burden and progression in patients.

Publications listed on page 67

PROSTATE ONCOBIOLOGY

Group Leader
Esther Baena

Postdoctoral Fellows
Valentina Ubertini[2]
Maria Roel Sánchez

Scientific Officer
Pengbo Wang

Graduate Students
Ivana Steiner
Alexander Du Feu
Alexandra Hendry[1]

Clinical Fellow
Amin Ali[1]

[1] Joined in 2019
[2] Left in 2019

Prostate cancer is a heterogeneous disease, both clinically and biologically. New therapies have produced some clinical successes but a substantial subset of patients progress to incurable castration-resistant PCa (CRPC). Importantly, it is yet not possible to predict which patient will develop aggressive tumours versus more indolent cases. Therefore, the work of our group aims at understanding the early stages of PCa development and to identify and characterise cells of PCa origin to facilitate the development of better therapies.

The initiation of aggressive tumours and CRPC involves the existence of so-called cancer-initiating cells, with the ability to self-renew, to survive anti-tumoural treatments and to interact with niche-cells. These properties are required for asymmetric cell divisions, ultimately contributing to the heterogeneity of PCa. Irrespective of recurrent mutations found in PCa cells, recent evidence suggests a key role of cell-of-origin and their reprogrammed niche for disease progression.

We have identified inherently castration-resistant cellular subpopulations in the prostate, defined by their unique cell-surface markers, using single cell profiling and functional characterisation by organoid-culture and in situ lineage tracing analysis in mouse models. In particular, our studies define LY6D as a marker for prostate progenitors and castration-resistant luminal cells, which may serve as a prognostic marker for aggressive prostate cancer (Figure 1). For this, we compared the mouse prostate of non-treated mice (hormone-naïve) 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 also to a lesser degree in the basal lineage. We found that a subset of prostate cells in the luminal lineage co-express multiple basal and luminal markers including androgen-driven genes together with prostate stem/progenitor marker genes. 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.

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. We are currently defining the functional role of LY6D for tumorigenesis and tumour maintenance, which so far 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 enriched organoid-forming multipotent luminal progenitors. Taken together, these findings suggest that LY6D expression correlates with PCa initiation and progression to castration-resistant growth from the luminal lineage. Importantly, in support of this hypothesis, analysis of human PCa cohorts revealed that higher LY6D expression levels is associated with more aggressive disease and worse outcomes, suggesting that LY6D may serve as a prognostic biomarker for advanced PCa.

In a complimentary study combining genomic and multiparametric imaging analysis of high-risk prostate cancer patients, we have characterised the radiogenomic landscape of multifocal prostate cancer. 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

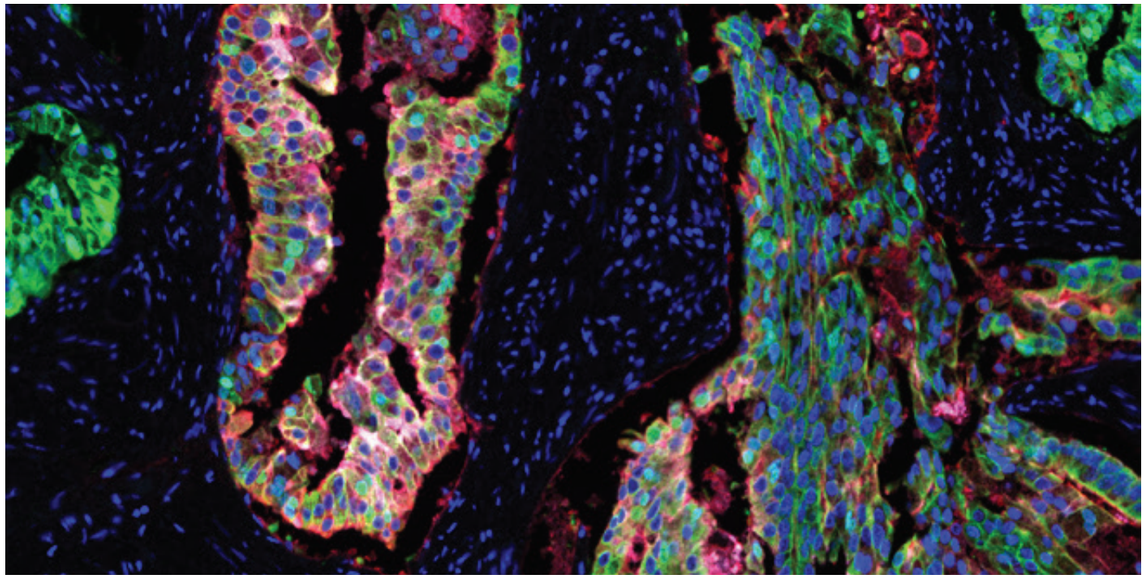


Figure 1:
LY6D is expressed from early stages of human prostate cancer. Magenta (LY6D), pan-cytokeratin (green), nuclear (Dapi).

than ultrasound. Importantly, our study identified that even with this superior technique >10% potentially significant PCas are missed by this approach because they are not detected by mpMRI. We 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, 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 lesion 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 two cores obtained from the same visible lesion, one of the cores 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. Contrarily, 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.

Our studies thereby advance patient stratification and set a pipeline to develop novel therapeutics. 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 prognostic marker for patient stratification, and to tailor specific therapies targeting CR LY6D+ cells as novel therapeutic target for patients with aggressive disease.

SKIN CANCER AND AGEING

Institute Fellow
Amaya Virós

Postdoctoral Fellow
Tim Budden

Clinical Fellow
Sarah Craig

Graduate Student
Shilpa Gurung

Pre-Doctoral Clinical Academic Fellow
Charles Earnshaw

The primary focus of our studies is melanoma and squamous cell carcinoma (SCC), two forms of skin cancer that predominate in the elderly population and usually arise over skin that has endured sun exposure and/or ageing. 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 of the melanoma patient at diagnosis 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, with a less clear-cut relationship to chronic UV exposure. 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 what drives skin ageing (intrinsic, due to chronological age, and extrinsic due to UV) and how these differences affect skin cancer onset, skin cancer progression and outcome. We are exploring how these specific changes in the architecture and cellularity of aged skin can be exploited therapeutically. We have new evidence that the earliest stages of disease, and the tumour microenvironment, are critical to plan patient surveillance and care, and we are hoping to contribute new strategies. Previous work has shown that multiple cells composing the melanoma tumour environment, co-opted from the healthy aged skin, can contribute to disease severity. Fibroblasts, innate immune cells, adaptive immune cells, keratinocytes and adipocytes are all known to crosstalk with melanocytes and/or melanoma cells. Our lab is addressing the role of healthy aged cutaneous cells in promoting or inhibiting melanoma progression at the earliest stages of disease, and investigating how their contribution varies according to anatomic site. We are particularly interested in the primary tumour 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 from the deeper cutaneous structures. One intriguing aspect of melanoma progression in the elderly is the higher likelihood of spreading via the vascular system, and one possibility could be the structural and functional changes in the aged cutaneous microenvironment are responsible for this phenomenon. We are investigating how aged keratinocytes, fibroblasts and adipocytes modify early cutaneous melanoma proliferation, invasion, migration and metastasis. Skin from sun-protected sites becomes paucicellular across the 3 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 cell loss and dermal connective tissue degradation, where collagen fibre networks disappear to form globules of degraded collagen and other fibres that form the connective extracellular matrix.

Further to our on-going work on how aged skin cells in the microenvironment contribute to melanoma, we have completed a study dissecting how the physical degradation of the extracellular matrix that occurs during ageing affects early tumour cell movement and skin homeostasis. We have now established multinational clinical collaborations that will allow us to explore the aged microenvironment in human tissue.

In addition to the aged skin microenvironment, we focus on how aged melanoma cells differ in their behaviour from young melanoma cells. Melanoma comprises multiple epidemiological and molecular subtypes, and we have found each subtype presents unique features of ageing. Additionally, we have now described the molecular aspects that define melanoma in the elderly patient, a work that is under revision. For this major aim of our lab, we have looked at the mutation rates, mutation types, pattern of DNA damage accumulated in melanoma cells during ageing, the sex of patients and the relationship to survival. We have identified and patented the unique DNA features that reliably identify old patients at high risk of melanoma-specific death. We are working to validate this in a larger human cohort, using our markers as a predictor tool in clinical practice, to stratify patients at high risk of death. Moreover, we are investigating if these markers can identify elderly patients who will better respond to checkpoint inhibitor treatment.

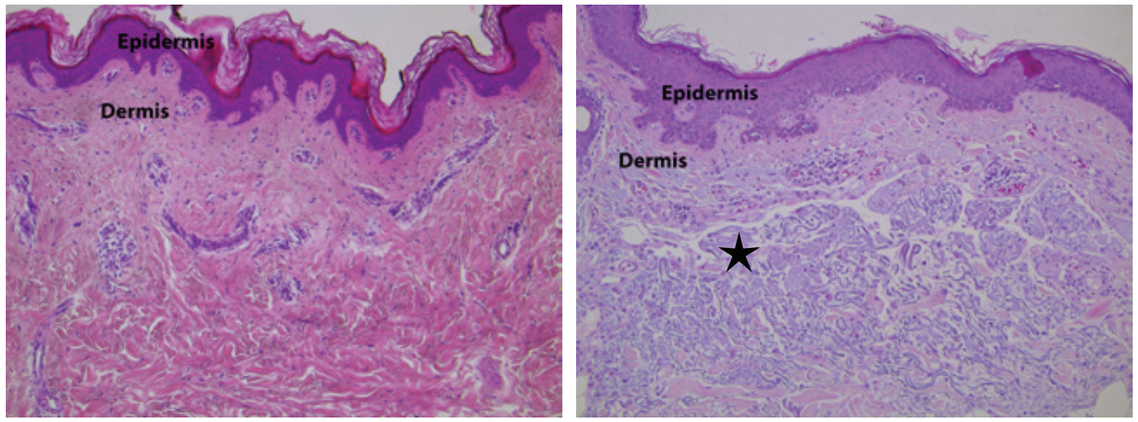


Figure 1:
Left panel: Non-exposed skin from the arm of a 20 year old. The dermis shows abundant, dense collagen (light pink bundles). The epidermis projects multiple epithelial extensions into the underlying connective tissue of the dermis. Right panel: Chronically sun damaged skin of the lower limb of a 70 year-old female. The epidermis is flattened, the amount of collagen is greatly reduced in the dermis, and instead of the architecturally preserved collagen bundles we observe degraded connective tissue (solar elastosis, star).

We have established a new line of research investigating the sex bias in the molecular landscape of cutaneous squamous cell carcinoma (cuSCC). Approximately 50,000 new cases of cuSCC are diagnosed yearly in the UK, representing a significant cost to healthcare. Patients present cuSCC at anatomic sites that have accumulated greater level of UV damage. The capacity of primary cuSCC to metastasise is low, but primary SCC incidence is so high in our population that the overall number of metastatic SCC cases is considerable. The incidence of SCC is 2-fold greater in men than women, and this bias is assumed to arise from greater male exposure to UV, from increased surface skin area of the ears and scalp of men compared to women, and due to a delay in aged men seeking clinical care. We have performed a large human audit investigating sex bias in the metastatic incidence and course of disease, and suspect an even stronger bias towards more aggressive male disease during the latter stages of progression. We are therefore performing comprehensive analyses in mouse and human cuSCC to understand the clinical differences we observe.

Publications listed on page 67

STEM CELL BIOLOGY

Group Leader
Georges Lacaud

Postdoctoral Fellows
Neo Wen Hao
Divya Malik
Muhammad Maqbool

Scientific Officers
Michael Lie-a-Ling
Rahima Patel[1]

Graduate Students
Renaud Mevel
Ewan Selkirk
Muhammad Fadlullah Wilmot

[1]Joined in 2019

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. Beyond its major regulatory role in haematopoiesis, RUNX1 has been more recently implicated in various non-haematopoietic cancers. In this context, we investigated the expression and potential role of RUNX1 in normal prostate and prostate cancer in collaboration with the groups of Dr Esther Baena at the CRUK Manchester Institute and Prof Karen Blyth at the CRUK Beatson Institute.

Prostate Cancer

Prostate Cancer (PCa) is the most commonly diagnosed cancer in men, and while its incidence continues to rise, it is currently impossible to discriminate with accuracy the difference between indolent and more aggressive forms of PCa, such as the development of metastatic castration-resistant PCa. This lack of prognostic biomarkers represents a central clinical challenge to avoid a worsening problem of over-diagnosis and -treatment. In PCa patients, androgen deprivation therapy (ADT) is frequently used as an adjunct to radiation therapy by targeting tumour cells that rely on androgens for their growth. However, despite the therapeutic benefits of targeting androgen receptor signalling, cancer cells are able to adapt and survive, resulting in disease recurrence after a few months or years. Understanding the origin of these resistant cells and the mechanisms underlying the acquisition of castration-resistance represent therefore major goals to improve PCa treatment.

The epithelium of the prostate is composed of three epithelial cell types: luminal, basal and rare neuroendocrine cells. Luminal cells form a layer of polarised tall columnar cells producing prostatic secretions and are largely dependent on androgen receptor signalling, unlike the supportive basal cell layer which is located between the luminal cells and the surrounding stroma. Surprisingly, while surgical castration of wild-type mice results in considerable cell death within the luminal layer, a small cellular subset is able to survive the process. This approach has allowed the identification of progenitor 'castration-resistant' cell populations within the adult murine prostate, characterised by their ability to survive in the absence of androgens and to fully regenerate an intact adult prostate after re-administration of testosterone. Thus, there is currently a need to better characterise the extensive heterogeneity within the epithelial compartment of the prostate, in order to improve our understanding of the processes associated with prostate tumorigenesis.

RUNX1 in normal prostate

Using fluorescent reporter mouse models, we found that *Runx1* was expressed in a large proportion of basal cells, but also strongly expressed in a subset of luminal cells. *In situ* analysis revealed that *Runx1+* luminal cells are particularly abundant in the peri-urethral region of the mouse prostate, which is thought to be enriched in stem and progenitor cells. To further evaluate the biological potential of *Runx1* expressing cells we used an e*x vivo* prostate organoid culture assay. We found that RUNX1$^+$ luminal cells were consistently forming organoids more efficiently than the remainder fractions. Also, RUNX1$^+$ luminal cells presented a strong potential at forming organoids with a hollow structure, rich in Keratin 8 expression (Figure 1), suggesting that *Runx1* is marking a specific subpopulation of luminal cells with biased differentiation potential.

In order to further characterise the biological potential of R*unx1* expressing cells, we challenged prostate epithelial plasticity by performing surgical castration experiments. Strikingly, we found *Runx1* expression in the majority of castration resistant cells. In addition, *Runx1* was expressed, both in intact and regressed prostate, and in a subset of cells which does not express *Nkx3.1*, a widely studied regulator of prostate development also known to mark cells with both castration-resistant and regenerative properties. Molecular profiling of the different compartment of the prostate by single cell RNA sequencing before and after surgical castration revealed that high Runx1 expression marks an independent luminal lineage in intact prostates, which has a close transcriptomic profile with castration resistant luminal cells. We then hypothesised that the increased proportion of RUNX1$^+$ cells following castration could be a consequence of either an induction of *Runx1* expression or the existence of intrinsically castration resistant RUNX1$^+$ cells present in the epithelium of intact mice. Using an *in vivo* lineage-marking approach, we were able to demonstrate that RUNX1$^+$ luminal cells are intrinsically castration resistant and maintain their identity after androgen deprivation. However, it was also evident that the regressed prostate is composed of cells which were not initially expressing *Runx1* in the epithelium of intact mice, meaning that androgen deprivation also leads to upregulation of *Runx1* expression in these other castration resistant populations. We then administered ectopic testosterone to the mice back to physiological levels to stimulate epithelial regeneration and evaluated the consequences on lineage marked RUNX1$^+$ cells. In this setup, we were unable to identify clonal expansion in the fully regenerated prostate, suggesting that intrinsically castration-resistant *Runx1* expressing cells do not proliferate in response to androgen signalling.

Our results led us to interrogate the pattern of Runx1 expression during the establishment of the prostate epithelial lineages. During *in vivo* embryonic prostate development, we found strong *Runx1* expression in the stratified urogenital epithelium, and lower levels in a specified subset of prostatic epithelial cells characterised by high levels of *Nkx3.1* and *p63*. Throughout the development of the prostate, RUNX1+ cells were mainly localised in the proximal region of the ducts, which later corresponds to the peri-urethral region. Using a urogenital sinus explant culture model to study embryonic prostate development *ex vivo*, we were able to show that the majority of epithelial expansion takes place in the NKX3.1$^+$ compartment, found in the distal part of the ducts, while RUNX1$^+$ cells remain largely quiescent at the centre/proximal region of the explant (Figure 2). These results show that during prostate development, *Runx1* marks a population of cells already distinct from the NKX3.1$^+$ lineage, enriched in the peri-urethral area, which is likely to correspond to the mature peri-urethral RUNX1$^+$ luminal cells found in the adult prostate.

Overall, the study of *Runx1* expression in the mouse prostate has allowed us to characterise specific lineages that reside in the prostate epithelium. In particular, we found that *Runx1* marks a population of adult luminal cells with intrinsic castration resistant potential. Further studies are now warranted to assess whether oncogenic events occurring within this cellular subset would lead to aggressive castration resistant tumours. Also, the potential functional role played by *Runx1* in prostate epithelial cells would need to be studied further, notably to evaluate to which extent it participates in prostate tumorigenesis. Finally, it would be important to determine whether RUNX1 could be used as a clinical marker of PCa in patient samples.

Publications listed on page 67

Figure 1

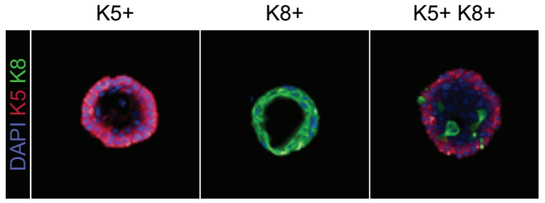


Figure 2

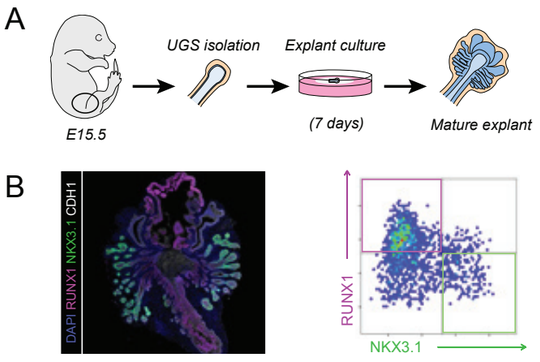


Figure 1:
Whole-mount immunofluorescent staining of mouse prostate organoids derived from Runx1 expressing cells.

Figure 2.
(A) Schematic of the UGS explant culture model to study early events of prostate organogenesis (B) Co-immunofluorescent staining for RUNX1/NKX3.1/CDH1 after 7 days in UGS explant culture. RUNX1 and NKX3.1 mark distinct cellular subsets.

SYSTEMS ONCOLOGY

Group Leader
Claus Jørgensen

Postdoctoral Fellows
Celia Cintas[1]
Adrian Gomez[1]
Brian Lee
Nasir Haider[1]
Joanna Kelly[1]
Stephen Kershaw[3]
Alexander Kononov[1]
Giulia Veluscek[2]

Scientific Officer
Xiaohong Zhang

Graduate Students
Christopher Below[4]
Catherine Felton[1]
Felix Heider[1]
Elizabeth Hogg[3]
Colin Hutton
Amy McCarthy[2]

Clinical Fellow
Konstatinos Georgiadis[5]

[1]Joined in 2019
[2]Left in 2019
[3]Joint with Caroline Springer, DDU
[4]Joint with Martin Humphries, University of Manchester
[5]Joint with Caroline Dive, CBC and Juan Valle, Christie NHS Foundation Trust

Tumours are complex ecosystems where cancer cells are embedded within a complex stromal reaction comprising multiple infiltrating cell types and pathological changes to the extracellular matrix. The aim of the Systems Oncology laboratory is to determine and define the rule-set by which tumour cells conscribe host cells to support tumour growth and resistance to therapies. Understanding these rules will enable development of synergistic combination therapies targeting both tumour cell intrinsic dependencies as well as their extrinsic dependencies on stromal reciprocal signals.

Pancreatic Ductal Adenocarcinoma

Pancreatic Ductal Adenocarcinoma (PDA) is a dismal disease with an average five-year survival rate of 9%. This is due to a combination of late diagnosis, the aggressive nature of the cancer and a lack of effective therapy. Consequently, while PDA only is 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 suppressors CDKN2A, SMAD4 and TP53.

PDA is characterised by an extensive desmoplastic reaction, which makes up 80% of the tumour volume on average. Here, an abundant and pathological remodelled extracellular matrix increases the tissue stiffness and restricts perfusion to limit therapeutic delivery. In addition, there is an abundant infiltrate of fibroblasts and myeloid cells, which together create an immune-privileged environment and confer resistance to therapy. Genetic heterogeneity of tumours is associated with aggressive behaviour and rapid development of therapeutic resistance. However, it is less clear whether heterogeneous populations of tumour cells also establish distinct reciprocal interactions with stromal cells. This is an important question as heterogeneous interactions across tumour and stromal cell populations increase functional plasticity and may therefore impact development and implementation strategies for stromal-targeting therapies. In order to interrogate such interactions we recently studied a set of single cell derived populations of pancreatic cancer cells and their interdependencies with stromal fibroblasts (Figure 1). We observed that individual tumour cell clones instigate diverse stromal behaviour, where some tumour cell clones induce fibroblasts to regulate the extracellular matrix and others induce fibroblasts to be more immune regulatory. Interestingly, while stromal cells appeared more diverse, the ensuing reciprocal effect on individual tumour cells lead to normalisation (phenotypic convergence). For example, the transcriptional programmes regulated in the tumour cells were less diverse (more similar) in the presence of stromal fibroblasts. Moreover the activity of the regulatory MAPK signalling pathway was normalised between tumour cell clones, when they were allowed to interact with stromal fibroblasts. Consequently stromal interactions had diverse impact on the effect of specific therapeutic inhibitors, such as MEK inhibitors. Together these data suggest that interactions between tumour and stromal cells need to be carefully considered when devising therapeutic strategies and that stromal targeting may have an heterogeneous effect in a complex tumour ecosystem.

Defining and targeting the tumour microenvironment in PDA

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-selective approach can be taken. In order to define interdependencies between tumour and stromal cells it is critical to map the cellular and extracellular components of 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.

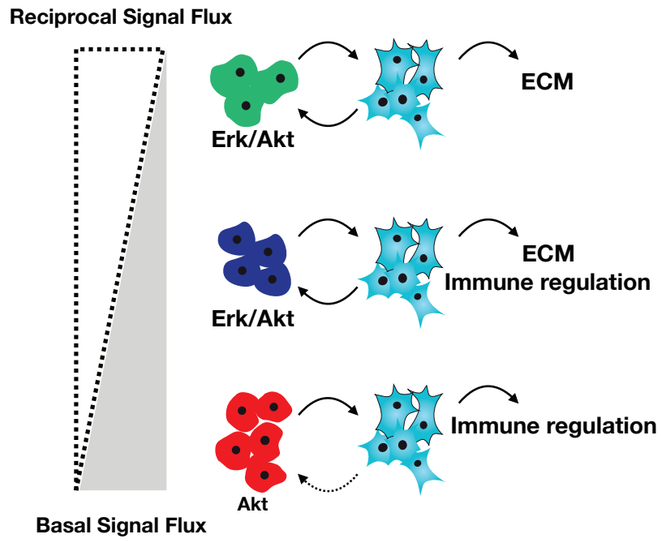
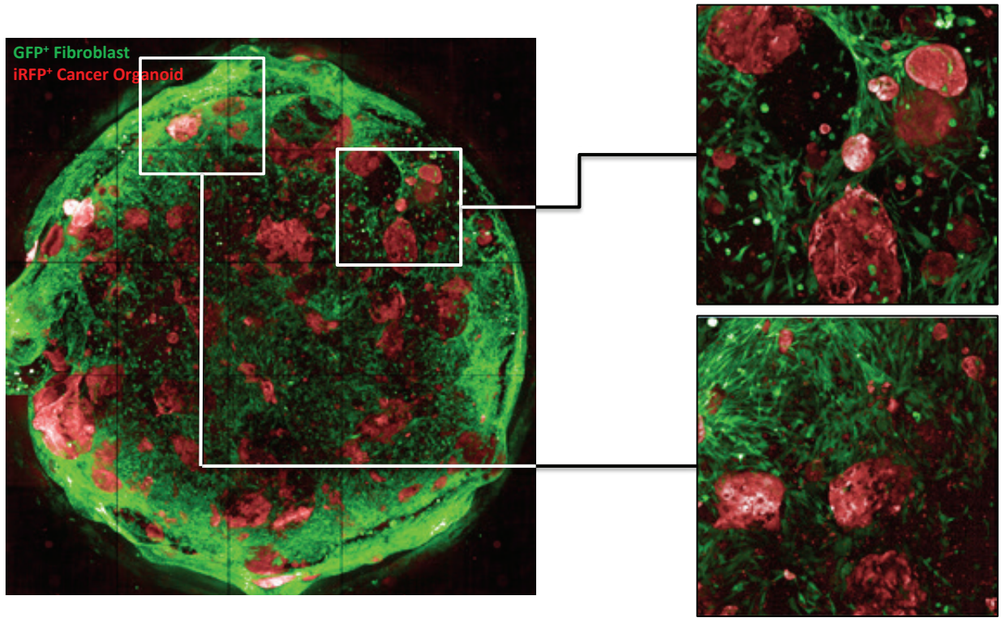


Figure 1:
Model of heterotypic interactions across heterogeneous tumour cells and stromal fibroblasts. Clonal tumour cell populations drive diverse phenotypic responses in stromal fibroblasts, which in turn differentially engage tumour cell signalling to normalise output.

Delivering personalised medicine in PDA

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, PRECISION-Panc, where individual tumours are subjected to molecular profiling such that patients can be matched with selected treatments. These clinical trials are underpinned by the development of biomarkers and pre-clinical research to further refine treatment strategies targeting specific dependencies. Together with collaborators at The Christie NHS Foundation Trust and Central Manchester NHS Foundation Trust we have implemented methodologies for isolation and expansion of primary tumour cells in 3-dimensional cultures (also known as organoids). Matching the tumour microenvironment to mimic the cellular and biophysical constraints of the patient tumour, these models may be used to define optimal combination of therapies and biomarkers to further develop clinical trials.

Figure 2:
Pancreatic cancer organoids and stromal cells.



TRANSCRIPTIONAL NETWORKS IN LUNG CANCER

Group Leader
Michela Garofalo

Postdoctoral Fellows
Lei Shi
Tiziana Monteverde

Scientific Officer
Peter Magee

Graduate Students
Lucy Ginn[1]
Manuela La Montagna
Athanasios Paliouras[2]

[1]Joined in 2019
[2]Left in 2019

Lung cancer is the primary cause of cancer-related mortality worldwide. The major focus of 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%) and in lung adenocarcinoma harbouring K-Ras mutations, although so far, no specific drug demonstrated efficacy. 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.

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. 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.

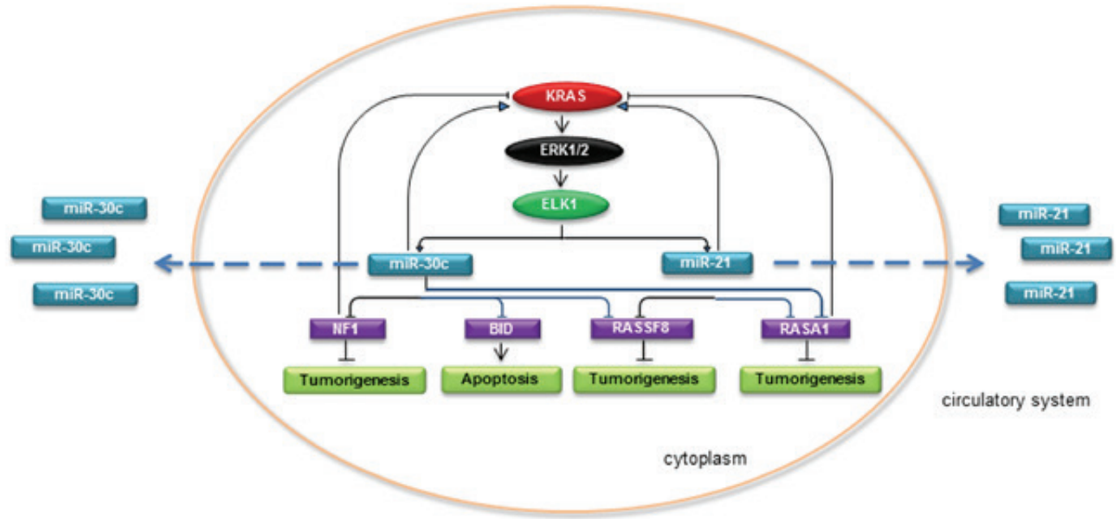


Figure 1. Working Model.
KRAS activates miR-30c and miR-21 through the transcription factor ELK1, which in turn by downregulating NF1, RASA1, RASSF8 and BID, regulates KRAS, NF-kB and the intrinsic apoptotic pathway, inducing lung tumorigenesis and inhibiting apoptosis in NSCLC. MiR-30c and miR-21 are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

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. These compounds robustly induce apoptosis through downregulation of anti-apoptotic genes. Importantly, alvocidib reduced tumour progression in vivo in xenograft mouse models. Furthermore, we found that two microRNAs, miR-25 and miR-30c, are upregulated in crizotinib-resistant cells and in plasma of patients who developed resistance in the clinic and therefore they could be potential biomarkers of resistance to ALK inhibitors. In summary, our study takes advantage of the transcriptional addiction hypothesis to propose a new treatment strategy for a subset of patients with acquired resistance to first, second and third-generation ALK inhibitors.

TRANSLATIONAL ONCOGENOMICS

Group Leader
Robert Bristow

Scientific Officers
Steve Lyons
Alicja Partridge[1]

Postdoctoral Fellows
Richard Rebello
Christoph Oing[2]

Graduate Students
Alexandru Suvac
Ronnie Rodrigues
Jack Ashton

[1]Joined in 2019
[2]Left in 2019

With an estimated 1.3 million new cases identified worldwide in 2018, prostate cancer is the most frequently diagnosed male cancer in the Western world, and in the UK, there are approximately 11,700 prostate cancer deaths per year.

The key challenge in management of this disease is to accurately assess the risk of progression to metastasis in each patient. Low risk, indolent tumours with favourable pathology may never progress, and in such cases active surveillance is a treatment option. Intermediate risk cancers that remain localised to the prostate are potentially curable with either surgery or radiotherapy approaches. However, high risk cancers have an increased probability of producing incurable metastases which may be undetectable at the point of diagnosis. Here, localised therapy (radiotherapy/surgery) is combined with systemic treatments to target secondary disease. Although initially effective, androgen deprivation therapy is often evaded, and an incurable metastatic castrate-resistant prostate cancer emerges.

The current method of quantifying risk in prostate cancer involves measurement of PSA (Prostate Specific Antigen) levels in serum, pathological assessment of biopsy material to produce a Gleason Score, and staging of the disease using the internationally recognised TNM (Tumour, Node, Metastasis) staging system. Yet despite the use of stringent clinical criteria to place patients into prognostic groups, 30-50% of men can still fail precision radiotherapy or surgery due to local resistance and/or systemic spread. Clearly, there is a need to develop new biomarkers that give an insight into heterogeneity of outcomes in prostate cancer patients. In addition, new biomarkers that predict patient responses to the various treatment options will also be required. To this end, recent advances have shown that detailed understanding of the genomic/transcriptomic landscape of prostate cancer together with knowledge of the degree of hypoxia within tumours can provide important insights into the aggressiveness and likely prognosis of localised prostate cancer.

Hypoxia and genome instability – signs of aggression in prostate cancer

In many solid tumours, regions of acute or chronic hypoxia develop as proliferating tumour cells outstrip the ability of a poorly organised vasculature to supply oxygen. Hypoxia correlates with a poor prognosis in prostate cancer, and this could be due to acquisition of a more metastatic phenotype and/or resistance to radiotherapy. Our work has shown that hypoxia is frequently associated with a range of unfavourable pathophysiological features. These include the presence of an adverse sub-pathology, IDC-CA (intraductal carcinoma with cribriform architecture), a propensity to metastasise, and high rates of copy-number aberration. Indeed we have shown previously that combined indices of genomic instability and hypoxia can improve accuracy of prognosis in following either radical prostatectomy or radiotherapy. Therefore, the relationship of hypoxia to genome stability is a major theme in our work.

In our latest bioinformatic analysis (Bhandari et al. Nature Genetics, 2019), we have taken advantage of well characterised gene expression scoring systems to estimate levels of hypoxia in a cohort of 1188 tumours from 27 cancer types together with matched normal tissue controls. This analysis detected subsets of patients with elevated hypoxia in 23 of the 27 cancer types, and tumours consistently had higher hypoxia scores than normal tissue. Notably, we found that the extent of hypoxia varies substantially across patients within cancer types. This striking variability suggests assessment of hypoxia could provide valuable information for use in personalised medicine strategies. Correlating hypoxia scores with genomic features, we found hypoxia associated with increased genome instability as measured by the percent genome alteration criterion. Alterations such as deletions and duplications were significantly higher in hypoxic tumours, as was the number of single nucleotide variants/Mb. At the individual gene level, hypoxia was associated with significantly more frequent alteration of several oncogenes and tumour suppressors including *TP53*, *MYC* and *PTEN*. Furthermore, the mutation profile of hypoxic tumours is consistent with inefficient mismatch repair and homologous recombination repair of DNA. This hypoxia-associated genome instability is in agreement with our previous analysis of a separate cohort of

Figure 1.

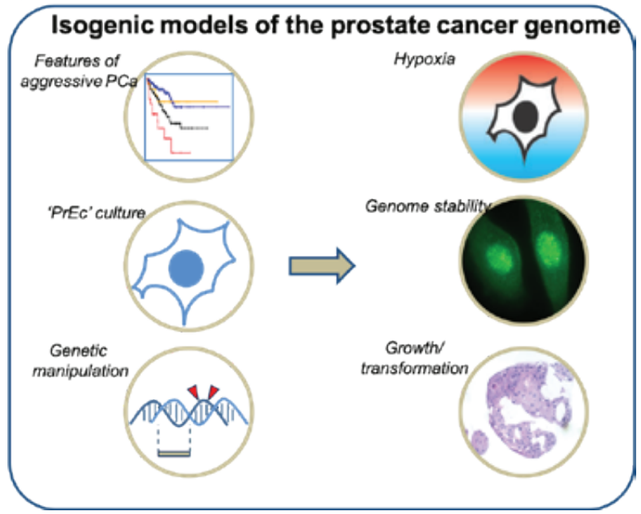


Figure 1.
Next generation sequencing studies have identified the genomic alterations correlated with poor outcomes in prostate cancer. By working with primary cell cultures isolated from patients, we aim to characterise the roles of specific gene alterations in driving phenotypes relevant to the disease. Immortalised primary cells will be engineered to recapitulate the prostate cancer genome using a range of molecular techniques. The engineered cells can then be used to provide insights into the responses to hypoxia, genome stability and DNA repair, tumorigenesis and drug resistance.

Figure 2.
The HYPROGEN study will provide unique insight into prostate cancer metastasis. By taking biopsies from patients with oligo-metastatic disease prior to treatment, we aim to comprehensively characterise the processes involved in the transition from localised to systemic cancer. The tracer molecule Pimonidazole will be used to assess the impact of hypoxia in driving extra-prostatic spread and genomic instability. We will compare the molecular profiles of matched primary and metastatic tumours, to identify novel features and correlate these with hypoxia levels. The prevalence of circulating tumour cells and their contribution to secondary tumour formation will also be assessed.

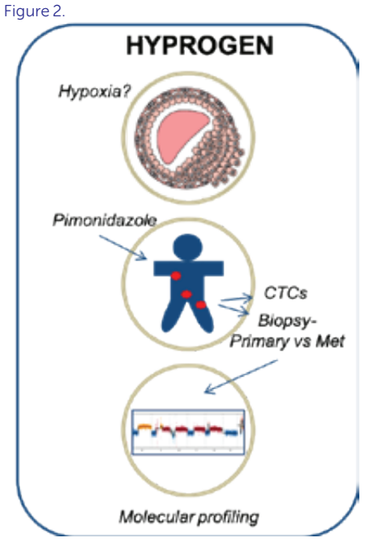
tumours, and raises the intriguing possibility that hypoxia may be a feature of the tumour microenvironment that impacts tumour evolution, and perhaps selects for specific alterations. This process may be facilitated by inefficient DNA repair since hypoxia is known to lead to downregulation of several repair proteins including *MLH1*, *MSH2*, *RAD51* and *BRCA2*. Further work involving experimental studies is now required to shed light onto the molecular mechanisms at play in hypoxic tumours.

Our laboratory studies aim to address the interplay between hypoxia and the function of potentially oncogenic genomic aberrations. Primary prostate epithelial cells form the basis of our isogenic cell culture models. Once immortalised by expression of the telomerase gene, these cells provide the ideal basis for further genetic engineering since they have an unaltered genome that remains stable during culture. We have used the latest genome engineering techniques to exogenously express, or mutate those genes we identified as being more frequently altered in hypoxic cells, prioritising *PTEN* and *MYC*. In addition, we are also investigating the role of *BRCA2*, a DNA repair gene that when mutated in carrier families, significantly increases the risk of developing aggressive prostate cancer. We are currently analysing the phenotypes of these cells with respect to genome stability, DNA repair, transformation and the response to hypoxia. Given the clear correlation between hypoxia and genome instability observed in patient biopsy material, it will be important to test whether exposure to chronic or cycling hypoxia is sufficient to drive mutational processes.

Illuminating the genomic landscape of hypoxia-driven early metastatic prostate cancer – HYPROGEN (HYpoxia-driven PROstate cancer GENomics)

Patients unlucky enough to have metastatic prostate cancer at the point of diagnosis are rarely

Figure 2.



offered treatments specifically targeting the primary tumour. Since the disease has spread, systemic treatments are required to control the disseminated malignancies. However, recent findings from the STAMPEDE trial demonstrate a significant improvement in disease-free survival in patients with oligo-metastatic disease (≤4 detectable metastases), who receive radiotherapy to the prostate. This striking finding suggests that the primary tumour continues to contribute to metastatic spread over a long period – perhaps by releasing further tumour cells into the circulation, or by expressing soluble factors that prime pre-metastatic niches. Our lab previously reported that the co-presence of tumour hypoxia (based on mRNA signatures or needle electrode measurements) and genomic instability synergistically portend rapid relapse after primary treatment for prostate cancer, supporting the concept that a hostile tumour microenvironment may select for or drive adaptation of a distinctive genomic profile and rapid failure due to occult metastases. This raises the possibility that hypoxic regions present in primary tumours play an important role in the development of metastatic disease. The HYPROGEN trial aims to characterise the role of hypoxia in driving the early metastatic spread of tumours. Patients with oligo-metastatic disease will be recruited to the study prior to receiving treatment. Hypoxia will be assessed by means of the tracer molecule, pimonidazole, which will be administered before biopsies are taken from both the primary tumour and selected bone metastases. Genomics and transcriptomics will then be applied to provide insights into the relationship between hypoxia, genomic instability and metastatic spread. We will test whether early metastases, circulating tumour cells and hypoxic regions of the primary tumour share distinct genomic and/or gene expression profiles.

Publications listed on page 68

TUMOUR SUPPRESSORS

Institute Fellow
Patricia Muller

Postdoctoral Fellow
Yannick von Grabowiecki

Graduate Student
Callum Hall

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.

P53 is a tumour suppressor that plays a pivotal role in detecting a variety of stresses and helps with deciding how cells are to react to cellular stress. If the stress is minimal, p53 will help in allowing for cell survival and repair of stress-related damage. Alternatively, if the stress is too much, p53 will initiate an apoptotic program to destruct the damaged cell. In order to orchestrate this, p53 acts as a transcription factor that can regulate hundreds, if not thousands of genes dependent on its levels of expression, epigenetic modifications and a variety of other factors. Ultimately, these p53 functions prevent the formation of DNA mutations and therefore prevent tumour formation. However, TP53 itself is the most frequently mutated gene in human cancers, illustrating its importance as a tumour suppressor. Remarkably, almost any amino acid in the p53 protein can be found mutated in human cancers, but mutations are more common in the DNA binding region of the molecule. In addition, there are a few hotspots that have been more thoroughly studied, although these comprise only 10-25% of all the p53 missense mutations that are found in cancers.

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, many 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 (see review Hall & Muller, Int J Mol Sci 2019) 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). In a screen to detect mutant p53 dependent RCP-interacting proteins, we identified drug transporters that mediate the efflux of chemotoxic drugs out of cancer cells. We are following up on these data and are investigating a role for these proteins and RCP in mediating mutant p53-dependent chemoresistance.

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). When looking at the mutation frequency in SCLC compared to all cancers, there are clear differences 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

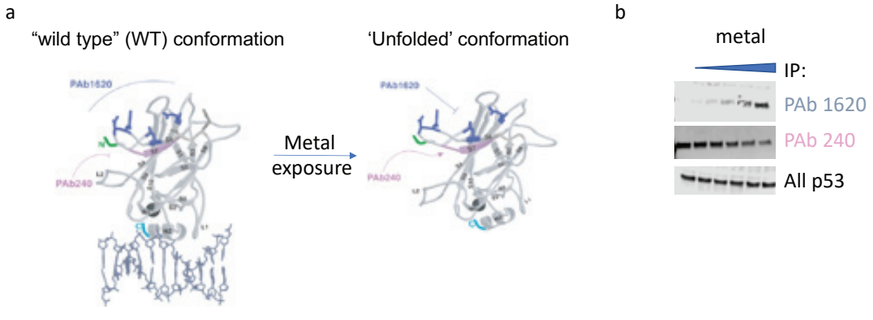
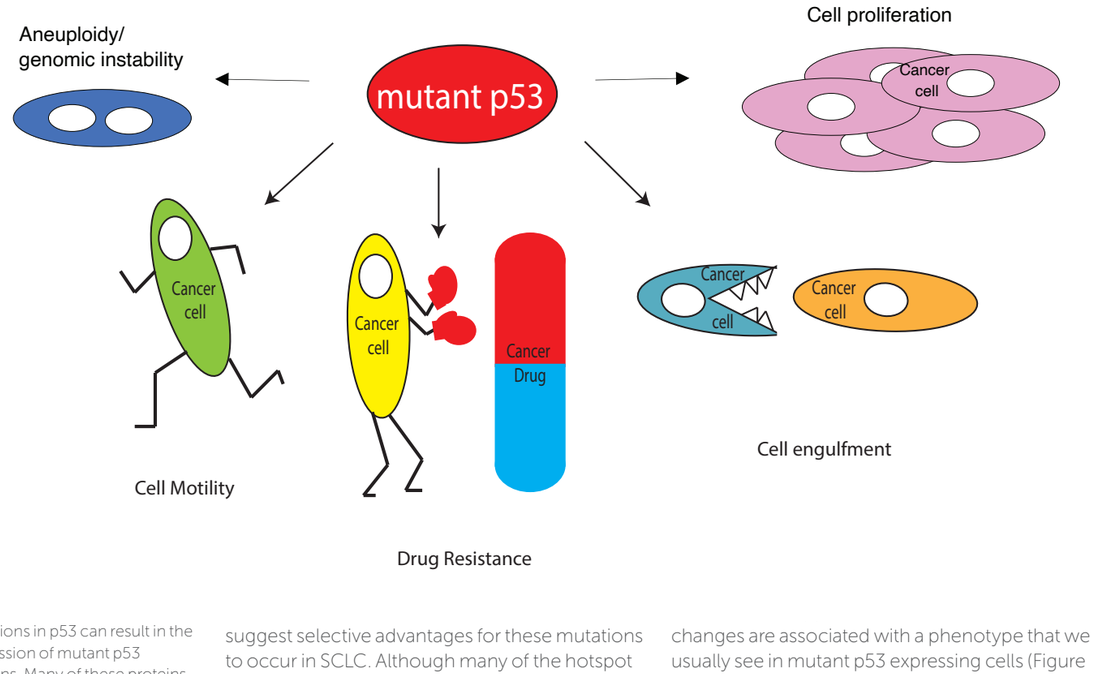


Figure 1.
The folding states of p53
a) Under normal conditions, p53 is folded to interact with the DNA. Upon exposure to certain metals, p53 is partially unfolded, which causes it to lose DNA binding capacity and makes it inactive for normal WT function. The folding states can be detected by two antibodies PAb1620 and PAb240. PAb1620 recognises multiple epitopes that are in close proximity when folded, but too far apart to be recognised by this antibody upon unfolding. PAb240 recognises a cryptic epitope that is only accessible when the p53 molecule is unfolded. Picture adapted from Wang PL et al (Oncogene, 2001). b) Western blots of immunoprecipitations with these antibodies under native conditions, allowing to detect folded and unfolded p53 upon metal exposure.



Mutations in p53 can result in the expression of mutant p53 proteins. Many of these proteins have lost some or all of their WT functions. In addition, many gain novel functions involved in various aspects of tumourigenesis. Depicted are those functions that we are studying in the lab.

suggest selective advantages for these mutations to occur in SCLC. Although many of the hotspot mutations have been thoroughly researched for their role in invasion, chemoresistance and metastasis, not much is known about these mutations specifically. In collaboration with CBC, we have created a library of these selected mutations and we are investigating if the mutations that occur in SCLC are beneficial to the growth, metastasis and survival of these tumour cells.

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 binding to mutant-specific interacting proteins. Smokers' lungs have been exposed to a variety of metals and in many cancers increased metal levels can be detected. Certain metals can cause a conformational change in p53 that renders it impaired in normal changes are associated with a phenotype that we usually see in mutant p53 expressing cells (Figure 2). Certain mutants have been characterised as 'unfolded', whereas others appear normally folded. Most likely this differentiation is too black and white and gradations of unfolding exist. This could mean that certain p53 mutant proteins might be more vulnerable to metal-mediated unfolding than others, which will have functional implications in cancers in which abnormal metal levels are present. We are currently characterising which metals affect p53, which mutants are most vulnerable for this and what are the functional consequences of metal exposure on tumours in vivo.

Publications listed on page 68

CANCER RESEARCH UK MANCHESTER INSTITUTE

RESEARCH SERVICES

RESEARCH SERVICES

Chief Laboratory Officer
Stuart Pepper

As the following reports show, 2019 has been a very productive year for the scientific core facilities. Achievements have been in a variety of different areas, which demonstrates the breadth of activity in the core facilities.

Chief Laboratory Officer
Stuart Pepper

In both Biological Mass Spectrometry and Scientific Computing there has been a focus on developing workflows and services on new hardware, leading to some exciting new capabilities, including an enhanced virtualisation infrastructure. These two teams have also worked closely with the newly expanded Computational Biology team to develop end to end solutions for mass spec data generation, storage and analysis. As technologies have evolved this close interaction between the traditional core facility areas has become vital and it has been great to see over the last year how closely the teams in IT, Sci Com, Mass Spec and the Molecular Biology Core Facility have worked together to provide seamless end to end support for research projects.

The collaborative approach is also well developed within the Visualisation, Imaging and Irradiation team who work closely with both Histology and the BRU Experimental Facility to support research activity. A new high throughput scanner has come online this year, and the work has begun with Histology to further expand our capability for multiplex staining during 2020. Support for multiplex analysis of single cells has also developed in the FACS service as the Helios platform has been used as the basis for a robust immuno-profiling platform.

The Experimental Facility have also carried out some excellent work developing ultrasound guided injection of tumour cells, a technique that delivers 3Rs benefits and scientific benefits in equal measure. The BRU Transgenic Breeding team have had an overhaul of operations with the introduction of Tick@LAb, software that improves the efficiency of operation of the entire facility. Our Transgenic Production Facility has continued to be highly successful at delivering new mouse models, but has also contributed to the broader scientific community by co-organising a national meeting on CRISPR technologies.

Next generation sequencing has been a core service for some time, but there is continued development on specific applications. Over the last year there has been a significant expansion in single cell sequencing projects at the Institute, which the Molecular Biology Core Facility have supported. Towards the end of the year the MBCF took delivery of a NovaSeq instrument, which will be used to further develop sequencing services in 2020.

A key consideration when developing services is balancing the provision of full services, where the facility do all sample processing and training for users to gain expertise in particular techniques. The Histology facility blends these two approaches particularly well; by combining provision of services with extensive training, our early career scientists gain valuable expertise, and we are able to achieve sample throughput on expensive equipment that could not be achieved with a small Histology team.

Overall the core facilities have been involved in a diverse range of activities supporting research projects across the Institute. With new equipment and newly appointed staff the next year should also prove to be highly successful.

Biological Mass Spectrometry
Duncan Smith, Yvonne Connolly

2019 saw the re-launch our in-house MS services on the Alderley Park site. The facility benefits from access to three cutting-edge mass spectrometers (Orbitrap Lumos, Q-Exactive HFX and a 6600 Q-ToF). Both Orbitrap based systems were new acquisitions at the end of 2018, bringing the facility right up to date with the latest technology available. Q1 of 2019 saw the extensive optimisation of key proteomic application workflows on the new systems. Excitingly, the expected performance enhancements these systems offer over the previous generation of instruments they replaced were exceeded in practice. For example, our absolute sensitivity of protein detection was improved by more than a magnitude using the Orbitrap Lumos, allowing us to characterise proteins whose abundance was too low to detect at all previously. We also exploited a new type of liquid chromatography chemistry, micro Pillar Array, in collaboration with PharmaFluidics. The use of these highly ordered laser etched micro pillars containing an outer porous shell grafted with C18 groups resulted in a significant increase in our peak capacity (the number of analytes we can fully resolve in a given time) and boosted sensitivity by approximately three-fold. The characteristics of this new chemistry allow us to more comprehensively profile complex and low abundance proteomes. The hardware and chemistry enhancements were carefully exploited with a bespoke set of method development projects applied to our suite of applications for qualitative and quantitative analyses of proteins and their post-translational modifications. This has resulted in additional applications being offered to our users, taking advantage of the full abilities of the new hardware.

This year saw the uptake of label-free, TMT and SILAC based quantitative applications in both protein profiling and phospho-protein profiling domains to great affect. 2019 also saw a step change in the way proteomic datasets are computationally processed. The Scientific Computing team have performed a wonderful job of migrating our data processing pipelines onto a new virtual machine environment they built and manage on our behalf. Their hard work and application has resulted in our ability to share high performance computing resources across multiple workflows without the need to replicate this resource for every software pipeline we use. We can therefore run our data analysis applications at exceptionally high performance levels and trial new software applications without costly outlay for new hardware. Timely to this job's completion, was the appointment of a new Bioinformatician to the Computational Biology Support team in late 2019. The new role is shared between MBCF and MS and the role will provide professional bioinformatics support to the MS facility users proteomic data analysis needs.

Biological Resources Unit
Transgenic Breeding
Jennifer Hughes, Daniel Bennett, Tim Bloor, Carl Conway[1], Ali Jammoul[1], James McDonald, Wesley Moore[1], Kerry O'Shea, Rose Storey, Natalie Varley[1], Martin Vincent, Mark Willington[1,2]

[1]Joined in 2019 [2]Left in 2019

The BRU Transgenic Breeding Team breeds mice to meet the requirements of CRUK MI researchers. Services offered in the last year have included 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.

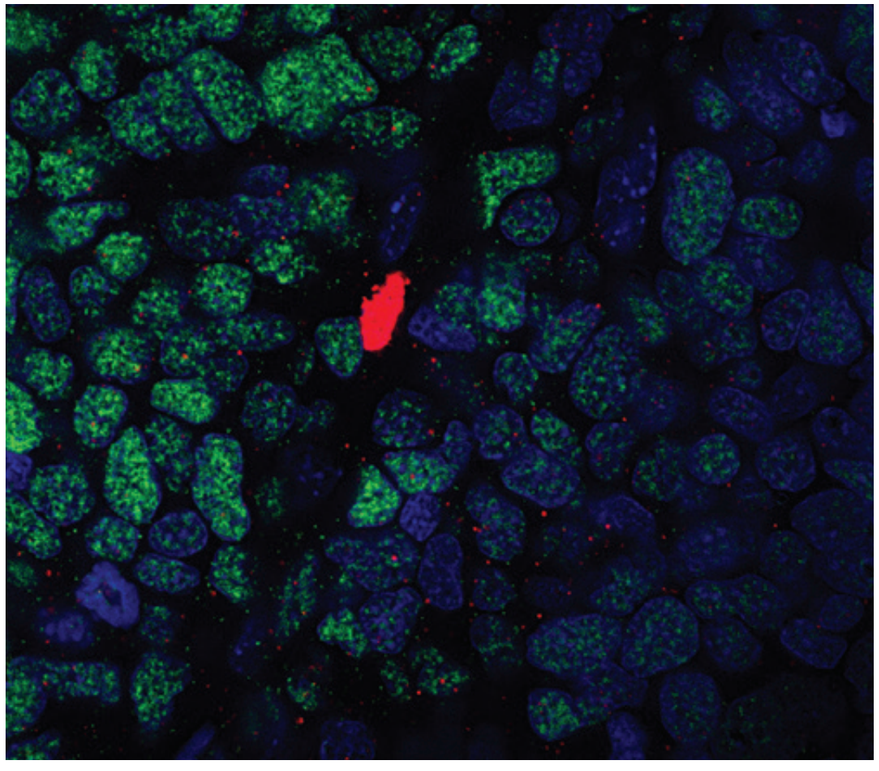
The breeding facility is housed in a clean unit with a high health status and is kept free from common mouse pathogens, which means that new transgenic mouse lines from external sources cannot be brought in directly as live mice. New lines coming from external sources instead have to be transferred in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

Twelve staff members currently provide day-to-day care for 141 different transgenic mouse lines spread across approximately 2,300 cages in a facility located within The University of Manchester main campus. The last year has been busy with 52 new breeding lines being started and 44 lines being closed. Thirteen of the 52 new lines have been rederived, using embryos and live mice sent from the USA and UK. Another four of the new lines have been transferred from TPF, three have been rederived from in-house frozen stocks 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 one week acclimatisation is required before mice can be enrolled in experiments. As well as shipping to Alderley Park, this year we have also shipped live mice to Switzerland, USA and North Korea, and have sent frozen embryos to USA and Netherlands.

2019 saw the introduction of the specialist mouse management system, going live in February 2020. Records for all live mice in established transgenic colonies were transferred in the initial upload, with remainder of records being added soon after. The system is now being used for keeping detailed animal stock records, including breeding details and genotype results, tracking health problems, and is also used for sending and reporting completion of tasks. We are moving over to using the system for semi-automated transfer of genotyping results and other functionalities may be explored. One major advantage of moving over to the new system will be that mouse details should be much more easily searchable across all lines, allowing easy identification of subsets within the population. Similarly tasks can also be tracked or searched, allowing both researchers and technicians to track progress more easily. Although edits can be made to the data on the system, changes will be tracked and viewable within the history functionality, leading to both greater transparency and greater security.

A CDX derived from a patient with small cell lung cancer was screened for the most commonly expressed transcription factors. It is shown to mostly express ASCL1 (in green), but also a few dispersed single cells expressing NEUROD1 (in red), that do not express ASCL1.

Image supplied by Alessia Catozzi (Cancer Biomarker Centre)



Experimental Services

Team Leader: Lisa Doar

2019 has been a busy year for the BRU Experimental Facility, with the workload continuing to increase, which is a great outcome following the disruption of the past two years since the fire.

Outside of the day-to-day workload, the team has been involved in giving talks, attending training and symposiums, and developing 3Rs initiatives (reduction, refinement and replacement). We have given talks on some of our interesting models and model development work, which is an important part of our work because the development of a good refinement should be shared more widely with other institutes. The team have also taken part in some public engagement events and have been involved in training organised by Understanding Animal Research (UAR) in how to talk to school children about animal research.

In terms of 3Rs initiatives, we are currently working on rolling out tunnel handling of mice across the facility. It is thought that handling the mice in plastic tunnels causes them less stress than the traditional method of picking them up by the tail so this is something we want to strongly consider as a welfare improvement. Another initiative is refining our injectable anaesthetic doses for our tumour-targeted irradiation (X-ray) work and also developing the equipment we use to restrain the mice for the procedure so that we can use inhalation anaesthesia instead of injectable anaesthesia, which has a much faster recovery time and is better tolerated by the mice.

In terms of technical development, we have also been working on utilising our ultrasound system to enable us to carry out image guided injection of tumour cells into different organs as an alternative to relying on surgery. We are yet to trial this in a live study but the developmental work is going well and we are looking at carrying out injections into the pancreas and also into the bladder wall via this method. The process is considerably quicker to carry out than surgery as well as being much less invasive for the mice, however it does require great technical skill so training will need to be undertaken.

Alongside our normal day job, the team have been putting time and effort into the plans for our new BRU facility which will be built as part of the Paterson Redevelopment Project. We have had support from some companies experienced in designing and building several other animal facilities. Following much discussion, we now have the facility design detailing exactly where every piece of equipment will be located in each room. The opportunity to design our animal facility and the planning process has been a rewarding experience.

Molecular Biology Core and Computational Biology Support Facility

Wolfgang Breitwieser, Andzhela Abu Rashed, Chris Clark, Bonnie Evans, Rachel Horner, Dave Lee[1], Amy Priestman, Sudhakar Sahoo, Robert Sellers, and John Weightman

[1]Joined in 2019

Cell culture is a fundamental tool of many molecular biology research activities. It is therefore crucial that cultured cells are unmistakably identified and are free from common pathogens such as mycoplasma. All scientists at the MI are strongly encouraged to test their cells upon receipt of new lines and regularly during culturing. The Molecular Biology Core has been offering a mycoplasma screening service for a number of years using a PCR method and has, in the last year, switched to a highly reliable QPCR detection method. In addition, prior to injection into mice, all cell line samples are routinely screened for both mycoplasma and mouse hepatitis virus (MHV). Detection is carried out using highly sensitive and specific reverse transcription coupled QPCR. Apart from the obvious reason of wanting to be certain which cell line one is working with journals and funding bodies frequently ask for evidence that cell lines used in research have been authenticated. The most common approach to authenticating cell lines is with a multiplex PCR assay, which detects highly variable short tandem repeat (STR) regions in the genome. The Core's Cell Line Authentication Service currently uses a commercial kit that examines a total of 21 loci across the genome. In addition, we have recently explored the application of Next Generation Sequencing (NGS) in the analysis of STRs as well as single nucleotide polymorphisms (SNPs) for cell authentication.

NGS related method development and validation remain a major focus for the Core's services. Over the last year especially, we experienced a rapid uptake of single cell RNA sequencing projects. The service currently offers two high throughput SC-RNA sequencing methods, SMART-Seq2 as well as 10x Genomics Technology. The latter, based on single cell compartmentalisation using nano-liter sized gel beads in emulsion (GEM) has proved very successful as it enables the simultaneous analysis of thousands of single cells in every experiment. Through comparative validation tests we showed that the GEM based single cell partition results in highly reproducible gene expression profiles. To date the service has processed a wide variety of input samples, ranging from cultured cell lines to primary tissue including blood, bone marrow and tumour biopsies. In further developments in SC-RNA sequencing the service also incorporated methods for sample multiplexing (cell hashing) as well as feature barcoding (CITE-Seq) that enables the simultaneous analysis of the transcriptome and panels of protein markers in single cell populations. The annotation of the heterogeneous cell types from primary tissue poses interesting new challenges. To this end, a range of novel bioinformatics analysis tools has been assessed and acquired by Computational Biology Support.

The Computational Biology Support team is responsible for genomics and transcriptomics analysis for projects undertaken by groups at the MI. Crucially, the team is also tasked with the establishment and benchmarking of bioinformatics workflows for analysis pipelines, e.g. for bulk and single cell RNA, ChIP, whole genome, and targeted sequencing. Starting at the end of 2018, the CBS has undergone substantial changes in the team structure that has resulted in the merger with the Molecular Biology Core. Apart from the continued support

for the Institute's research groups, the merger has produced a marked improvement in speed of quality control and validation of sequence run data coming off the instruments. This has proved of enormous benefit to maintain quality in standard services and in the validation of new methods. In addition to providing a helpful bridge between the wet lab and the service users, the integration of the Computational Biology Support has also helped in the interactions with other services, e.g. Scientific Computing. Pleasingly, the team has, over the course of the last year, grown in number and has completed a multitude of analysis projects and contributed to numerous publications. Crucially, the service has recruited a Senior Computational Biologist to fill a gap in support for tandem mass spectrometry data generated by the Institute's Biological Mass Spectrometry Facility. This post assesses and maintains existing analysis workflows, e.g. Mascot, with a mission to develop new pipelines and guide users in the analysis of all proteomics data.

Transgenic Production Facility

Natalia Moncaut, Athina Papaemmanouil, Satish Arcot-Jayaram[1], Mark Willington[2]

[1]Joined in 2019 [2]Left in 2019

The Transgenic Production Facility (TPF) is a core facility that offers a comprehensive service in the generation of genetically engineered mouse lines. TPF mission is to assist the researcher not only by providing cutting-edge transgenic services but to also advise them and if desired carry out all steps of transgenic model design and generation on collaborative basis.

The way we understand, classify and treat cancer has been transformed by next generation sequencing of cancer patient genomes. In this context, the discovery and development of CRISPR-Cas-based genome editing has provided a powerful means to test the functional relevance of human cancer-associated alterations. In this context, we use this versatile technology to achieve the targeted introduction of patient-specific mutations in genetically engineered cancer mouse models in a cost-effective and straightforward manner. This year, we have collaborated with a number of research groups using this approach and generated several mouse models with constitutive and conditional point mutations that will allow the researchers to evaluate the impact of such mutations in cancer development, progression and response to treatment. Also, using random integration and targeted transgenesis we generated reporter mouse models that enable the studies of live cell imaging, cell lineage tracing, etc.

Together with other transgenic facilities within the UK, we started the LASA Animal Science Transgenic Section. During 2019, we organised two technical workshops about surgical innovations and CRISPR technology in the generation of transgenic mouse models. These two technical meetings, and the others to come next year, provide the right environment to build an active community of transgenic technologists in the UK.

Histology

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

[1]Joined in 2019 [2]Left in 2019

By offering a full range of both routine and advanced histological services, the Histology core facility underpins oncology research across the CRUK MI. In addition to the Alderley Park facility there is also a small facility housed within the OCRB. Together both of these facilities allow for the adoption of tissue-based experimental approaches across a large number of both basic and translational research groups.

In 2019 recruitment continued with a new scientific officer appointed, continuing to expand and develop the laser capture microdissection service with downstream extraction of both RNA and DNA. Both archival FFPE and fresh frozen samples are routinely evaluated. Like previous years, focus remains on the training and continued professional development of staff ensuring the unit continues to be at the forefront with technological developments whilst also offering a comprehensive and flexible service relevant across all research themes.

In routine practice, both human and mouse tissues, in addition to organotypic assays, spheroids, agar plugs and cell pellets, continue to be evaluated together with fresh vibratome tissue sections (50–250µm) for *ex vivo* cultures of tumours to evaluate and develop three dimensional studies. Requests for special stains have seen an increase with Masson Trichrome, PAS and reticulin stains still commonly requested.

Sophisticated labelling techniques including the development of multiplex panels of antibodies, mRNA *in situ* hybridisation and the use of both mRNA in situ hybridisation and protein immunohistochemistry on single tissue sections are routinely available and automated. The use of DNA barcoded antibodies and their simultaneous amplification prior to imaging is another labelling technology the unit is currently evaluating. This allows for spatial, quantitative and tumor immune-profiling studies.

The high throughput routine immunohistochemistry service, troubleshooting and antibody validation services once again continue to see high demand, together with an increase in the numbers of researchers accessing the automated platforms individually. The unit continues to be used routinely for phenotyping of CDX models on our automated platforms ensuring consistency, reproducibility and standardisation.

Research projects involving the use of biobank material processed through the facility continues to increase. A dedicated scientific officer is responsible for ensuring the unit is compliant with current human tissue legislation. In addition to FFPE and frozen tissue samples, the number of blood, bone marrow and plasma samples from haematological malignancy patients continues to increase.

One interest of the Molecular Oncology group is uveal melanoma, cancer of the eye arising from the melanocytes of the uveal tract inside the eye. A new mouse model has been established and histologically characterised. In addition, the environment of the eye and the presence of tumour infiltration is being studied requiring whole skull processing in addition to cranial-caudal spatial information from the spinal cord. Specialised tissue processing and multiplex immunohistochemistry and the development of panels of antibodies have been instrumental in this study.

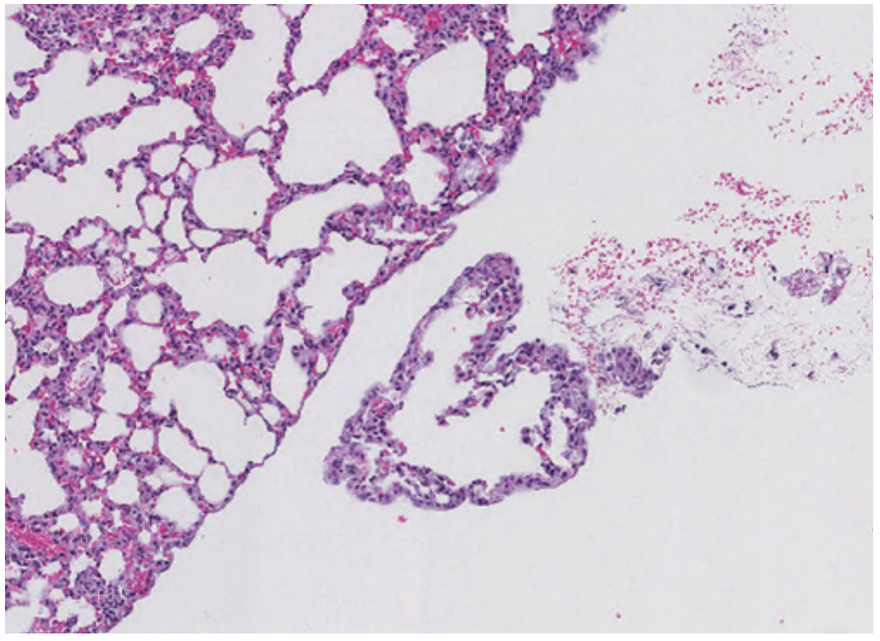
The Stem Cell Biology group have continued to apply multiplex immunohistochemistry to a variety of tissue samples, including mouse prostate organoids and urogenital systems, and human patient tissue microarrays in order to further characterise the expression of RUNX1 in the context of other markers.

Another project looking at the assessment of metastatic incidence and molecular traits of brain metastases from melanoma in xenograft models of cutaneous melanoma has used the immunohistochemistry service. Working in the core facility the PhD student was able to identify a unique melanoma cell population with relatively high ability to colonise the brain (compared to its isogenic counterpart). Additionally, laser capture microdissection allowed the extraction of genetic material specifically from metastatic tissue regions allowing gene expression analysis.

More generally the Histology core facility has been involved in determining the invasion of cells in organotypic assays in addition to

Hematoxylin and eosin (H&E) staining of mouse lung tissue showing 'heart' shape formed by lung epithelial cells.

Image supplied by Charlotte Bell (Cancer Inflammation and Immunity)



verifying cellular findings in mouse models and *ex vivo* models for several groups.

Finally, the Prostate Oncobiology group are interested in characterising a subpopulation of luminal prostate progenitors and their role in prostate tumourigenesis and treatment resistance. Multiplexing has again been used to immunophenotype and study cellular spatial distribution. In addition, *in situ* hybridisation allowing for gene expression analysis or the study of translocation events in the murine prostate tissue has also been employed.

Scientific Computing

Marek Dynowski, Kevin Doyle, Nadeem Baig[1], Stephen Kitcatt[1], Rishi Ramgolam[2], ZhiCheng Wang

[1]Joined in 2019 [2]Left in 2019

The high staff turnover, currently very common in IT, continues to be a major challenge for the Scientific Computing core facility (SciCom). Therefore, we were pleased to hire two new software architects in 2019. In January, Nadeem Baig joined the facility to work on the Octopus Pre-processing framework, and Stephen Kitcatt joined in October. Stephen is a Senior Software Architect and provides programming as well as HPC support and assists scientists in implementing bioinformatic analysis pipelines. In April, Rishi Ramgolam left the Institute after he successfully implemented the analysis pipelines for the clinical TARGET trial and migrated them from the former Troodon HPC system to Phoenix.

This year, SciCom focused on improving the infrastructure and initiating new projects to build the CRUK MI scientific computing platform for the future. At the beginning of the year we expanded the storage capacity of the central research storage by 1PB and its backup capacity by 1.5PB, increasing its total capacity to 2PB and 3PB respectively. Afterwards, we took crucial steps to develop Phoenix, CRUK MI's High Performance Computing Cluster, into a comprehensive data analysis platform. It now provides convenient tools for every aspect of the data analysis life cycle and gives users the option to subsequently analyse their data, which was processed and stored on Phoenix using an R-Web GUI (http://rstudio.scicom.cruk.manchester.ac.uk). This reduces risks and speeds up the analysis process, since data no longer has to be transferred between local computers and Phoenix. The pre-processing and downstream analysis of sequencing data has been accelerated by the integration of a new FPGA (Field-Programmable Gate Array) into Phoenix. The Illumina DRAGEN (Dynamic Read Analysis for GENomics) Bio-IT Platform was purchased in cooperation with the Molecular Biology core facility and provides accurate, ultra-rapid secondary analysis of sequencing data, making it a perfect addition to the existing platform.

In recent years, it has emerged that the steadily growing demand for virtual servers for compute, data and memory intensive workloads necessitated the acquisition of a more powerful and scalable virtualisation solution. Thus, SciCom designed and purchased a virtualisation platform using high performance computing hardware and Open Virtualisation (oVirt) VM management software. After the successful installation, a project was initiated for migrating all VMs to the new platform, including the virtual servers for the HD-SCA project between the Cancer Biomarker Centre and Peter Kuhn's lab at the University of Southern California. The opportunity was also used for consolidating the virtual machines with high availability but low performance requirements, by transferring them to the Core IT infrastructure. The new technology enables SciCom to replace some costly Windows workstations with powerful VMs that users access through remote desktop connection. Several of these Windows VMs were set up for the Biological Mass Spectrometry and the Visualisation, Irradiation & Analysis core facilities in close cooperation with the Core IT team. For example, the server component of the HALO image analysis platform, which is administered by the IT team on SciCom IT infrastructure. The cooperation between the teams was further intensified by the publication of a common service catalogue for CRUK MI.

Flow Cytometry

Jeff Barry, Antonia Banyard, Helen Carlin[1], Jack Eastham[1], Michele Fresneda Alarcon[1], Yorsa Elagili[2]

[1]Left in 2019 [2]Joined in 2019

The Flow Cytometry facility's remit is to provide state-of-the-art instrumentation for both basic science and translational cancer studies. The facility also has a wider remit to work alongside researchers to develop new approaches and to provide expertise and training.

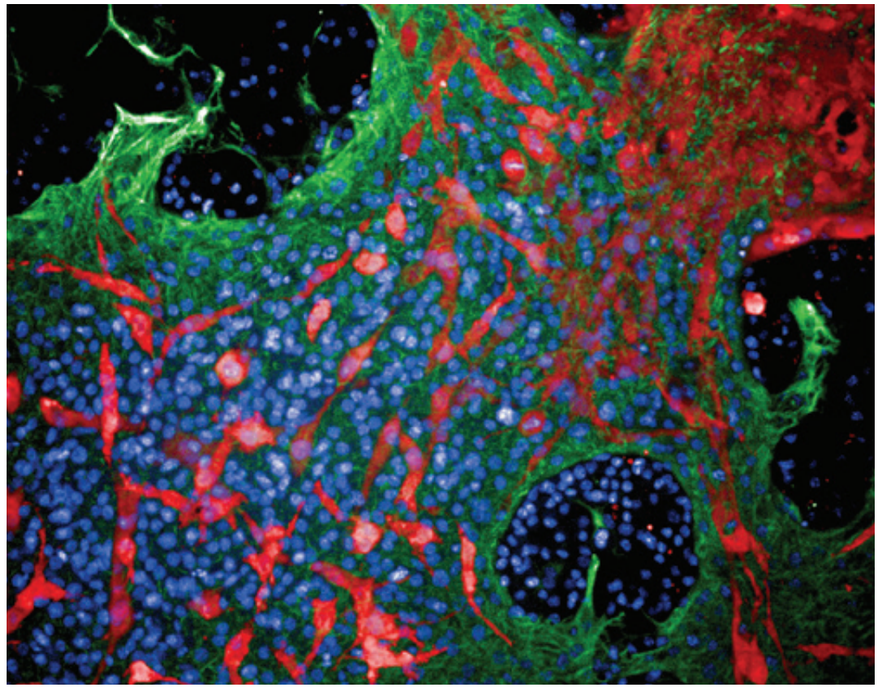
This year the facility has undergone a significant re-organisation. From April 2019 the Flow Cytometry facility separated from Advanced Imaging (now Visualisation, Irradiation & Analysis), with Jeff Barry becoming the service manager. The operation and development of mass cytometry (Helios/CyTOF) within the facility became the primary role of Antonia Banyard.

Education and staff training became a strong theme, with staff training a high priority. In addition to our usual training commitments, the facility took a strategic decision to give researchers greater access to the facility's cell sorters. Through an in-house "super user" training scheme, users were trained to operate the facility's cell sorters allowing the extension of the hours of operation.

During the past year 146 researchers and 32 groups have used the facility's resources. In addition to supporting researchers, the facility has invested significant development time in improving sample preparation and workflows, particularly those used in the newly established mass cytometry service.
The sorting service spent a significant amount of time establishing optimal sorting conditions for problematic tissues such as prostate tissue. These sample preparations contain an assortment of cells encompassing huge ranges of cell sizes that are difficult to resolve from cellular debris and cell aggregates, making obtaining pure populations challenging. We have also worked closely with the Molecular Biology Core Facility to optimise single cell sorting procedures, dramatically reducing assay costs.

Flow Cytometry is considered the gold standard for evaluation of marker expression in immunological samples and recently the addition of the Helios has considerably strengthened our ability to probe deeply into which immune cells respond in the presence of cancerous cells. This technology allows researchers to investigate how these immune cells are recruited and how they interact with each other and with cancer cells. We have developed phenotyping antibody panels for murine and human cells. This powerful technique uses over 40 markers, producing information rich datasets. We are working closely with bioinformaticians to create powerful workflows to phenotype blood cells and tissue cells for clinical trials and mouse models. This technique will enable our scientists to potentially determine patient stratification for clinical treatments and elucidate pathways in mouse models to better understand progression of many different cancer types.

In the forthcoming year, the facility's aims are to build a strong, responsive flow team and to continue to collaborate with researchers to develop and apply high content flow and mass cytometry applications. To further these objectives, the facility is looking to renew some

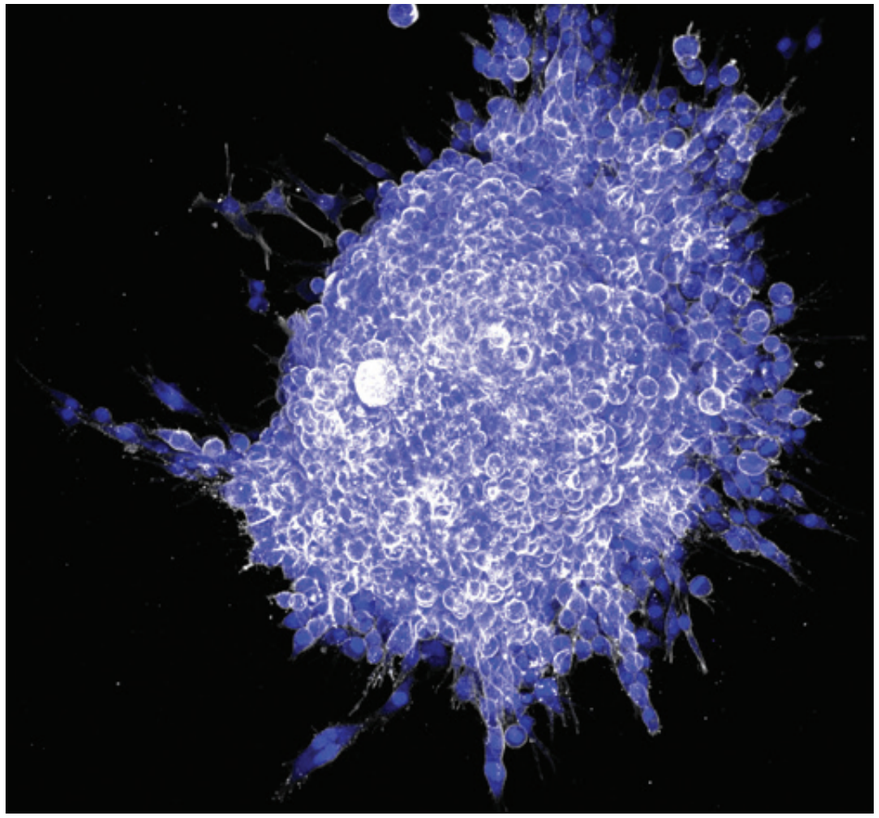


Organotypic brain slice invasion by patient-derived melanoma cells tagged. Red: melanoma cells, green: brain-resident astrocytes, blue: cell nuclei (DAPI). Illustrating melanoma-brain interactions during brain invasion by melanoma cells.

Image supplied by Denys Holovanchuk (Molecular Oncology)

Live image of melanoma cells invading into collagen

Image supplied by Andrew Porter (Cell Signalling)



of its older systems to benefit from new and novel instrumentation, ensuring we remain at the fore front of technological advancements.

Visualisation, Irradiation & Analysis

Steve Bagley, Alex Baker, Isabel Peset Martin[1], Heather Woodhouse[1], Kang Zeng

[1]Left in 2019

The facility has undergone expansion and repositioning in response to both fundamental and translational research requirements, being concerned not just with photon imaging but also a range of other modalities. A large proportion of the facility's efforts have always occupied the space between the Institute facilities, such as with Histology with whole slide imaging and using high content screening to validate flow cytometry sorting. However, in vivo support for imaging and irradiation has occupied a large proportion of this year.

The purchase of an additional histology whole slide imaging system has aided throughput and permitted new forms of imaging tissue, such as extended focus and polarisation. There are plans to introduce another system to automate mega-slide imaging, a technology that enables larger tissue sections to be accessed and evaluated that will permit a greater understanding of phenotype and heterogeneity. With the assistance of Scientific Computing and core IT, additional licenses of histological image analysis software have been introduced as the majority of histology that is digitised is processed using tissue-based and single cell phenotypic analysis.

As automated imaging and screening becomes vital to research output, an additional incubator-based microscope was purchased. Within the Facility there has been a migration from routine microscopy where a researcher is in front of a microscope to capture a few fields of view, to systems that permit the generation of large 2D and 3D data sets, statistical relevant analysis and in a format that is collaborative. As a consequence, the use of widefield environmental chamber-based microscopy is on the decline and so equipment that has become surplus has been reconditioned and, in some cases repurposed. The ability of the Facility to accommodate new equipment modalities and respond to research requirements is essential as it endeavours to continually adapt to new demands in automation and analysis.

In vivo and in vitro irradiation was enhanced in 2017 with the purchase of cabinet irradiators that permit customisation of dose and allow use of filtering to attenuate the beam. In collaboration with BRU and Targeted Therapy (within Division of Cancer Sciences), a recent development has been the introduction of aluminium filters to permit modification of the X-ray beam profile, reducing the penetrating ability of the beam and hence a measurable reduction in dose with depth. The application of beam filtering refines research efforts and improves welfare of in vivo model systems held under an X-ray. Over the coming year in collaboration with BRU new methods of location-based dosimetry will be explored, techniques for comparing irradiation systems across research sites and completion of a novel shielding system.

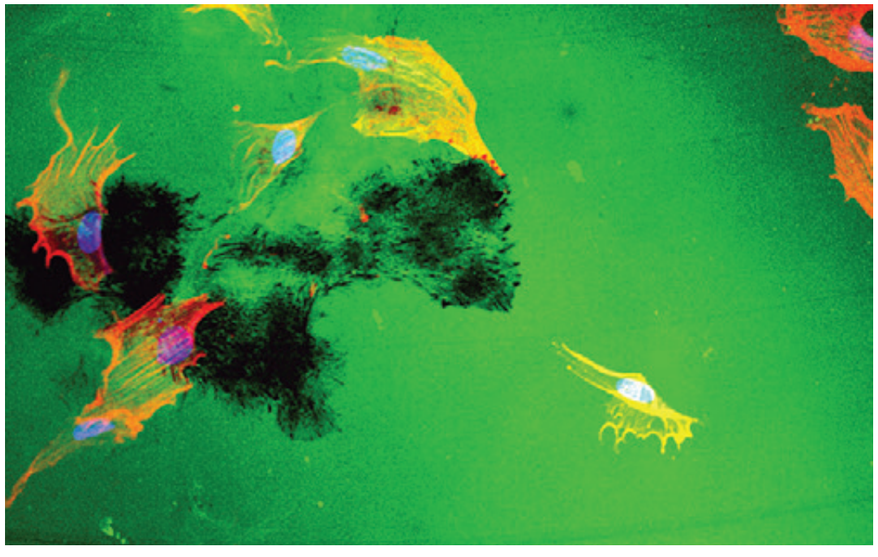
The Facility operates under the philosophy of training and enabling researchers to be able to operate the equipment and to assist in performing data processing and analysis. The extent of training this year has increased in response to demand, for levels of training from basic through to advanced. Super-resolution imaging is becoming a commonplace enabling technology and 3D high content screening has rapidly become the convention for a wide range of research questions. The Facility team have had a busy year, as demonstrated by the extent to which instruments have been utilised: over 2,000 hours for high-end microscopy; 34,000 hours for incubator-based microscopy; 1,800 hours for high content screening; over 24,000 slides processed for whole-slide histology imaging; and over 80 training sessions were held.

Design of the new laboratories within the plans for the redevelopment of the Paterson site has taken a considerable amount of effort this year, however we are coming to the end of the planning phase and look forward to the start of building work next year.

For the coming year, plans are in place to develop technology to increase the levels of multiplexing tissues from the routine five to seven labels, through a collaboration between the research groups and the Histology facility. This technology will enable complex pathways and interdependences to be mathematically modelled across a range of tissue and cancer types. In addition, there are also new avenues of investigation into corelative technologies, such as imaging histology and combining mass cytometry data, molecular biology outputs and histology mapping, developments and refinements in irradiation technologies and working with Scientific Computing for the implementation of more machine learning algorithms.

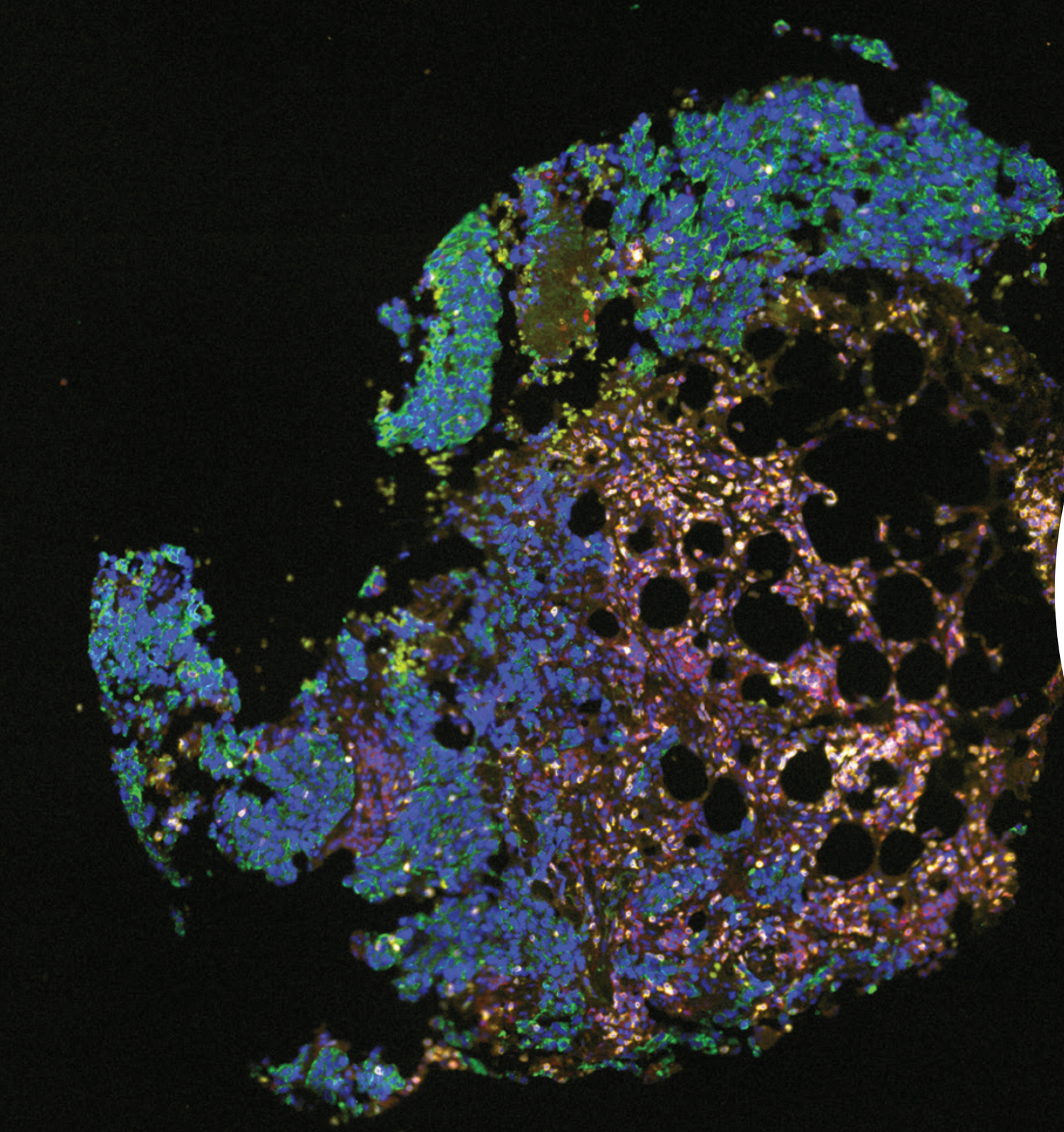
Populations of chronically UV-damaged dermal fibroblasts are plated on FITC-labelled collagen (green) coated wells and stained with Hoechst (Nuclei - blue) and Phalloidin (Actin – red) to assess their collagen degradation activity. 20x

Image supplied by Charles Earnshaw (Skin Cancer and Ageing)



CANCER RESEARCH UK MANCHESTER INSTITUTE

PUBLICATIONS AND ADMINISTRATION



Tissue microarray cores from triple-negative breast cancer needle biopsies were subjected to multiplex immunofluorescence. Nuclei are labelled in blue; cancer cells labelled in green with a pan-cytokeratin antibody. CD45, expressed by all immune cells, is in red, and CD3, expressed only by T cells, is in yellow.

Image supplied by Christian Bromley (Cancer Inflammation and Immunity)

RESEARCH PUBLICATIONS

Cancer Biomarker Centre
(page 14)
Caroline Dive

Refereed research publications

Chemi F, Rothwell DG, McGranahan N, Gulati S, Abbosh C, Pearce SP, Zhou C, Wilson GA, Jamal-Hanjani M, Birkbak N, Pierce J, Kim CS, Ferdous S, Burt DJ, Slane-Tan D, Gomes F, Moore D, Shah R, Al Bakir M, Hiley C, Veeriah S, Summers Y, Crosbie P, Ward S, Mesquita B, Dynowski M, Biswas D, Tugwood J, Blackhall F, Miller C, Hackshaw A, Brady G, Swanton C, Dive C; TRACERx Consortium. (2019)
Pulmonary venous circulating tumor cell dissemination before tumor resection and disease relapse.
Nature Medicine, 25(10):1534-1539.

Kirwan CC, Descamps T, Castle J.
Circulating tumour cells and hypercoagulability: a lethal relationship in metastatic breast cancer.
Clinical and Translational Oncology [Epub 31 August 2019]

Geary B, Walker MJ, Snow JT, Lee DCH, Pernemalm M, Maleki-Dizaji S, Azadbakht N, Apostolidou S, Barnes J, Krysiak P, Shah R, Booton R, Dive C, Crosbie PA, Whetton AD. (2019)
Identification of a biomarker panel for early detection of lung cancer patients.
Journal of Proteome Research, 18(9):3369-3382.

Mohan S, Ayub M, Rothwell DG, Gulati S, Kilerci B, Hollebecque A, Sun Leong H, Smith NK, Sahoo S, Descamps T, Zhou C, Hubner RA, McNamara MG, Lamarca A, Valle JW, Dive C, Brady G. (2019)
Analysis of circulating cell-free DNA identifies KRAS copy number gain and mutation as a novel prognostic marker in Pancreatic cancer.
Scientific Reports, 9(1):11610.

Siravegna G, Mussolin B, Venesio T, Marsoni S, Seoane J, Dive C, Papadopoulos N, Kopetz S, Corcoran RB, Siu LL, Bardelli A. (2019)
How liquid biopsies can change clinical practice in oncology.
Annals of Oncology, 30(10):1580-1590.

Tinsley N, Zhou C, Tan G, Rack S, Lorigan P, Blackhall F, Krebs M, Carter L, Thistlethwaite F, Graham D, Cook N.
Cumulative antibiotic use significantly decreases efficacy of checkpoint inhibitors in patients with advanced cancer.
Oncologist [Epub 10 July 2019]

Lindsay CR, Blackhall FH, Carmel A, Fernandez-Gutierrez F, Gazzaniga P, Groen HJM, Hiltermann TJN, Krebs MG, Loges S, López-López R, Muinelo-Romay L, Pantel K, Priest L, Riethdorf S, Rossi E, Terstappen L, Wikman H, Soria JC, Farace F, Renehan A, Dive C, Besse B, Michiels S. (2019)
EPAC-lung: pooled analysis of circulating tumour cells in advanced non-small cell lung cancer.
European Journal of Cancer, 117:60-68.

Marti FEM, Jayson GC, Manoharan P, O'Connor J, Renehan AG, Backen AC, Mistry H, Ortega F, Li K, Simpson KL, Allen J, Connell J, Underhill S, Misra V, Williams KJ, Stratford I, Jackson A, Dive C, Saunders MP. (2019)
Novel phase I trial design to evaluate the addition of cediranib or selumetinib to preoperative chemoradiotherapy for locally advanced rectal cancer: the DREAMtherapy trial.
European Journal of Cancer, 117:48-59.

Tay RY, Fernández-Gutiérrez F, Foy V, Burns K, Pierce J, Morris K, Priest L, Tugwood J, Ashcroft L, Lindsay CR, Faivre-Finn C, Dive C, Blackhall F. (2019)
Prognostic value of circulating tumour cells in limited-stage small-cell lung cancer: analysis of the concurrent once-daily versus twice-daily radiotherapy (CONVERT) randomised controlled trial.
Annals of Oncology, 30(7):1114-1120.

Zhou C, Taylor S, Tugwood J, Simpson K, Jayson GC, Symonds P, Paul J, Davidson S, Carty K, McCartney E, Rai D, Dive C, West C. (2019)
Dynamics of circulating vascular endothelial growth factor-A predict benefit from antiangiogenic cediranib in metastatic or recurrent cervical cancer patients.
British Journal of Clinical Pharmacology, 85(8):1781-1789.

Vassileva V, Stribbling SM, Barnes C, Carroll L, Braga M, Abrahams J, Heinzmann K, Haegeman C, MacFarlane M, Simpson KL, Dive C, Honeychurch J, Illidge TM, Aboagye EO. (2019)
Evaluation of apoptosis imaging biomarkers in a genetic model of cell death.
EJNMMI Research, 9(1):18.

Harle ASM, Blackhall FH, Molassiotis A, Yorke J, Dockry R, Holt KJ, Yuill D, Baker K, Smith JA. (2019)
Cough in patients with lung cancer: a longitudinal observational study of characterization and clinical associations.
Chest, 155(1):103-113.

Mohan S, Foy V, Ayub M, Leong HS, Schofield P, Sahoo S, Descamps T, Kilerci B, Smith NK, Carter M, Priest L, Zhou C, Carr TH, Miller C, Faivre-Finn C, Blackhall F, Rothwell DG, Dive C, Brady G.
Profiling of circulating free DNA using targeted and genome wide sequencing in patients with Small Cell Lung Cancer.
Journal of Thoracic Oncology [Epub 16 October 2019]

Rothwell DG, Ayub M, Cook N, Thistlethwaite F, Carter L, Dean E, Smith N, Villa S, Dransfield J, Clipson A, White D, Nessa K, Ferdous S, Howell M, Gupta A, Kilerci B, Mohan S, Frese K, Gulati S, Miller C, Jordan A, Eaton H, Hickson N, O'Brien C, Graham D, Kelly C, Aruketty S, Metcalf R, Chiramel J, Tinsley N, Vickers AJ, Kurup R, Frost H, Stevenson J, Southam S, Landers D, Wallace A, Marais R, Hughes AM, Brady G, Dive C, Krebs MG. (2019)
Utility of ctDNA to support patient selection for early phase clinical trials: the TARGET study.
Nature Medicine, 25(5):738-743.

Other publications

Conway AM, Mitchell C, Cook N. (2019)
Challenge of the unknown: how can we improve clinical outcomes in cancer of unknown primary?
Journal of Clinical Oncology, 37(23):2089-2090.

Rudin CM, Poirier JT, Byers LA, Dive C, Dowlati A, George J, Heymach JV, Johnson JE, Lehman JM, MacPherson D, Massion PP, Minna JD, Oliver TG, Quaranta V, Sage J, Thomas RK, Vakoc CR, Gazdar AF. (2019)
Molecular subtypes of small cell lung cancer: a synthesis of human and mouse model data.
Nature Review Cancer, 19(5):289-297.

Cell Division (page 22)
Iain Hagan

Refereed research publications

Forte GM, Davie E, Lie S, Franz-Wachtel M, Ovens AJ, Wang T, Oakhill JS, Maček B, Hagan IM, Petersen J. (2019)
Import of extracellular ATP in yeast and man modulates AMPK and TORC1 signalling.
Journal of Cell Science, 132(7). pii: jcs223925.

Cell Signalling (page 26)
Angeliki Malliri

Refereed research publications

Porter AP, White GRM, Mack NA, Malliri A. (2019)
The interaction between CASK and the tumour suppressor Dlg1 regulates mitotic spindle orientation in mammalian epithelia.
Journal of Cell Science, 132(14). pii: jcs230086.

Drug Discovery (page 28)
Caroline Springer

Refereed research publications

Cornwell MJ, Thomson GJ, Coates J, Belotserkovskaya R, Waddell ID, Jackson SP, Galanty Y. (2019)
Small-molecule inhibition of UBE2T/FANCL-mediated ubiquitylation in the fanconi anemia pathway.
ACS Chemical Biology, 14(10):2148-2154.

Smithen DA, Leung LMH, Challinor M, Lawrence R, Tang H, Niculescu-Duvaz D, Pearce SP, Mcleary R, Lopes F, Aljarah M, Brown M, Johnson L, Thomson G, Marais R, Springer C.
2-Aminomethylene-5-sulfonylthiazole Inhibitors of Lysyl Oxidase (LOX) and LOXL2 Show Significant Efficacy in Delaying Tumor Growth.
Journal of Medicinal Chemistry, [Epub 4 September 2019]

Leung L, Niculescu-Duvaz D, Smithen D, Lopes F, Callens C, McLeary R, Saturno G,

Davies L, Aljarah M, Brown M, Johnson L, Zambon A, Chambers T, Ménard D, Bayliss N, Knight R, Fish L, Lawrence R, Challinor M, Tang H, Marais R, Springer C. (2019)
Anti-metastatic inhibitors of lysyl oxidase (LOX): design and structure-activity relationships.
Journal of Medicinal Chemistry, 62(12):5863-5884.

Jain A, Agostini LC, McCarthy GA, Chand SN, Ramirez A, Nevler A, Cozzitorto J, Schultz CW, Lowder CY, Smith KM, Waddell ID, Raitses-Gurevich M, Stossel C, Gorman YG, Atias D, Yeo CJ, Winter JM, Olive KP, Golan T, Pishvaian MJ, Ogilvie D, James DI, Jordan AM, Brody JR. (2019)
Poly (ADP) ribose glycohydrolase can be effectively targeted in pancreatic cancer.
Cancer Research, 79(17):4491-4502.

Pillay N, Tighe A, Nelson L, Littler S, Coulson-Gilmer C, Bah N, Golder A, Bakker B, Spierings DCJ, James DI, Smith KM, Jordan AM, Morgan RD, Ogilvie DJ, Foijer F, Jackson DA, Taylor SS. (2019)
DNA replication vulnerabilities render ovarian cancer cells sensitive to poly(adp-ribose) glycohydrolase inhibitors.
Cancer Cell, 35(3):519-533.

Acton B, Small HF, Smith KM, McGonagle A, Stowell AIJ, James DI, Hamilton NM, Hamilton N, Hitchin JR, Hutton CP, Waddell ID, Ogilvie DJ, Jordan AM. (2019)
Fluoromethylcyclopropylamine derivatives as potential in vivo toxicophores - A cautionary disclosure.
Bioorganic & Medicinal Chemistry Letters, 29(4):560-562.

Head and Neck Cancer Biology (page 30)

Robert Metcalf

Refereed research publications

Rothwell DG, Ayub M, Cook N, Thistlethwaite F, Carter L, Dean E, Smith N, Villa S, Dransfield J, Clipson A, White D, Nessa K, Ferdous S, Howell M, Gupta A, Kilerci B, Mohan S, Frese K, Gulati S, Miller C, Jordan A, Eaton H, Hickson N, O'Brien C, Graham D, Kelly C, Aruketty S, Metcalf R, Chiramel J, Tinsley N, Vickers AJ, Kurup R, Frost H, Stevenson J, Southam S, Landers D, Wallace A, Marais R, Hughes AM, Brady G, Dive C, Krebs MG. (2019)
Utility of ctDNA to support patient selection for early phase clinical trials: the TARGET study.
Nature Medicine, 25(5):738-743.

Leukaemia Biology (page 32)

Tim Somervaille

Refereed research publications

Williams MS, Amaral FM, Simeoni F, Somervaille TC.
A stress-responsive enhancer induces dynamic drug resistance in acute myeloid leukemia.
Journal of Clinical Investigation [Epub 26 November 2019]

Deb G, Wingelhofer B, Amaral FMR, Maiques-Diaz A, Chadwick JA, Spencer GJ, Williams EL, Leong HS, Maes T, Somervaille TCP.
Pre-clinical activity of combined LSD1 and mTORC1 inhibition in MLL-translocated acute myeloid leukaemia.
Leukemia [Epub 28 November 2019]

Yoshimi A, Lin KT, Wiseman DH, Rahman MA, Pastore A, Wang B, Lee SC, Micol JB, Zhang XJ, de Botton S, Penard-Lacronique V, Stein EM, Cho H, Miles RE, Inoue D, Albrecht TR, Somervaille TCP, Batta K, Amaral F, Simeoni F, Wilks DP, Cargo C, Intlekofer AM, Levine RL, Dvinge H, Bradley RK, Wagner EJ, Krainer AR, Abdel-Wahab O. (2019)
Coordinated alterations in RNA splicing and epigenetic regulation drive leukaemogenesis.
Nature, 574(7777):273-277.

Paris J, Morgan M, Campos J, Spencer GJ, Shmakova A, Ivanova I, Mapperley C, Lawson H, Wotherspoon DA, Sepulveda C, Vukovic M, Allen L, Sarapuu A, Tavosanis A, Guitart AV, Villacreces A, Much C, Choe J, Azar A, van de Lagemaat LN, Vernimmen D, Nehme A, Mazurier F, Somervaille TCP, Gregory RI, O'Carroll D, Kranc KR. (2019)
Targeting the RNA m6A reader YTHDF2 selectively compromises cancer stem cells in acute myeloid leukemia.
Cell Stem Cell, 25(1):137-148.e6.

Lin CH, Wang Z, Duque-Afonso J, Wong SH, Demeter J, Loktev AV, Somervaille TCP, Jackson PK, Cleary ML. (2019)
Oligomeric self-association contributes to E2A-PBX1-mediated oncogenesis.
Scientific Reports, 9(1):4915.

Pearson S, Blance R, Somervaille TCP, Whetton AD, Pierce A. (2019)
AXL inhibition extinguishes primitive JAK2 mutated myeloproliferative neoplasm progenitor cells.
HemaSphere, 3(3):e233.

Other publications

Wingelhofer B, Somervaille TCP. (2019)
Emerging epigenetic therapeutic targets in acute myeloid leukemia.
Frontiers in Oncology, 9:850.

Gilding LN, Somervaille TCP. (2019)
The diverse consequences of FOXC1 deregulation in cancer.
Cancers (Basel), 11(2). pii: E184.

Molecular Oncology (page 34)

Richard Marais

Refereed research publications

Smithen DA, Leung LMH, Challinor M, Lawrence R, Tang H, Niculescu-Duvaz D, Pearce SP, Mcleary R, Lopes F, Aljarah M, Brown M, Johnson L, Thomson G, Marais R, Springer C.
2-Aminomethylene-5-sulfonylthiazole Inhibitors of Lysyl Oxidase (LOX) and LOXL2 Show Significant Efficacy in Delaying Tumor Growth.
Journal of Medicinal Chemistry [Epub 4 September 2019]

Leung L, Niculescu-Duvaz D, Smithen D, Lopes F, Callens C, McLeary R, Saturno G, Davies L, Aljarah M, Brown M, Johnson L, Zambon A, Chambers T, Ménard D, Bayliss N, Knight R, Fish L, Lawrence R, Challinor M, Tang H, Marais R, Springer C. (2019)
Anti-metastatic inhibitors of lysyl oxidase (LOX): design and structure-activity relationships.
Journal of Medicinal Chemistry, 62(12):5863-5884.

Rodrigues M, Koning L, Coupland SE, Jochemsen AG, Marais R, Stern MH, Valente A, Barnhill R, Cassoux N, Evans A, Galloway I, Jager MJ, Kapiteijn E, Romanowska-Dixon B, Ryll B, Roman-Roman S, Piperno-Neumann S; UM Cure 2020 Consortium. (2019)
So Close, yet so Far: Discrepancies between Uveal and Other Melanomas. A Position Paper from UM Cure 2020.
Cancers (Basel), 11(7). pii: E1032.

Tan L, Sandhu S, Lee RJ, Li J, Callahan J, Ftouni S, Dhomen N, Middlehurst P, Wallace A, Raleigh J, Hatzimihalis A, Henderson MA, Shackleton M, Haydon A, Mar V, Gyorki DE, Oudit D, Dawson MA, Hicks RJ, Lorigan P, McArthur GA, Marais R, Wong SQ, Dawson SJ. (2019)
Prediction and monitoring of relapse in stage III melanoma using circulating tumor DNA.
Annals of Oncology, 30(5):804-814.

Rothwell DG, Ayub M, Cook N, Thistlethwaite F, Carter L, Dean E, Smith N, Villa S, Dransfield J, Clipson A, White D, Nessa K, Ferdous S, Howell M, Gupta A, Kilerci B, Mohan S, Frese K, Gulati S, Miller C, Jordan A, Eaton H, Hickson N, O'Brien C, Graham D, Kelly C, Aruketty S, Metcalf R, Chiramel J, Tinsley N, Vickers AJ, Kurup R, Frost H, Stevenson J, Southam S, Landers D, Wallace A, Marais R, Hughes AM, Brady G, Dive C, Krebs MG. (2019)
Utility of ctDNA to support patient selection for early phase clinical trials: the TARGET study.
Nature Medicine, 25(5):738-743.

Skin Cancer and Ageing (page 38)

Amaya Virós

Refereed research publications

Angela Moreno, Esperanza Manrique-Silva, Amaya Virós, Celia Requena, Onofre Sanmartín, Víctor Traves, Eduardo Nagore. (2019)
Histologic features associated with an invasive component in lentigo maligna lesions.
JAMA Dermatology, 155(7): 782–788.

Stem Cell Biology (page 40)

Georges Lacaud

Refereed research publications

Miyamoto C, Kojo S, Yamashita M, Moro K, Lacaud G, Shiroguchi K, Taniuchi I, Ebihara T. (2019)
Runx/Cbfβ complexes protect group 2 innate lymphoid cells from exhausted-like hyporesponsiveness during allergic airway inflammation.
Nature Communications, 10(1):447.

Vijayabaskar MS, Goode DK, Obier N, Lichtinger M, Emmett AML, Abidin FNZ, Shar N, Hannah R, Assi SA, Lie-A-Ling M, Gottgens B, Lacaud G, Kouskoff V, Bonifer C, Westhead DR. (2019)
Identification of gene specific cis-regulatory elements during differentiation of mouse embryonic stem cells: An integrative approach using high-throughput datasets.
PLoS Computational Biology, 15(11):e1007337.

Other publications

Mevel R, Draper JE, Lie-A-Ling M, Kouskoff V, Lacaud G. (2019)
RUNX transcription factors: orchestrators of development.
Development, 146(17). pii: dev148296.

Menegatti S, de Kruijf M, Garcia-Alegria E, Lacaud G, Kouskoff V.
Transcriptional control of blood cell emergence.
FEBS Letters 593(23): 3304–3315

Translational Oncogenomics

(page 46)
Rob Bristow

Refereed research publications

Salem A, Little RA, Latif A, Featherstone AK, Babur M, Peset I, Cheung S, Watson Y, Tessyman V, Mistry H, Ashton G, Behan C, Matthews JC, Asselin MC, Bristow RG, Jackson A, Parker GJM, Faivre-Finn C, Williams KJ, O'Connor JPB. (2019)
Oxygen-enhanced MRI is feasible, repeatable, and detects radiotherapy-induced change in hypoxia in xenograft models and in patients with non-small cell lung cancer.
Clinical Cancer Research, 25(13):3818-3829.

Bhandari V, Hoey C, Liu LY, Lalonde E, Ray J, Livingstone J, Lesurf R, Shiah YJ, Vujcic T, Huang X, Espiritu SMG, Heisler LE, Yousif F, Huang V, Yamaguchi TN, Yao CQ, Sabelnykova VY, Fraser M, Chua MLK, van der Kwast T, Liu SK, Boutros PC, Bristow RG. (2019)
Molecular landmarks of tumor hypoxia across cancer types.
Nature Genetics, 51(2):308-318.

Other publications

Taylor RA, Fraser M, Rebello RJ, Boutros PC, Murphy DG, Bristow RG, Risbridger GP. (2019)
The influence of BRCA2 mutation on localized prostate cancer.
Nature Reviews Urology, 16(5):281-290.

Rebello RJ, Oing C, Gillessen S, Bristow RG. (2019)
TP53 and Prognosis in mCRPC Survival: Biology or Coincidence?
Clinical Cancer Research, 25(6):1699-1701.

Tumour Supressors (page 48)

Patricia Muller

Other publications

Mackay HL, Muller PAJ. (2019)
Biological relevance of cell-in-cell in cancers.
Biochemical Society Transactions, 47(2):725-732.

Hall C, Muller PAJ. (2019)
The diverse functions of mutant 53, its family members and isoforms in cancer.
International Journal of Molecular Sciences, 20(24), 6188.

Select additional publications

Memon D, Bi J, Miller CJ. (2019)
In silico prediction of housekeeping long intergenic non-coding RNAs reveals HKlincR1 as an essential player in lung cancer cell survival.
Scientific Reports, 9(1):7372.

Hadjidemetriou M, McAdam S, Garner G, Thackeray C, Knight D, Smith D, Al-Ahmady Z, Mazza M, Rogan J, Clamp A, Kostarelos K. (2019)
The human in vivo biomolecule corona onto PEGylated liposomes: a proof-of-concept clinical study.
Advanced Materials, 31(4):e1803335.

Matsumura Y, Ito Y, Mezawa Y, Sulidan K, Daigo Y, Hiraga T, Mogushi K, Wali N, Suzuki H, Itoh T, Miyagi Y, Yokose T, Shimizu S, Takano A, Terao Y, Saeki H, Ozawa M, Abe M, Takeda S, Okumura K, Habu S, Hino O, Takeda K, Hamada M, Orimo A. (2019)
Stromal fibroblasts induce metastatic tumor cell clusters via epithelial-mesenchymal plasticity.
Life Science Alliance, 2(4). pii: e201900425.

von Schuckmann LA, Hughes MCB, Lee R, Lorigan P, Khosrotehrani K, Smithers BM, Green AC. (2019)
Survival of patients with early invasive melanoma down-staged under the new eighth edition of the American Joint Committee on Cancer staging system.
Journal of the American Academy of Dermatology, 80(1):272-274.

THESES

There was a reduced number of PhD theses submitted in 2019 due to extensions to the studies of a number students as a consequence of the Paterson Building fire in 2017.

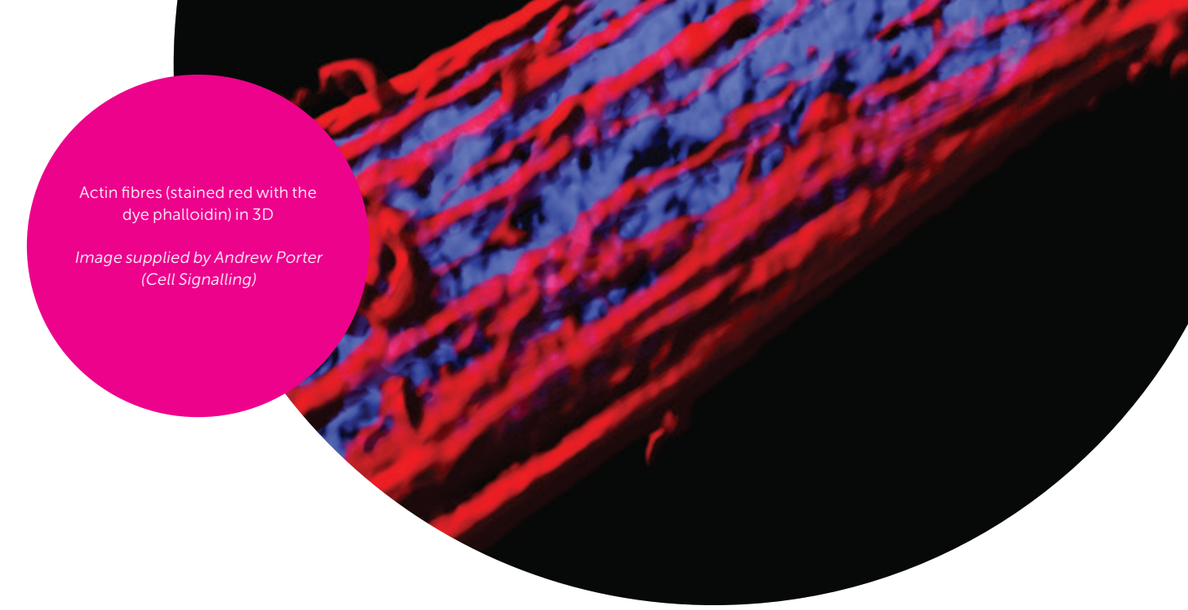
Amy McCarthy
Systems Oncology

Investigating heterogeneity in tumour-stroma interactions in pancreatic ductal adenocarcinoma

Athanasios Rafail (Sakis) Paliouras
Transcriptional Networks in Lung Cancer

Investigating the mechanisms of acquired resistance to ALK inhibitors in EML4-ALK-driven lung cancer

EXTERNAL SEMINAR SPEAKERS 2019



Actin fibres (stained red with the dye phalloidin) in 3D

Image supplied by Andrew Porter (Cell Signalling)

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

Massimiliano Mazzone
VIB Leuven Centre for Cancer Biology

Kairbaan Hodivala-Dilke
Barts Cancer Institute

René Bernards
Netherlands Cancer Institute

Pierre Close
GIGA Institute

Manuela Tosin
University of Warwick

Julian Downward
Francis Crick Institute

Miguel Ángel del Pozo
Spanish National Centre for Cardiovascular Research

Richard Bayliss
University of Leeds

Sam Butterworth
University of Manchester

David Tuveson
Cold Spring Harbour Laboratory

Pamela Kreeger
University of Wisconsin-Madison

Andrew Fry
University of Leicester

Gerard Evan
University of Cambridge

Steve Jackson
The Gurdon Institute

Shozeb Haider
UCL

Kenneth Pienta
Johns Hopkins University Hospital

Elke van Oudenhove
Perlmutter Cancer Centre

Qi Chen
University of California, Riverside

Anton Berns
Netherlands Cancer Institute

Walid Khaled
University of Cambridge

Lukas Dow
Weill Cornell Medicine

James O'Connor
University of Manchester

Madelon Maurice
UMC Utrecht

Sara Zanivan
Beatson Institute

Marleen Kok
Netherlands Cancer Institute

Maite Huarte
CIMA, University of Navarra

Breast Cancer Now Seminars

Therese Sørlie
Oslo University Hospital

Cathrin Brisken
Swiss Federal Institute of Technology Lausanne

Jeff Pollard
University of Edinburgh

Zuzana Koledová
Masaryk University

Hasan Korkaya
Augusta University

María del Mar Vivanco
CIC bioGUNE

Carla van Gils
UMC Utrecht

Karin E. de Visser
Netherlands Cancer Institute

Cristina Branco
Queen's University Belfast

Charlotte Coles
Cambridge University Hospitals

OPERATIONS

Chief Operating Officer
Caroline Wilkinson

Chief Laboratory Officer
Stuart Pepper

Chief Finance Officer
Mike Berne

Chief Human Resources Officer
Rachel Powell

The Operations team provides a suite of support services that facilitate the smooth running of the Institute. There are four senior 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 the Chief Human Resources Officer and oversees a team providing general human resources support and recruitment services. Stuart Pepper is the Chief Laboratory Officer and has oversight of general laboratory management across the Institute as well as responsibility for the IT and Health and Safety teams. Mike Berne is the Chief Finance Officer who manages the work of the Institute's finance and purchasing team.

This year we welcomed a number of new members to the team. Chris Bamber joined us as a Health and Safety Advisor; Samantha Brandolani was recruited as maternity cover for Ruth Cox; Andrew Haines started as a Recruitment Officer; and Wayne Howarth joined the Logistics team. We said goodbye to Hong Mach, Tom Bolton, Stephen Keane and Tony Dawson. We wish them well in their future endeavours and thank them for their many years of excellent service at the Institute. The Operations team has integrated well into life at our interim home of Alderley Park whilst continuing to support activity at our other sites as we work on arrangements for our eventual return to our main base in Withington. Major projects across the team for the year included examining the Institute's results from the biannual University staff survey, holding feedback sessions with staff and preparing an action plan in response. We developed and deployed a new online portal to streamline our recruitment process. The team continued to work on operational preparations for a possible no-deal scenario following the exit of the UK from the EU and carried out a rolling review and update of our general business continuity plans.

General Administration Team

Ruth Cox, Samantha Brandolani[1], Maria Belen Conti[2], Jayne Fowler, Delydd Jones

[1] Joined in 2019
[2] Joint with the Scientific Administration team

This year we welcomed Samantha Brandolani as Executive Assistant to the Institute Director as she covers the maternity leave of Ruth Cox. Samantha also manages the Institute Administration team which comprises of Belen Conti, who undertakes the role of Executive Assistant to the Senior Management Team, Jayne Fowler as Executive Assistant to the Director of the Drug Discovery Unit, and Delydd Jones who is our Administration Services Coordinator.

As a team they support the Director and the Institute Faculty in the current, temporary home of Alderley Park. They are responsible for the organisation of several annual events, including the Summer Party, Institute Christmas party, and a whole host of guest speakers to deliver lectures over the course of the year along with the Institute Colloquium.

They 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. They support a varied programme of national and international speakers. Details can be found at www.cruk.manchester.ac.uk/seminars.

Finance and Purchasing

Mike Berne, David Jenkins, Denise Owen, Muhammad Raja, Vikki Rosheski, Debbie Trunkfield

With the transition to Alderley Park finally complete, operational activity began to return to pre-fire levels which brought with it increased financial activity. The finance team began a restructure of activity and responsibilities as we look to provide a more comprehensive and efficient financial service to the group leaders.

The Institute continues to support the Director and the management of the £30m budget while providing costs and advice for new research proposals and contracts for all of our groups. We have also started to review activity within the core facilities to try and improve the financial management and systems currently implemented. We have been awarded 10 new research grants totalling nearly £4m within the year as well as a number of new commercial contracts and renewed funding against existing grants

The exit from the European Union has had an extensive impact on the finance team this year with work being carried out around avoiding disruption of service during the Brexit period. In addition to this, we have a continuing responsibility to adhere to financial regulations and procedures, including those outlined in our research grants and contracts. Given that we collaborate with a number of European entities, there is a lot of work to be undertaken to assess the impact of research funding changes stemming from the UK's exit.

Human Resources

Rachel Powell, Laura Bayliff, Rachel Craven, Andrew Haines[1], Julie Jarratt, Laura Jones, Emma Lloyd, David Stanier[2]

[1] Joined in 2019
[2] Joint with the Scientific Administration team

Over the past year, the HR Department 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, onboarding, policy guidance, employment legislation and best practice.

The department's main focus in 2019 was the development and launch of our online candidate management system JobMarker, which went live in June. The implementation of this system has streamlined the recruitment and selection processes at the Institute, therefore enhancing the candidates' overall experience.

The department has also transferred wholly over to e-personnel files to ensure that we continue to align to GDPR requirements and best practice.

The department has been visible at a number of career fairs such as Nature Careers and recruitment events held at Alderley Park. During 2019, we completed 136 recruitment rounds and successfully appointed 79 individuals to enhance the work of the Institute. This was compared to 126 recruitment rounds in 2018; an increase of 7.5% in one year. In addition, we have coordinated 148 security screenings for individuals at Alderley Park and inducted 116 individuals on the Alderley Park Site Induction.

In 2019 the department facilitated the successful promotion of 10 individuals. The Institute has continued our commitment to develop our staff and ensure that Personal Development Reviews (Contribution Reviews) are undertaken.

We have continued our commitment to joint partnership working with the union, which has resulted in the revision of several HR policies and procedures. We have also worked closely with CRUK and The University of Manchester, plus the Institute is also a member of a research-based Pay Club, which consists of 11 other research institutes. We also held a series of staff meetings along with the Senior Management Team to discuss the results from

the staff survey; this provided an opportunity for staff to understand the work undertaken by the Institute and enable further developments in employee relations at the Institute.

In the meantime, the Institute's Gender Pay Gap has decreased this year from 12.4 % to 10.5% and we will continue to monitor this closely. 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 our EU staff during the uncertain time as the UK prepares to leave the European Union.

Next year, the focus will be on the Athena Swan accreditation, review of the Personal Development Reviews and the recruitment of new research groups in line with the Institute's research strategy.

Information Technology

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

[1] Left in 2019

The CRUK Manchester Institute core IT team provides a wide range of IT support services to over 400 research and support staff, currently spread across several sites. This includes staffed service desks on our two main sites at Alderley Park and the Oglesby Cancer Research Building, where we provide 'drop-in' Service Desks providing hardware and software support and advice.

We manage over 600 desktop computers, comprising a mixture of Windows PC's and Laptops, Apple Macs and Mac Book's, plus a growing number of 'tablet' devices, mainly Apple iPad's and iPhones. All centrally authenticated, with access to a central file-store, a server farm and network printing. All desktop, portable and handheld devices are built on Windows 10, Mac-OS Mojave or OSX standard images to facilitate their on-going management.

Our core IT infrastructure comprises an enterprise-class file storage facility with a capacity in the region of 400Tb for our research data. This is based on a replicated design, hosted in two geographically separate datacentres to provide a resilient, high availability, redundant, and fit for purpose, storage facility. This is connected by a dedicated CRUK MI resilient wired and wireless network infrastructure across all CRUK MI research facilities at Alderley Park and the OCRB.

Our email service is MS-Exchange based and is currently hosted by The University of Manchester. This will transition to O365 during the coming year as part of the University's digital transformation project.Supporting multi-site operation and remote working is a challenge, however, we have deployed network monitoring to rapidly identify the source of any outages. We also make greater use of automated deployment tools to deploy new client computers. Also, our adoption of 'self-service' application installation now enables research staff to resolve a significant number of IT Service Requests themselves.

In the past year, we also developed further collaboration with our Scientific Computing and Visualisation, Irradiation and Analysis groups on various aspects of IT service provision with a view to adopting a more unified approach going forward.

Planning work is now progressing at pace to shape our return to our former 'home' in Withington based on The Christie NHS Foundation Trust site, in a new custom-built cancer research building to be shared between The University of Manchester, including CRUK MI, and The Christie.

Safety and Facilities Management

Colin Gleeson

Health and Safety

Colin Gleeson, Chris Bamber[1]

[1] Joined in 2019

We have focused on a range of issues over the past year. These have included the development of a risk control assurance programme with particular focus on the services provided by the landlord of Alderley Park. In so doing, we have secured the provision of the landlord's building fire risk assessments, including the testing of fire alarms, planned fire evacuations, the testing of cold room (+4°C and -20°C) alarms, liquid nitrogen room low-oxygen alarms, RCD testing, the testing of fixed LEV, the removal of waste by the landlord's contractors, and the maintenance of critical equipment controlling environmental parameters such as the cooling in our large -80°C freezer room (which houses many valuable research samples). We have also reviewed the risk control measures in relation to CRUK MI activities, including MSC testing, isoflurane monitoring in the Biological Resources Unit and airborne xylene levels in the Histology facility areas.

We also have been involved in the Paterson replacement building design process, attending meetings and advising and discussing our requirements with the designers. The range of issues discussed has been large and included ventilation and cooling requirements, fume cupboard specifications, drainage needs, electrical supply requirements, space requirements for services and the general layout of laboratories and offices. This will continue in the forthcoming months.

We have also undertaken more routine health and safety activities, including the implementation of a full inspection schedule of our laboratory areas, the production of remedial action plans and their close-out. We will further analyse these shortly to identify any Institute wide learning opportunities and report them to our Health and Safety Committee.

Laboratory Services

Mark Craven, Busola Atuegbe[1], Corinne Hand, Tony Dawson[2], Petra Kubinova and Christine Whitehurst

[1] Joined in 2019 [2] Left in 2019

During 2019, Lab Services continued to support the research buildings across the site with a main base at the OCRB where the glass washers and autoclaves are based. From here they supply sterile glassware, plastics and bespoke microbiological media to scientists at the OCRB and the expanded sites at the Kay Kendall laboratory, WMIC Building and Incubator Building.

The team support the scientists at AP, alongside the on-site VWR team, with sterile plastics and bespoke microbiological media.

In partnership with the Logistics team, Lab Services continue to deliver items from OCRB to other sites via the daily shuttle van service. They also continue to support the research groups in other ways:

- Maintaining and servicing two photographic dark rooms at OCRB and AP
- Providing a drop in monthly pipette clinic at OCRB and AP
- Organising the delivery of clean general and tissue culture lab coats across the AP site

Working with the Health and Safety Manager, the Lab Services Head coordinates the maintenance and testing of the microbiological safety cabinets and the lab water systems at AP.

In conjunction with the Chief Laboratory Officer, the Lab Services Head reports and tracks facility management concerns raised in laboratories and in shared spaces such as cold rooms, freezer rooms, dark rooms and the microbiology room. Over the last year, the Freezer Monitoring System that protects our stored samples was expanded to include more freezers and fridges.

In addition, the Lab Services Head continues to administer the revised lab waste removal account at AP and manages the cleaning team based at OCRB.

Logistics

Andrew Lloyd, Michael Alcock, Edward Fitzroy, Nigel Fletcher, Sedia Fofana, William Glover, Wayne Howarth[1], Stephen Keane[2], Jonathan Lloyd, Robin Sherratt, Tony Woollam

[1] Joined in 2019 [2] Left in 2019

Over the past year, the Logistics teams based at Oglesby Cacner Research Building and Alderley Park have continued to deliver successfully a high quality and reactive service to the Institute.

The service includes the receipting, checking, booking in and distribution of goods ordered by staff. In the OCRB, the team facilitate the delivery of dry ice where they also monitor the gas cylinders and replace them as necessary. The team also monitor the liquid nitrogen levels in the cell storage tanks and will replenish these when required.

Researchers can order central stores stock items via the intranet, which can be collected 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). New products have been introduced throughout the year. We have continued to make savings by buying in bulk from our suppliers.

The team based at OCRB support waste removal and are implementing new recycling initiatives. Recycling at Alderley Park continues

to develop with the CRUK MI Logistics team working together with APL waste teams.

One of the key tasks carried out by the team over the past year is the transport of samples and goods. Using the OCRB as the main base and drop off point, the team have supported the movement of samples and goods between various research locations/core facility groups. The team continue to collect samples from the Christie NHS Foundation Trust CTU on a daily basis and support the collection of mice from the Incubator Building twice weekly. The team have also worked alongside the Lab Service team in delivering sterile media and glassware and returning empty items. The team are currently supporting staff based in the Incubator Building, WMIC building, Michael Smith building, Tumour Implantation Facility and the Christie Biobank team located in the KK laboratories. Next year there are plans to support the research lab based in the Proton Beam facility.

Electronics

Yunis Al-hassan, Tony Woollam

As part of the Institute's electrical and fire safety strategies, the Electronics Team have PAT tested well over a thousand pieces of electrical equipment across multiple sites. This includes PAT testing newly acquired equipment. In addition, the Institute's electronics engineer has assembled many items of new equipment and repaired and calibrated multiple items of scientific research equipment. In a number of 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 is avoided. The Institute electronics engineer also tracks Institute equipment which is under warranty, service contract or in-house repair. Again, this provides a significant economic benefit to the Institute.

Scientific Administration

Caroline Wilkinson, Tom Bolton[1], Gillian Campbell, Maria Belen Conti[3], Julie Edwards, Steve Morgan, David Stanier[2]

[1]Left in 2019 [2]Joint with HR [3]Joint with the General Administration team

The Institute has a small Scientific Administration team that is overseen by Caroline Wilkinson, Chief Operating Officer for the Institute, who also acts as the main point of contact for both The University of Manchester and Cancer Research UK. The team have continued to work with colleagues at Alderley Park to ensure that we are integrated with processes at our interim location.

Gill Campbell is our Grants Adviser who provides support for the Institute's researchers to apply for external funding to supplement their core CRUK allocation. A total of 39 grant applications were submitted in 2019, with 10 being successfully funded, bringing in the additional sum of over £4m to the Institute. Of particular note is the CRUK Experimental Medicine Award to Richard Marais and Christie NHS Foundation Trust Consultant Medical Oncologist Paul Lorigan, which will fund their DETECTION trial to use circulating tumour DNA to guide treatment for metastatic melanoma. Another significant success was the CRUK Accelerator Award to Caroline Dive and Andrew Hughes, funding their programme "UpSMART" that aims to digitalise experimental cancer medicine centres across the UK, Italy and Spain. Georges Lacaud also received funding from the charity Bloodwise, to investigate MOZ histone acetyl transferase activity in leukaemia. The grant application process is ably supported by our Grants Committee, chaired by Iain Hagan, who review all proposals and provide critical feedback.

Tom Bolton is the Institute's Web Developer. In 2019, he worked closely with the Human Resources team to produce a bespoke online application system for staff recruitment for the Institute, which was successfully deployed resulting in streamlining the Institute's recruitment process. Tom moved on in the autumn after several successful years at the Institute and we wish him all the best in the next phase of his career.

Julie Edwards is the Postgraduate Education Manager and has continued to help 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. Julie attended a successful careers event at Alderley Park for University of Manchester students and co-ordinated successful rounds of recruitment for our next cohort of graduate students.

David Stanier has continued to oversee 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. He also co-ordinates several aspects of on-boarding new starters including data protection training and health and safety induction sessions. David is the Institute's Information Governance Co-ordinator supporting Caroline Wilkinson as the Institute's Information Governance Guardian. This year David has worked closely with colleagues at The University of Manchester's Information Governance Office to implement a new information governance software tool to track information assets. He has also conducted several audits to ensure that the Institute continues to follow best practice with respect to protecting our data.

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

Caroline Wilkinson, Establishment Licence Holder, Simon Poucher, Regulatory Liaison and Training Officer, Janet Watson, Animal Welfare & Ethical Review Body (AWERB) Chair, 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 Ethical 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. One of our Group Leader scientists has been appointed this year to the Review Panel for grant awarding at the National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3Rs).

In 2019, our AWERB met formally on five occasions with two additional meetings involving all licensees at the Institute. Two of these meetings were also attended by our Home Office Inspector. During the year, five applications to the Home Office for new Project Licences and 12 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 (e.g. ultrasound guided injection into the pancreas; injection of tumour cells into the brain of mice under anaesthesia). At our mouse breeding facility we have introduced a new database system to manage the tracking of the breeding programme. This will result in efficiencies in our breeding programme. We have actively participated in workshops, or given invited presentations at national level (involving the NC3Rs, RSPCA and LASA) on our practices and improvements that we have introduced. We are currently trialling a tunnel handling technique for mice, which is recommended to provide welfare advantages to the mice and the quality of data generated compared to traditional means of handling. The NVS provides invaluable advice on all animal husbandry and procedural technique matters, including this year improved anaesthesia practice, screening of biological agents to minimise risk of adverse reactions, after care following tumour removal and dosing to the lungs via the airways. A total of 30,413 mice were used at the Institute in regulated procedures under the Act in 2019.

Licensees are required by law to report any unforeseen adverse effects on animals or breaches of the controls and limits in their licence. Thirty-four incidents were self-reported to the Home Office in the year. From these incidents, the majority were mice found dead overnight unexpectedly after being assessed as being well the previous day (thirty in total). Eight mice failed to recover from short term general anaesthesia. All incidents were satisfactorily resolved with the inspector with suitable adjustments made where relevant. There were also incidents whereby twenty mice on a breeding programme were not culled within a specified time frame. There were no welfare issues for these mice however. The AWERB reviewed these reports and the learning shared across licensees.

The CRUK MI AWERB has continued to interact with other establishments through the North West 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 and also helps train new Establishment Licence Holders. The AWERBs of CRUK MI, AstraZeneca and Agenda Life Sciences, all based on the Alderley Park site, continue to hold joint meetings to share ideas, presentations and training opportunities – in October, the three organisations held a second 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 Research Spotlight event, as well as Institute Open Days; a workshop run by the Understanding Animal Research organisation helped our staff and students to communicate how we work with mice in our research, and the care with which we do this, to the general public at these events.

Cancer Research UK Commercial Partnerships

Martyn Bottomley

Cancer Research UK Commercial Partnerships (CP) Team 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, medical technologies 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.

Following on from a reorganisation in April 2018, the CP Team continues to work in 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 R&I with whom we collaborate to achieve our joint goals of progressing CRUK science. This is enabling us to build deeper and more strategic relationships with our funded centres, Institutes and Universities, as well as improving internal information flow and collaboration.

CRUK is aware that the ability to translate new discoveries in to patient benefit has not progressed at the same pace as discovery research. This disconnect is linked to several factors related to academic culture, entrepreneurial mindset and the skills required to move discoveries forward. The CRUK-PACE team was set up to understand how CRUK could *Promote an Academic Culture of Entrepreneurship* within our research community. The team has produced an entrepreneurial programme to promote an academic culture where entrepreneurship is incentivised, enabled and rewarded. As part of this initiative we organised a very successful CRUK Innovation Summit on the Alderley Park site in October 2019 to facilitate this within the CRUK Manchester Institute. To further build on this, an Innovation and Translation Officer is being recruited to focus specifically on building a culture of innovation and translation at the CRUK Manchester Institute. This post will work closely with the CP Team and the CRUK MI.

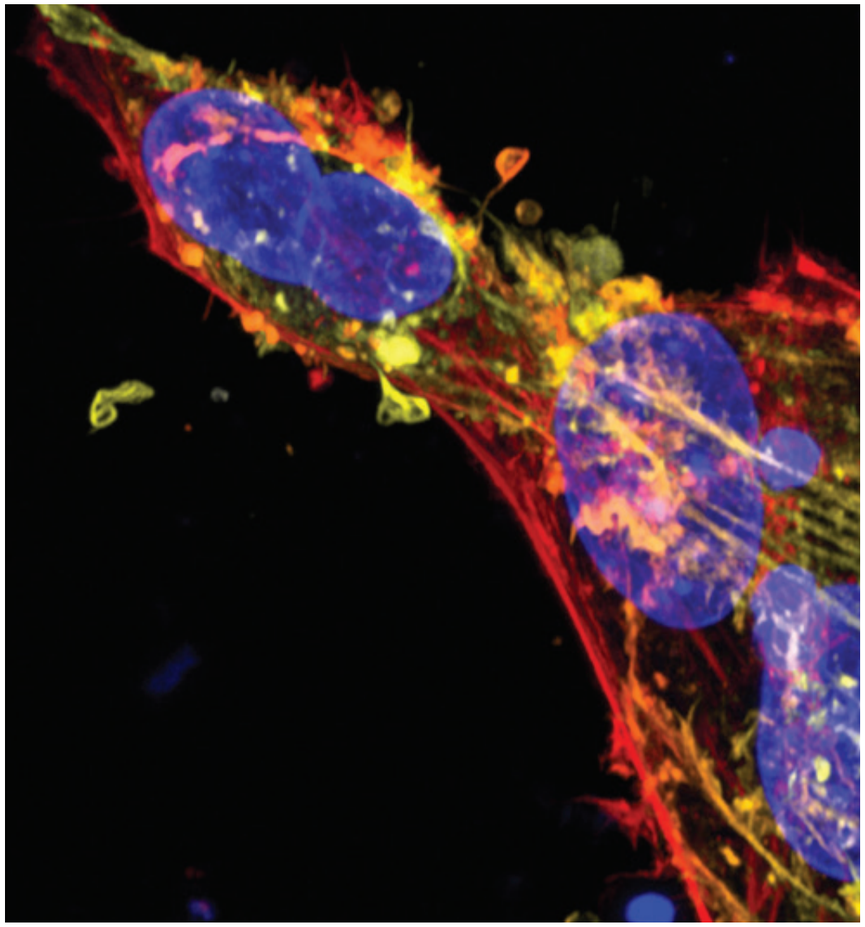
By arrangement with The University of Manchester, CRUK owns and is responsible for the development and commercialisation of intellectual property arising from CRUK funded research at The University of Manchester. To effectively facilitate this, Martyn Bottomley, a CRUK CP Translation Lead is based within Manchester and is currently hot desking at the OCRB, 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 AZ and also the filing and management of a number of patent applications for the Drug Discovery Unit to protect novel compounds resulting from their research.

CP 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.

Lung cancer cells in 3D

Image supplied by Andrew Porter (Cell Signalling)



POSTGRADUATE EDUCATION

Postgraduate Education Manager
Julie Edwards

Postgraduate Tutor
Angeliki Malliri

Postgraduate Director and Chair of the Education Committee
Tim Somervaille

The Cancer Research UK Manchester Institute (CRUK MI) offers a 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 2019, we welcomed nine graduate students and one clinical research fellow to our PhD programme, working in a variety of fields including leukaemia, pancreatic cancer, drug discovery, lung cancer, prostate cancer, signal transdution and cancer biomarkers. It was also particularly gratifying to see that, over the past twelve months, three of our PhD students and Clinical Research Fellows had published first author original papers in the British Journal of Pharmacology, Scientific Reports, and The Journal of Clinical Investigation. First author review articles were also published in FEBS Letters and Development.

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 leading scientists in cancer research, which our students are encouraged to attend. The speakers are internationally renowned scientists and we consider it essential that our students are exposed to outstanding research from leaders in different disciplines, which will give them a broad understanding of many aspects of cancer research and basic biology. In addition, we hold a series of weekly postdoctoral research seminars and attendance from PhD students is also an integral part of their learning. 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 (Science TakeAway) is a Committee run by junior scientists and students in the Institute with the aim of providing a forum for discussion and training related to research, communication of scientific engagement and development of social and networking opportunities. STAy are keen to encourage networking, career progression and personal growth of early-career researchers, and with this in mind, in January 2019 the STAy Committee were given the opportunity to host a number of external seminars. External speakers were invited to not only give a scientific talk on their research, but also a short talk about their own career pathway and hold small group discussions with audience members.

In 2019, STAy and healthcare professionals from the Christie NHS Foundation Trust jointly organised a World Cancer Day Event, bringing together people with a wide range of cancer experience, researchers, patients and health care teams. The evening presented a number of short talks, discussion activities with the opportunity to share perspectives on life, cancer and research. Other activities over the last year have included pub quizzes, bowling, speed networking, science media training, a grant writing seminar and a mini-research spotlight conference.

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 2019, the prize went to PhD student Fabrizio Simeoni from the Leukaemia Biology group for his PhD work describing how FOXC1 blocks terminal differentiation in acute myeloid leukaemia cells.

There was further success for Fabrizio who helped to organise the first BACR PhD cancer conference which took place in December 2018. He was one of six who formed the BACR PhD Student Conference Organising Committee compiling an exciting programme of talks by leading cancer scientists, attracting over 100 participants from across the UK. This was a fantastic opportunity for Fabrizio who contributed to the grant application resulting in a £5000 award from CRUK to help fund this event.

Cancer Research UK contributes towards an annual International PhD Student Cancer Conference (IPSCC) allowing high calibre students (typically in 2nd - 4th years) from top cancer research institutes across Europe to organise and present at their own scientific conference. The conference is organised by students for students from core participating institutes; 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).

The 13th IPSCC was organised by students at the National Cancer Institute, Amsterdam from 12-14 June 2019. The CRUK MI was represented by 23 students, the highest number of attendees from a single institute. The conference was attended by over 100 students from the participating institutes with 16 panel selected student talks and 86 posters over two and a half days. The forum provided a unique opportunity for PhD students to present their work via a poster or oral presentation, network with some of Europe's best cancer research students, and engage in organised career

sessions providing advice on future career options. Students enjoyed keynote lectures from Dr Andrea van Elsas, Aduro Biotech; Prof Karin de Visser, NKI; Dr Nicholas Navin, MD Anderson Cancer Center; and Prof Ben Feringa, Rijks Universiteit Broningen.

We were delighted that Maximilian Schenk from CRUK Manchester Institute Cancer Biomarker Centre was awarded the overall prize for best oral presentation showcasing his work on chemoresistance in small cell lung cancer. Christian Bromley based in the Cancer Inflammation and Immunity group, was awarded the prize for one of the top three poster presentations "Combining cancer promoting and cancer-inhibitory inflammation signatures to predict cancer patient outcome".

The social events during the conference included a pub quiz, networking social event and a walking dinner with a guided tour around Amsterdam, which were all well received. The event was a great success - especially for the CRUK MI Manchester Institute.

PhD studentships

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

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.

Education Committee 2019

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 to not only monitor progress, but also help to develop performance and presentations skills.

Education Committee Members

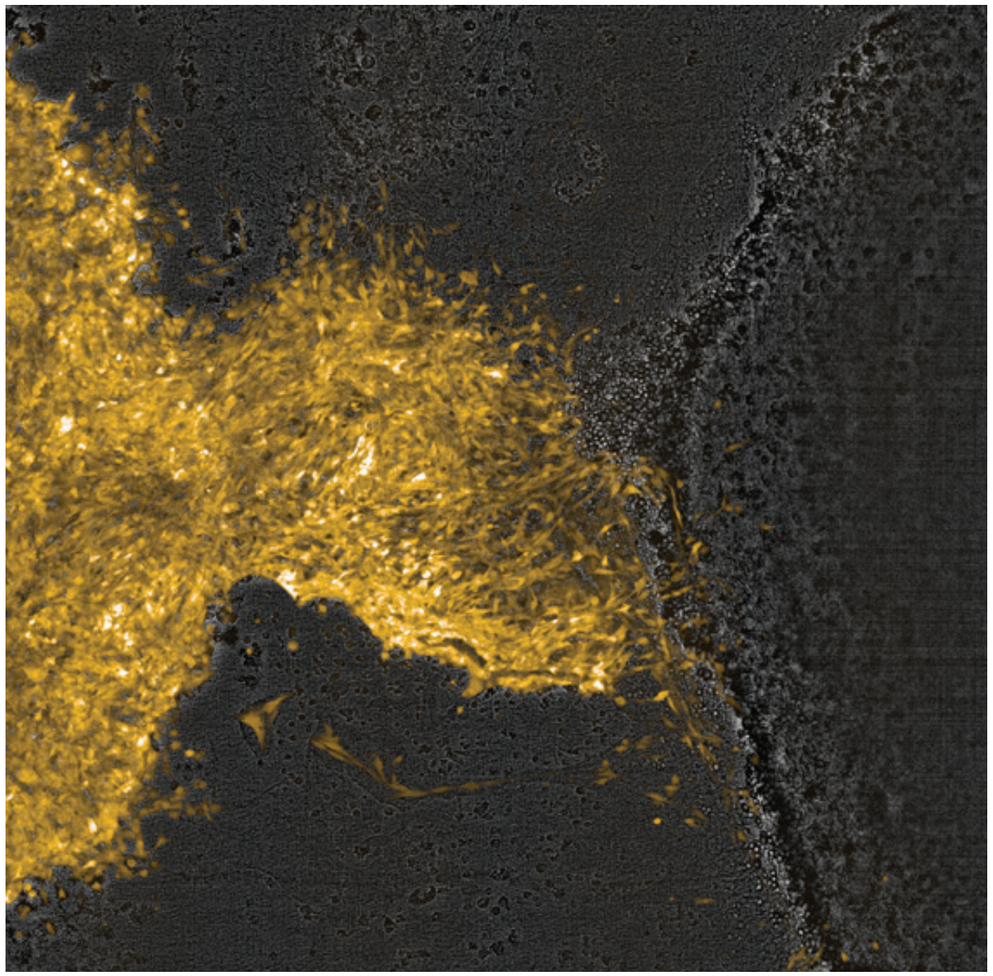
Tim Somervaille
Postgraduate Director and Chair
Angeliki Malliri
Postgraduate Tutor
Richard Marais
Ex-Officio Member
Wolfgang Breitwieser
Julie Edwards
Postgraduate Manager

Claus Jørgensen
Elaine Kilgour[1]
Georges Lacaud
Jonathan Tugwood[2]
Amaya Viros[1]
Caroline Wilkinson

Student Representatives

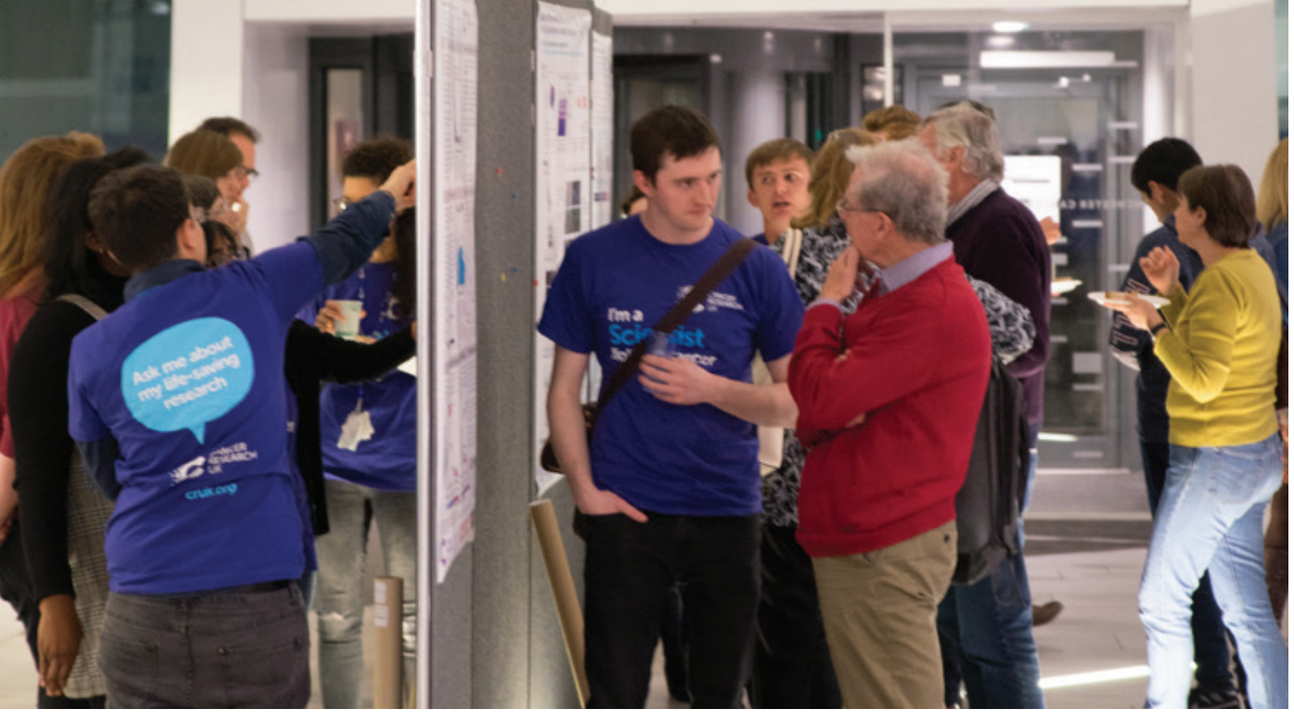
Jakub Chudziak[2]
Callum Hall
Ryan Guilbert[1]

[1] Joined in 2019
[2] Left in 2019



Organotypic brain slice (grey scale) invasion by patient-derived melanoma cells tagged with mCherry (orange). Illustrating melanoma cell capacity to invade into the brain tissue ex vivo.

Image supplied by Denys Holovanchuk (Molecular Oncology)



Research Spotlight: A public event organised in collaboration with the STAy Committee

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 drives local interaction with life-saving research through compelling research content.

Almost 2000 people interacted with the work of the Manchester Institute during 2019, through the work of the CRUK Research Engagement Manager based in Manchester.

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

A highlight came in October when, with support from CRUK, members of the MI STAy Committee staged Research Spotlight: Manchester's Early Career Researchers, a public event giving PhD students and Scientific Officers a chance to present their work to a lay audience of 60 members of the public via presentations and posters. Scientists from across the MI, together with other colleagues from The University of Manchester, spoke to the public about their research. The evening culminated in a keynote presentation by Institute Fellow, Patricia Muller.

Our scientists also took their work out to the community at 18 external events.

Relay for Life Stockport took place in the height of the summer, featuring the team of researchers from the Manchester Institute and University of Manchester, captained by Steve Lyons. As well as joining in the festivities on the day, MI scientists and staff shared stories about their research and hosted the CRUK Engagement Team's Escape the Lab activity.

Following the event, our scientists also attended CRUK's national Relay for Life Summit, inspiring supporters from across the UK through the research taking place at the Institute and thanking them for their support.

Senior Group Leader Rob Bristow celebrated with the Nelson & Barrowford Fundraising Committee in May, where he thanked them for 60 years of determined fundraising and on reaching a £1million milestone. Rob shared with supporters and guests the impact their fundraising efforts make to patients' lives and the latest progress happening in Manchester.

Following Rob's address, a member of the committee (who are all over 80 years old) commented that whilst they often think of stepping down, they now felt inspired to keep on fundraising.

Senior Group Leader Iain Hagan celebrated with volunteers from across the North West at a regional Flame of Hope event, during which Iain thanked the supporters for their contributions whilst illustrating how their efforts are translated in to critical research.

With the launch of the Re-Write Cancer campaign to raise £20million towards the rebuild of the Paterson Building, alongside The University of Manchester and The Christie Charitable Fund, the Institute was delighted to welcome fundraising colleagues from CRUK to Alderley Park for an exclusive tour of the labs to see our research in action. Scientists from the Cancer Biomarker Centre, the Drug Discovery Unit and the Imaging, Irradiation and Analysis team welcomed CRUK staff in to the labs, giving a crucial insight in to the work taking place which may help to inspire prospective donors of the campaign.

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.

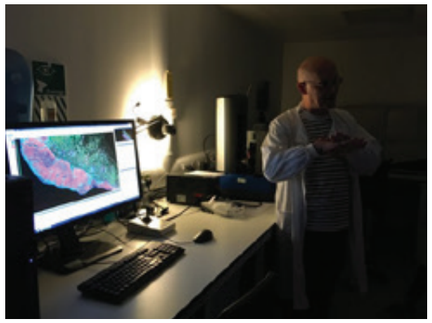
Images clockwise from top left:

Staff Engagement Day: Nic Jones, Caroline Dive and Rob Bristow chat to the Re-Write Cancer fundraising team about why the new building is critical to research progress

Staff Engagement 2: Steve Bagley explains the work of the Imaging, Irradiation and Analysis team to CRUK fundraising staff

60 Year Celebration: Rob Bristow celebrates 60 years of fundraising with the Nelson & Barrowford Fundraising Committee

Research Spotlight: A public event organised in collaboration with the STAy Committee



Relay for Life Stockport:
Some of the scientists and families who took part in the fundraising event

ACKNOWLEDGEMENT FOR FUNDING FOR THE CANCER RESEARCH UK MANCHESTER INSTITUTE

The total funding of the CRUK Manchester Institute for 2019 was £29.6m. The major source of this funding was awarded by Cancer Research UK (CRUK) via a core grant of £13.6m plus additional strategic funding of £6.3m. 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.

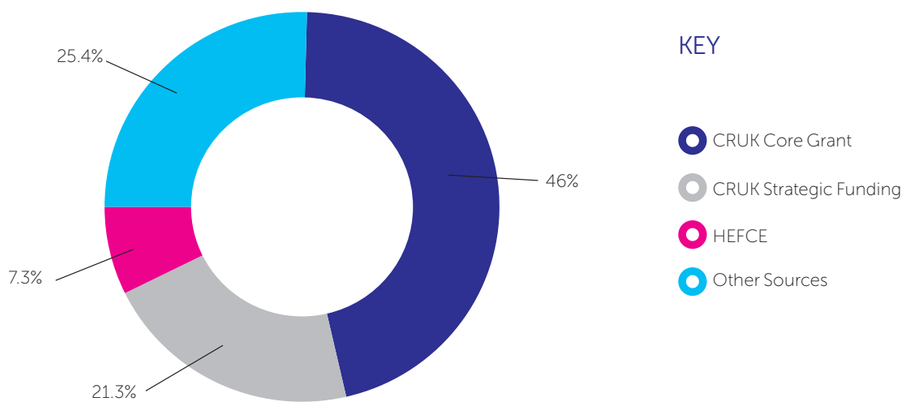
The balance of the Institute's funding is received from a number of additional sources. The research carried out through these additional projects enhances and supports the research undertaken by the core funding.

These sources are as follows:

- Amgen
- Angle Inc
- Astex Pharmaceuticals
- Astra Zeneca
- 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 Kendal 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 sponsors.

CRUK MANCHESTER INSTITUTE FUNDING 2019



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.

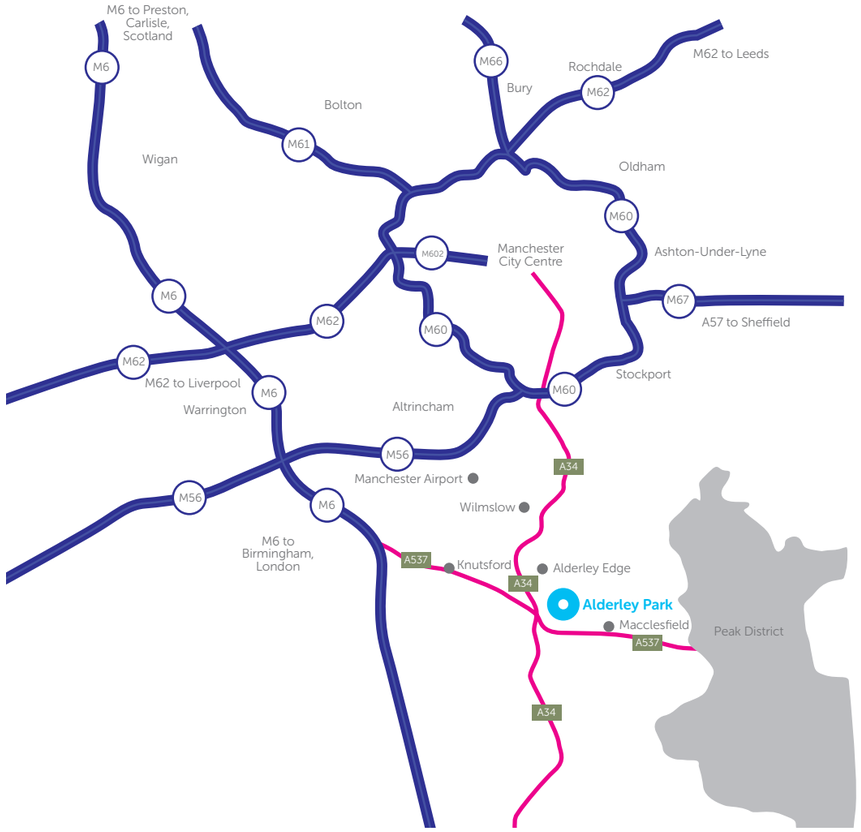
Opportunities exist at a number of levels in the Institute. We have a well-established programme of degrees by research which is described in the section on Postgraduate Education. We encourage applications from suitably qualified graduates to apply to join either the PhD or MD programmes. Graduates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



Cancer Research UK Manchester Institute
Director: Professor Richard Marais

Address
Cancer Research UK Manchester Institute
The University of Manchester
Alderley Park
SK10 4TG
United Kingdom

e-mail: enquiries@cruk.manchester.ac.uk
website: www.cruk.manchester.ac.uk

Tel +44(0) 161 306 0871

Cancer Research UK
Cancer Research UK is a registered charity in England and Wales (1089464), Scotland (SC041666) and the Isle of Man (1103). Registered address: Angel Building, 407 St John Street, London, EC1V 4AD.

Tel 44(0) 20 1234 5678
www.cruk.org

Electronic version of this report can be found at:
www.cruk.manchester.ac.uk/About/

ISSN 1479-0378

Edited by: Caroline Wilkinson
Gillian Campbell

CANCER RESEARCH UK
MANCHESTER INSTITUTE

The University of Manchester
Alderley Park
SK10 4TG
United Kingdom
Telephone +44(0) 161 446 3156

www.cruk.manchester.ac.uk