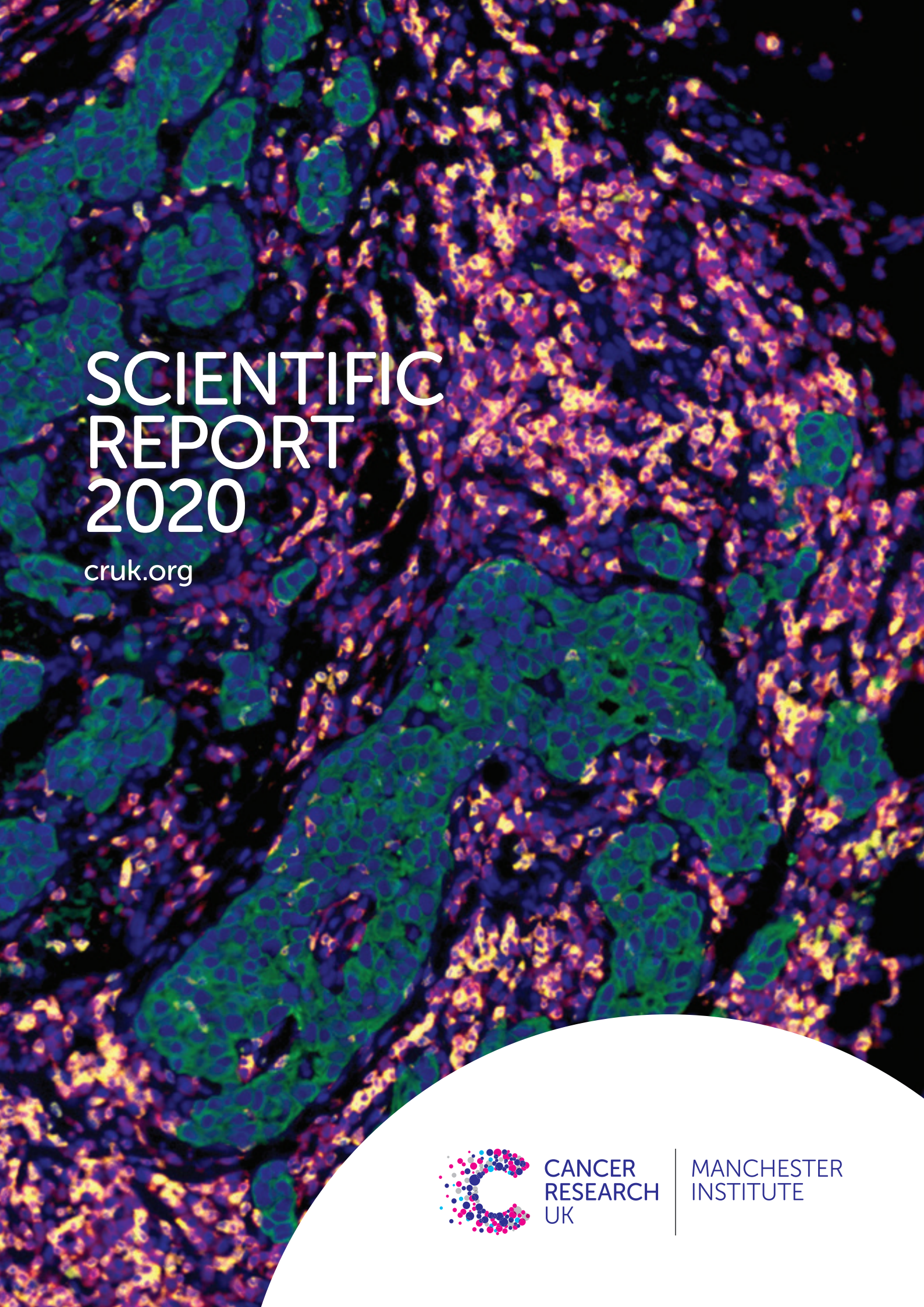

SCIENTIFIC REPORT 2020

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COVER IMAGE

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 Christopher Bromley (Cancer Inflammation and Immunity)

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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

In 2020 we experienced unprecedented challenges caused by the global COVID-19 pandemic. Our priority is the wellbeing and safety of our staff, so early in March we convened tri-weekly meetings of the Institute's Emergency Response and Business Continuity Committee (ERBCC) to manage our response to the emerging crisis. In common with other research institutes, we closed our laboratories in late-March to protect our staff and prevent the virus from spreading through the Institute and through our community.

Our laboratories were fully closed for 11 weeks, and we started reopening in early June, albeit with limited access and social distancing rules in place. During the closure, a core team remained on site to protect our research-critical activities, ensure that we maintained rigorous animal welfare standards, saved our vital experimental materials, and maintained our critical infrastructure. Let me therefore start by thanking the ERBCC for its exceptional guidance and help throughout the pandemic, and a huge thank you to our staff who remained on site during this very difficult time to protect our core functions. You showed exceptional team spirit, commitment and dedication, together ensuring that our response to the crisis was effective and proportional.

At the beginning of the lockdown a COVID-19 testing hub, the Lighthouse Laboratory, was established at Alderley Park. We entered a working partnership with the Lighthouse to get this essential national facility up and running. Several of our senior leaders joined the Lighthouse management team to help establish the laboratories and its important workflows, and we loaned the new facility equipment, including the vital PCR machines. Over 30 of our highly skilled staff and students volunteered to work in the Lighthouse Laboratories and were amongst the first to be trained to start testing samples from front-line NHS workers, allowing those with negative tests to quickly return to patient care. Our staff members also found other innovative ways to support front line NHS workers, using our specialist 3D printer to create over 200 plastic PPE headbands for hospitals across the region and donating our PPE to them. I am very grateful to all of our staff who helped to support the national effort, and I am proud of the magnificent response CRUK MI made. Although we had to pause our own research, our skills were used very effectively to fight the virus.

Most of our remaining staff worked from home, so it was important that we kept connected and supported each other. We held weekly virtual updates to keep staff informed on progress, the ever-changing situation, to share news and to take questions. We formed an Education and Engagement group to explore methods for motivating home-working staff, and this resulted in several very successful new online training sessions. Our STAy Committee once again stepped up to the plate with new ideas for boosting morale such as their Quarantine Quizzes. We held a very successful virtual Christmas Party (I believe my "how to make a no-cheese cheesecake" video is still available). We took our internal and external seminar series online to create opportunities for continued discussion of our research, and to learn from each other and colleagues from around the world. I am grateful to all those who embraced these very successful seminar series, particularly our invited speakers who committed so much of their time to the (initially) rather alien format of the virtual talk. International meetings also went online or, at best, adopted a hybrid format, so although attendance at these meetings became easier as it meant no travel and lower costs, we did have to learn how to get the most from the new format. I am delighted that many of our staff attended national and international meetings during the pandemic and that despite the challenges, they managed to present their work and get the all-important feedback on their research from the international community. I was also delighted the CRUK MI was invited to showcase our research at the prestigious Royal Society Summer Science Exhibition, so congratulations to the team that put in the hard work to win this accolade. Although the exhibition was postponed, our Tumour Microenvironment exhibit will be presented at next year's online Royal Society Summer Science Exhibition.

Despite the challenges, I am pleased that we continued to make excellent progress in our research. Our Drug Discovery Unit continues to develop exciting inhibitors to explore the therapeutic potential and biology of lysyl oxidase in cancer, published in the *Journal of Medicinal Chemistry, Cancer Research and Organic Process Research*. They have developed exciting new cancer targets with CRUK MI colleagues Iain Hagan (cancer cell cycle target) and Claus Jorgensen (tumour stroma target), and new interactions with Caroline Dive on biomarkers and with Stephen Taylor on PARG. They also published very exciting preclinical and clinical data in *Annals of Oncology*, showing that the orally available well-tolerated RAF+SRC inhibitor 3833 is effective in RAS-driven cancers. The Cancer Biomarker Centre published papers in *Nature Cancer* and the *Journal of Thoracic Oncology* that describe inter and intra-tumour heterogeneity in small cell lung cancer (SCLC), highlighting the potential of their CDX approach, now used worldwide, to advance SCLC research. Their T7 ctDNA pipeline now includes ctDNA methylation profiling and is being used to subtype SCLC patients, explore Cancers of Unknown Origin, and improve early detection of non-small cell lung cancer. They also launched their CRUK UpSMART programme to bring digital solutions to early clinical trials. On the biomarker theme, my own Molecular Oncology group reported signatures that predict which patients will respond to immune checkpoint blockade immunotherapy and which could therefore be used to refine patient care (published in *Nature Communications*). In *Nature Cancer*, we published that T cell evolution in response to immunotherapy can be monitored using human blood samples, providing a tractable and convenient approach to determine which patients are likely to respond to these treatments. In this vein, the Cancer Inflammation and Immunity group published in the journal *Immunity* that antagonistic inflammatory profiles can anticipate immune-dependent progressive or regressive tumour growth.

Embracing human cell studies, the Cell Division group developed an approach to synchronise human cell populations by exploiting the CDK4/6i drugs approved for breast cancer. Their approach, published in *Open Biology*, arrests cells at the restriction point but, importantly, without affecting DNA integrity. This means that it is now possible to study transcription, DNA replication, DNA repair and chromatin biology in synchronised cells, features that cannot be studied in double thymidine blocked cells (the traditional approach) because they are obscured by the accumulation of DNA damage. This important contribution will likely be widely adopted, and they are using the approach to refine our understanding of the G2/M control. Along these lines, the Cell Signalling group published an exciting study in the *Journal of Cell Science*, which revealed that the RAC guanine nucleotide exchange factor (GEF) TIAM1 also regulates centriole duplication. Centrosomes are composed to two centrioles, and they coordinate DNA segregation during cell division. The group found that TIAM1 associates with centrosomes, but when TIAM1 is depleted, PLK4 levels at the centrosome increase, causing centriole overduplication and chromosome lagging in anaphase, which is known to drive malignant progression. Curiously, PLK4 regulation by TIAM1 is independent of its GEF activity but does require binding to the F-box protein βTRCP, suggesting that TIAM1 promotes PLK4 degradation through βTRCP and independently of RAC1 activation.

Continuing their studies on high-risk prostate cancer, the Translational Oncogenomics group published several authoritative reviews that highlight the challenges faced by prostate patients and their treating clinicians (*Nature Disease Primers, Nature, Nature Comms*). Also moving into the prostate, the Stem Cell Biology group found that RUNX1 is highly expressed in a subpopulation of prostate proximal luminal cells that are castration-resistant, self-sustained and do not generate other luminal cells. Their work, published in *eLife*, identifies a cell type from the onset of prostate development and provides new insight into prostate biology.

The Leukaemia Biology group continued to co-develop the LSD1 inhibitor ORY-1001 (now called iadademstat) reporting in the *Journal of Clinical Oncology* that the compound has a good safety profile. The *Tumour Suppressors Group* identified an intriguing role for copper in

regulation of p53 (published in *Cell Death and Disease*) and developed powerful tools for their research (published in *Cancer Cell International*). Finally, the Skin Cancer & Ageing group reported an intriguing sex bias in skin cancer, with men presenting more aggressive disease (published in *Clinical Cancer Research*). Details of these discoveries and many more are in the specific reports from individual groups, so please read on.

Congratulations to all our staff who won prizes and awards last year. The 2020 Dexter award was awarded to Wendy Trotter (Cell Division). Bettina Wingelhofer (Leukaemia Biology) won a John Goldman Fellowship from Leukaemia UK, and Sara Valpione (Molecular Oncology) won a Career Development Award from the Harry J Lloyd Charitable Trust and was selected to participate in the ESMO Leaders Generation Programme 2020. Santiago Zelenay (Group Leader, Cancer Inflammation and Immunity) and I were awarded the inaugural BIAL Prize in Biomedicine together with other colleagues. Alex de Feu (Prostate Oncobiology) won the BioRad International Science writing competition, Denys Holovanchuk (Molecular Oncology) won an AACR Annual Meeting Travel Award, and Hannah Reed (Cell Signalling) won the prize for the best talk at the virtual Actin Meeting. Finally, Caroline Dive (Cancer Biomarker Centre) won the Johann Anton Merck Award for Outstanding Preclinical Research in Oncology, was elected an EMBO Member and became President of the European Association of Cancer Research.

Despite working from home, our staff and students continued to fundraise for CRUK. I joined a Drug Discovery Unit *Race for Life at Home Challenge* that raised over £3,000, and Steve Lyons and the 'Manchester Scientists' raised over £2,200 for the *Stockport Relay for Life*. These are impressive achievements, so thank you to everyone who took part and everyone who sponsored us.

We have faced adversity before and are still recovering from the 2017 Paterson Building fire, but challenge forces us to discover new and sometimes better ways of pursuing our goals and working together. The pandemic has reminded me that the Institute is its people and that you are strong, resilient, and productive. You continue to thrive, and I am delighted to see the dedication of our staff, now back in the laboratories. Thank you for toughing it out, for sticking together and for pursuing your projects with renewed vigour. It is testament to our core values and the strengths of the culture and community that is CRUK MI.

Finally, this is my last Annual Report, because after 9 years I decided to step down as Director to explore other opportunities. It is an exciting time for the Institute. The Paterson Building has been demolished and, at extraordinary speed, its replacement is emerging from the hole it left. Our move into this new facility is scheduled for early 2023 and will provide opportunities to build the Institute in new directions. I wish Caroline Dive and Iain Hagan all the best as they prepare for this important milestone in our history. As I step away, let me reflect that while I am sad to move on, I am proud of the Institute I have handed over. So let me end as I started, by thanking people. First, my thanks to CRUK and The University of Manchester for allowing me the privilege of leading CRUK MI; it was hard work, but it was also a lot of fun. My thanks to Caroline Dive for your friendship, hard work and guidance as Deputy Director; you contributed so much to the growth of the Institute over the last decade. Thank you to Caroline Wilkinson and Stuart Pepper for your friendship, unflinching dedication, and hard work; you made my job easier, and you make the Institute a fantastic place at which to do research. Thank you to Margaret Lowe and Mike Berne for managing our massively complex budgets and to Rachel Powell for your assistance and guidance throughout. Thank you to the Group Leaders and Core Facility Managers for guiding your units so productively. Thank you to my own group for your productivity and the impressive body of work we generated over the last decade. Thank you to Ruth Cox, my Executive Assistant for your unwavering support, for steadying the ship when needed and, with help from your assistants, for making sure I was always prepared and in the right place at the right time; without you it would have been chaos. Finally, thank you to everyone who worked at the Institute over the last decade; your hard work and scientific contributions have taken us closer to our aim of *beating cancer sooner.*

RESEARCH HIGHLIGHTS

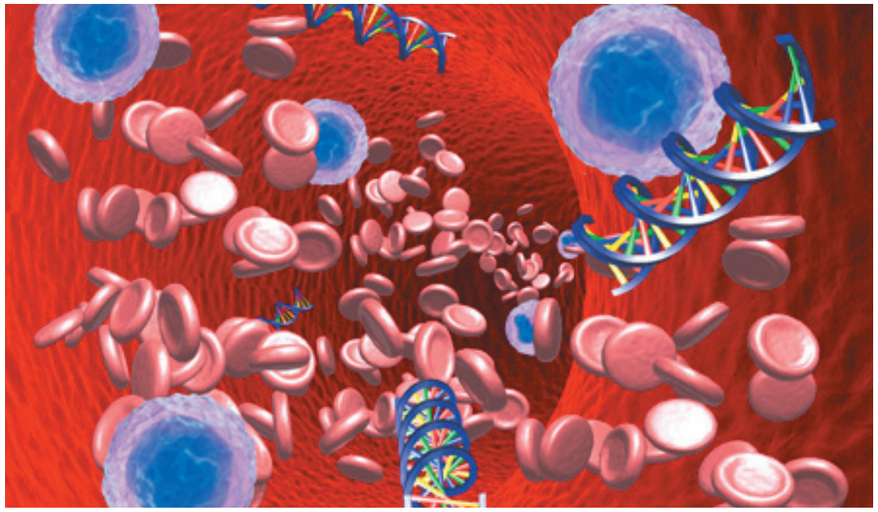
In this section we highlight some research publications from 2020 which report significant advances in specific areas. The selected papers demonstrate the breadth and the quality of the research being undertaken by the groups at the Cancer Research UK Manchester Institute.

Valpione S, Galvani E, Tweedy J, Mundra PA, Banyard A, Middlehurst P, Barry J, Mills S, Salih Z, Weightman J, Gupta A, Gremel G, Baenke F, Dhomen N, Lorigan PC, Marais R.
Immune awakening revealed by peripheral T cell dynamics after one cycle of immunotherapy.
Nature Cancer 2020; 1(2):210-221.

In this publication, the Molecular Oncology group show that liquid biopsies can be used to identify which patients will benefit from immunotherapy. They demonstrate that following immunotherapy, T cell receptors evolve to increase diversity or clonality in patients who will go on to respond, but not in patients who do not respond. They also identify a subset of circulating T cells that expand in patients who go on to respond, but not in patients who do not respond. These cells are characteristic of cells that fight infections, but this data suggests that the same cells are recruited to fight tumours in patients receiving some forms of immunotherapies, so the group called these cells T-immune effector or T_{IE} cells. Their studies contribute to improving our understanding of the mechanisms that mediate effective immune-responses to immune-checkpoint blockade drugs, so the data opens new opportunities to design effective immune-biomarkers for future clinical development. The study also opens new avenues to further exploit the immune system for therapeutic gain. Critically, because both parameters can be measured using patient blood, responding patients can be identified early during treatment, with all of the advantages associated with minimally invasive liquid biopsies. Early identification of responses will improve outcomes for patients, because it will allow therapies to be refined and personalised to the individual. This will also reduce toxicity, because non-responding patients can be spared extended treatments that will not provide any benefit.

The Molecular Oncology group identified an immune signature of response to anti-PD1 drugs analysing peripheral blood T cells and the T cell receptor DNA sequences in cfDNA of cancer patients receiving immunotherapy.

This artwork was designed for the publication. (Image supplied by Sara Valpione, Molecular Oncology)



Galvani E, Mundra PA, Valpione S, Garcia-Martinez P, Smith M, Greenall J, Thakur R, Helmink B, Andrews MC, Boon L, Chester C, Gremel G, Hogan K, Mandal A, Zeng K, Banyard A, Ashton G, Cook M, Lorigan P, Wargo JA, Dhomen N, Marais R.
Stroma remodeling and reduced cell division define durable response to PD-1 blockade in melanoma.
Nature Communications 2020; 11(1):853.

Immune checkpoint inhibitors (ICIs) have revolutionised the treatment of melanoma, with checkpoint blocking antibodies to CTLA-4 and PD-1/PD-L1 improving survival for many patients, and eliciting long-term durable responses for some. However, the biological features underlying these durable responses remain poorly understood. Efforts to identify markers of response in patient-derived materials have been limited by the diversity and plasticity of the immune system in a human population shaped by diverse variables such as genetic background, lifestyle and prior lines of treatment. In recent years, the Molecular Oncology group reported that its $BRAF^{V600E}$/UVR-driven mouse melanoma model recapitulates the cardinal pathological and genomic features of human melanoma including a UVR-induced mutational signature and high C-to-T mutation burden. Critically, this model retains the mouse's native immune system and tumour microenvironment (TME), while allowing control of genomic, behavioural and environmental variables. In this publication, the Molecular Oncology group used this controlled system to assess the genetic and phenotypic changes induced by PD-1 blockade and report that anti-PD-1 treatment yielded responses in ~35% of tumours, and prolonged survival in ~27% of the animals, similar to the responses observed in the human patient population. From RNA sequencing, the group identified genes whose expression correlated to response to PD-1 blockade, allowing the development of two signatures that were predictive of later response, one for stroma remodelling and one for proliferation. Their signatures were validated in two independent early on-treatment anti-PD-1 patient cohorts. Together, these data suggest that stroma remodelling and proliferation are features of response to immunotherapy that could be used to identify responding patients so that treatment can be tailored to achieve the best outcome for individual patients.

Pearsall SM, Humphrey S, Revill M, Morgan D, Frese KK, Galvin M, Kerr A, Carter M, Priest L, Blackhall F, Simpson KL, Dive C.
The rare YAP1 subtype of SCLC revisited in a biobank of 39 circulating tumor cell patient derived explant models: a brief report.
Journal of Thoracic Oncology 2020; 15(12):1836-1843.

Small cell lung cancer is an aggressive neuroendocrine (NE) cancer with poor prognosis, characterised by high circulating tumour cell burden and early widespread metastasis.

Four consensus subtypes were proposed recently for molecular classification of SCLC, defined by the expression of NE transcription factors *ASCL1* and *NEUROD1*, the *POU2F3* secretory tuft cell lineage marker and *YAP1*, a transcriptional activator with diverse roles culminating in pro-survival, pro-metastatic and chemoresistance oncogenic functions. SCLC cells predominantly express NE phenotypic markers, however it is known that a small minority of these NE cells can transition to a non-neuroendocrine (non-NE) phenotype, and many of the Cancer Biomarker Centre's CTC-derived explant (CDX) models of SCLC also contain low numbers of non-NE cells, which are marked by expression of the NE repressor protein, REST. Whilst the team were unable to identify the YAP1 subtype in their global transcriptomics analysis of 39 CDX models, they identified several models that had relatively high expression of *YAP1* transcript. In the majority of these models, YAP1 expression was co-localised with REST, and thus restricted to localised clusters of minority non-NE cell subpopulations. A rare, 'NE-low' (i.e. expressing low levels of NE markers by multiple metrics) SCLC phenotype was also observed in their CDX biobank and in these, YAP1 expression was more abundant and present both in NE and non-NE cells. When culturing the separated NE and non-NE cells from individual CDX models ex vivo, they found that the non-NE, YAP1-expressing cells were more chemoresistant than their NE counterpart cells. Together, these data indicate that YAP1 expression in SCLC represents an additional feature of SCLC phenotypic heterogeneity, and warrants further exploration in order to understand and exploit these differences for therapeutic gain.

Salamero O, Montesinos P, Willekens C, Pérez-Simón JA, Pigneux A, Récher C, Popat R, Carpio C, Molinero C, Mascaró C, Vila J, Arévalo MI, Maes T, Buesa C, Bosch F, Somervaille TCP.
First-in-Human Phase I study of Iadademstat (ORY-1001): A first-in-class lysine-specific histone demethylase 1a inhibitor, in relapsed or refractory acute myeloid leukemia.
Journal of Clinical Oncology 2020; 38(36):4260-4273.

Acute myeloid leukaemia is a haematological malignancy characterised by a block in myeloid lineage differentiation. It remains, for the most part, an incurable disease, especially in the elderly, and new approaches to treatment are required, including those that promote differentiation. Certain epigenetic regulators are under evaluation as therapeutic targets, including lysine-specific demethylase 1 (LSD1). LSD1 exhibits demethylase activity versus methylated lysine residues as well as a transcription factor scaffolding activity.

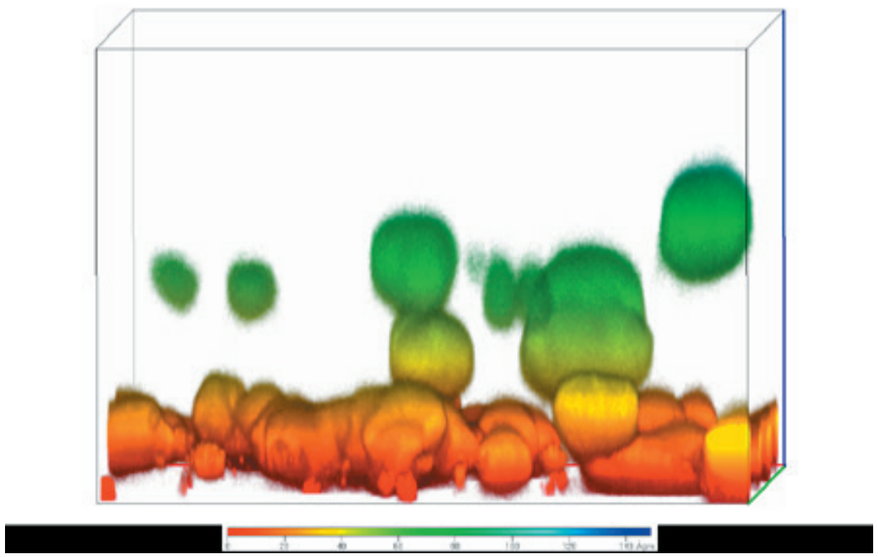
Preclinical studies from the Leukaemia Biology group revealed that LSD1 sustains the differentiation block in certain molecular subtypes of AML, in particular *MLL*-translocated AML. Iadademstat is a highly selective and potent covalent inhibitor of LSD1. Iadademstat was evaluated in a first-in-human dose-escalation and extension-cohort phase I study in patients with refractory or relapsed acute myeloid leukaemia. The primary objective was to assess safety and tolerability of iadademstat; secondary objectives were to study pharmacokinetics, pharmacodynamics and efficacy.

Twenty-seven patients were treated with iadademstat on days 1 to 5 of each week in 28-day cycles in a dose-escalation phase that resulted in a recommended dose of 140 $\mu g/m^2$/day. This dose was chosen to treat all patients in an extension-cohort enriched with patients with MLL-rearranged AML. Most adverse events were as expected and included myelosuppression and non-hematologic events, such as infections, asthenia, mucositis, and diarrhoea. Pharmacokinetic data demonstrated a dose-dependent increase in plasma exposure, and pharmacodynamic data confirmed a potent time- and exposure-dependent induction of differentiation biomarkers. Reductions in blood and bone marrow blast percentages were observed, together with induction of blast cell differentiation, in particular in patients with MLL translocations. One complete remission with incomplete count recovery was observed in the dose escalation arm. Thus, iadademstat exhibits a good safety profile together with signs of clinical and biologic activity as a single agent in patients with AML.

Williams MS, Basma NJ, Amaral FMR, Williams G, Weightman JP, Breitwieser W, Nelson L, Taylor SS, Wiseman DH, Somervaille TCP.
Targeted nanopore sequencing for the identification of ABCB1 promoter translocations in cancer.
BMC Cancer 2020; 20(1):1075.

A549 cells invading into artificial basement membrane following metal exposure.

Image supplied by Yannick von Grabowiecki (Tumour Suppressors)



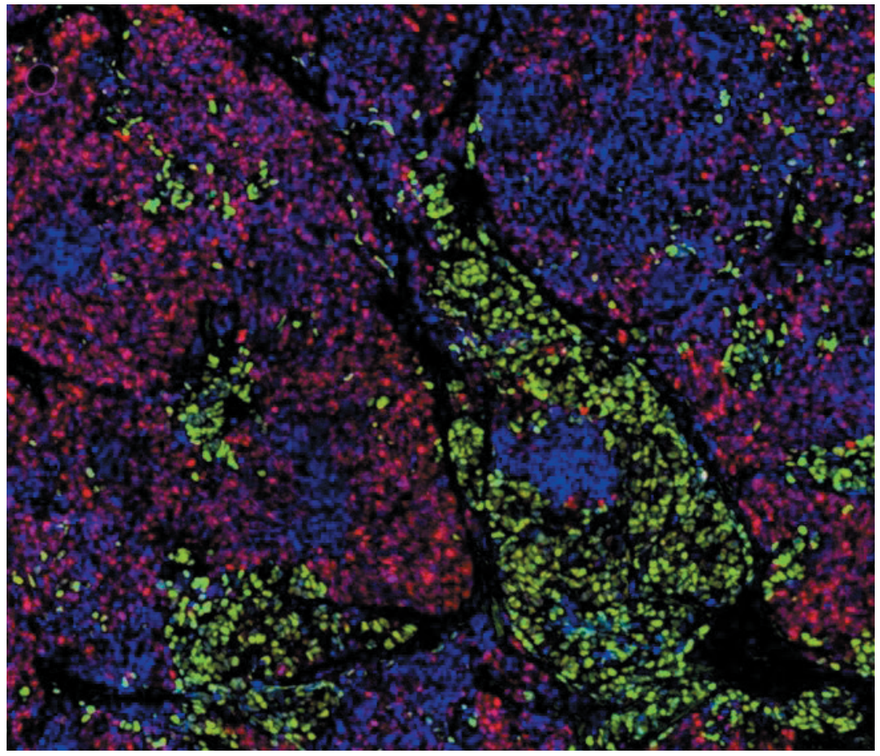
Resistance to chemotherapy is the most common cause of treatment failure in acute myeloid leukaemia (AML) and the drug efflux pump ABCB1 is a critical mediator. Recent studies have identified promoter translocations as common drivers of high *ABCB1* expression in recurrent, chemotherapy-treated high-grade serous ovarian cancer (HGSC) and breast cancer. These fusions place *ABCB1* under the control of a strong promoter while leaving its open reading frame intact. The mechanisms controlling high *ABCB1* expression in AML are largely unknown. The Leukaemia Biology group therefore established an experimental system and analysis pipeline to determine whether promoter translocations account for high *ABCB1* expression in cases of relapsed human AML.

The group first demonstrated that prolonged in vitro daunorubicin exposure could induce activating *ABCB1* promoter translocations in a human AML cell line (THP-1), similar to those recently described in recurrent high-grade serous ovarian and breast cancers. This model was used to establish a targeted nanopore long-read sequencing approach that was then applied to cases of *ABCB1*^{high} HGSC and AML.

This approach proved an efficient method for identifying *ABCB1* structural variants in THP-1 AML cells and HGSC, identifying both novel and previously described promoter translocations in HGSC. In contrast, activating *ABCB1* promoter translocations were not identified in *ABCB1*^{high} AML. H3K27Ac ChIP sequencing demonstrated significant activity of native promoters in all cases of *ABCB1*^{high} AML studied, consistent with endogenous regulation.

Despite frequent high-level expression of *ABCB1* in relapsed primary AML, they found no evidence of *ABCB1* translocations and instead confirmed activity of native *ABCB1* promoters. These findings are consistent with the group's recent work describing the regulation of *ABCB1* by a network of stress responsive enhancers in primary AML.

Paliouras AR, Buzzetti M, Shi L, Donaldson IJ, Magee P, Sahoo S, Leong HS, Fassan M, Carter M, Di Leva G, Krebs MG, Blackhall F, Lovly CM, Garofalo M.
Vulnerability of drug-resistant EML4-ALK rearranged lung cancer to transcriptional inhibition.
EMBO Molecular Medicine 2020; 12(7):e11099.



Mutually exclusive expression of the MYC family proteins MYC and MYCL in CDX17P via multiplex immunofluorescence. MYC (green), MYCL (red), DAPI (blue), scale bar, 200 µm).

Image supplied by Kathryn Simpson (Cancer Biomarker Centre)

In non-small cell lung cancer (NSCLC), small molecule inhibitors that target mutant kinases have offered unprecedented success in the management of the disease. One of the most successful examples is echinoderm microtubule like-4-anaplastic lymphoma kinase (EML4-ALK)-mutant lung cancer, which affects 4-5% of lung cancer patients. EML4 causes the constitutive activation of the ALK kinase domain which leads to cancer. To date, the first-generation ALK inhibitor crizotinib, second-generation ALK inhibitors ceritinib, alectinib and brigatinib, and the third-generation ALK inhibitor lorlatinib have been approved by the Food and Drug Administration (FDA) for the treatment of patients with lung cancer harbouring the EML4-ALK translocation.

While initial responses are excellent, patients eventually relapse due to the development of acquired resistance to these therapies. Exploring these mechanisms of resistance, the Transcriptional Networks in Lung Cancer group found that EML4-ALK cells parental or resistant to crizotinib, ceritinib or alectinib are remarkably sensitive to inhibition of genes involved in the cell cycle (namely, CDK7/12 with THZ1 and CDK9) with drugs called alvocidib and dinaciclib. This treatment induces cell death through transcriptional inhibition and downregulation of anti-apoptotic genes and reduces tumour growth not only in cancer cells but also in mouse models of the disease. In summary, patients with acquired resistance to ALK inhibitors and wild-type ALK have only chemotherapy left as a treatment option. The team's findings point to the possibility that CDK inhibition may be clinically tested as an alternative to help manage this disease.

Trotter EW, Hagan IM.
Release from cell cycle arrest with Cdk4/6 inhibitors generates highly synchronized cell cycle progression in human cell culture.
Open Biology 2020; 10(10):200200.

Iain Hagan and Wendy Trotter from the Cell Division group developed a new approach to synchronise the cell division cycle of an entire population of human cells in culture. This method, published in *Open Biology,* exploits the ability of CDK4/6 inhibitor drugs to arrest cell cycle progression at a natural pause point of the cycle: the restriction point. After synchronisation and re-initiation of the cycle, all the cells in the population go through it at the same time and rate, making it possible to perform biochemical and functional analyses of the changes that occur in cells at specific points of the cycle.

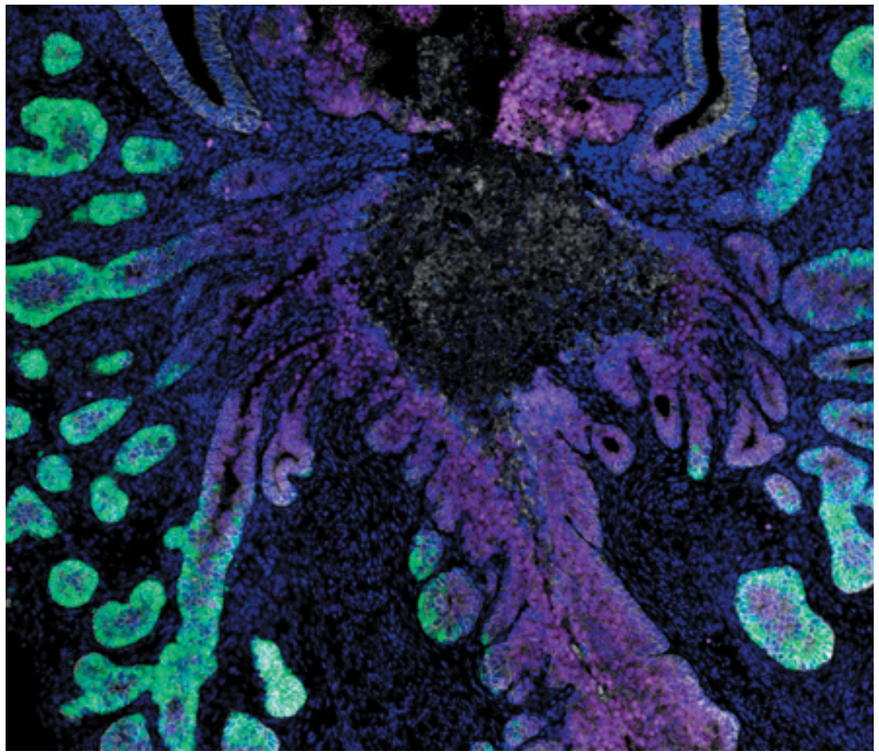
Importantly, cells can be removed from the cycle for up to 48 hours, during which they retain their ability to resume synchronous cell cycling. That time can be used to manipulate any molecule and when the cells are returned to the cycle, their fate can be observed and enable significant insights to be made into the function of molecules and the behaviour of cells within the actual cycle under study.

The gold standard for cell cycle synchronisation has long been the double thymidine block approach, which stops the cell cycle at the phase of DNA replication and causes DNA damage. The subsequent cell cycle is therefore far from normal, making the double thymidine block approach difficult to study the biochemistry of DNA replication or transcription, or chromatin biology. By contrast, using CDK4/6 inhibitors clinically approved to treat breast cancer have no impact upon DNA integrity, making the Cell Division group's approach a game changer for researchers studying DNA biology. Critically, this publication shows that a drug developed to treat cancer, and with great effect, can now advance our understanding of cell division to help identify further routes to cancer therapy.

Bonavita E, Bromley CP, Jonsson G, Pelly VS, Sahoo S, Walwyn-Brown K, Mensurado S, Moeini A, Flanagan E, Bell CR, Chiang SC, Chikkanna-Gowda CP, Rogers N, Silva-Santos B, Jaillon S, Mantovani A, Reis E Sousa C, Guerra N, Davis DM, Zelenay S.
Antagonistic inflammatory phenotypes dictate tumor fate and response to immune checkpoint blockade.
Immunity 2020; 53(6):1215-1229.e8.

Immunotherapy has emerged as an alternative anti-cancer therapy, which has revolutionised the field of cancer research and treatment. Unprecedented outcomes continue to be observed in multiple cancer types, including malignancies where conventional therapies such as chemotherapy, radiotherapy or targeted therapy have failed. Nevertheless, the immunotherapy excitement has been rapidly tempered by clinical data showing that only a minority of cancer patients achieve complete and long-lasting responses. This has underscored the need for extensive preclinical research to understand the basis of these remarkable but still rare outcomes.

Inflammation can fuel or inhibit cancer progression and the response to therapy. In this study, the Cancer Inflammation and Immunity group investigated the signals and pathways that regulate the establishment of tumour inflammatory microenvironments that support or restrain cancer progression. Combining the use of genetically engineered mouse cancer



The developing prostate of an embryo with RUNX1 shown in magenta.

Image supplied by Renaud Mével and Georges Lacaud (Stem Cell Biology)

models with the analysis of samples from cancer patients, the group identified Natural Killer (NK) cells as key drivers of cancer-inhibitory inflammation. In cancer models rendered immunogenic by genetic ablation of the cyclooxygenase (COX)-2 pathway, NK cells were essential for initiating an inflammatory response that preceded and stimulated cytotoxic T cell-dependent tumour growth control. The analysis of patient datasets suggested the COX-2 pathway regulates equally the cellular and molecular inflammatory profile across multiple human cancer types. Furthermore, the authors developed an approach that by combining tumour-promoting and anti-tumour mediators improves our ability to predict overall patient survival and the response to immunotherapy in a wide range of human cancers. Collectively, their findings established the COX-2 pathway and NK cells as critical orchestrators of T cell-mediated cancer immunity and demonstrate the value of integrating pro- and anti-tumorigenic inflammation to predict patient outcome.

Mevel R, Steiner I, Mason S, Galbraith LC, Patel R, Fadlullah MZ, Ahmad I, Leung HY, Oliveira P, Blyth K, Baena E, Lacaud G.
RUNX1 marks a luminal castration-resistant lineage established at the onset of prostate development.
Elife 2020; 9:e60225.

The prostate is a glandular organ of the mammalian male reproductive system. A prostate luminal secretory epithelial subpopulation is involved in fluid secretions that form part of the semen. One incredible property of the prostate epithelium is its plasticity. In the absence of hormones, such as chemical or surgical castration during prostate cancer treatment, the prostate shrinks dramatically and loses most of the luminal cells. Strikingly, the prostate can fully regenerate itself upon hormone replenishment. This impressive regenerative capacity has been extensively demonstrated in mouse models, suggesting the presence of a subpopulation of cells with specific regenerative properties. However, the precise nature of these cells and the mechanisms by which they survive hormone deprivation and subsequently regenerate the prostate's complex cellular content, or are involved in the emergence of castration-resistant cells, remain to be elucidated. The RUNX1 transcription factor is a master regulator of the blood system essential for hematopoietic development, homeostasis, and disease. Interestingly, there is increasing evidence implicating RUNX1 in the biology and pathology of hormone-regulated tissues. Thus, the Stem Cell Biology group systematically studied the presence and contribution of RUNX1 expressing cells during the development, homeostasis, and regeneration of the prostate gland.

The group found that RUNX1 is highly expressed in a subpopulation of proximal luminal cells located at the base of the adult mouse prostate, in the region next to the urethra. They characterised these RUNX1$^+$ luminal cells during development and castration-regeneration assays using single cells transcriptomics and genetic lineage-tracing. They showed that RUNX1$^+$ luminal cells are castration-resistant, self-sustained and do not regenerate other distinct luminal cells types. They found that a similar RUNX1$^+$ population emerges in the proximal region of the developing prostate ducts during embryonic prostate development, indicating that RUNX1$^+$ proximal luminal cells are an independent luminal cell type established at the onset of prostate development. This Stem Cell Biology study provides new insights into prostate development and the cellular composition of the mouse prostate epithelium. Investigating the functional relevance of these RUNX1$^+$ cells in prostate cancer, where the presence of castration-resistant cells is a critical therapeutic problem, may open the door to improved cancer treatments.

CANCER RESEARCH UK MANCHESTER INSTITUTE

RESEARCH GROUPS

Cancer Research UK Manchester Institute Cancer Biomarker Centre

The goals of the CRUK Manchester Institute Cancer Biomarker Centre (CBC) are a) to discover, develop, validate and implement biomarkers and digital solutions that optimise personalised cancer medicine; and b) to characterise and exploit our panel of CDX models derived from circulating tumour cells from small cell lung cancer patient to discover new targets and test new therapies.

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CBC showcase studies published in Nature Cancer and the Journal of Thoracic Oncology that describe the inter- and intra-tumoural heterogeneity of SCLC, highlighting the potential of the CDX approach, now used worldwide, to advance SCLC research. We submitted a patent on our workflow for our T7 ctDNA pipeline that incorporates ctDNA methylation profiling, and is being deployed to subtype SCLC patients, explore Cancers of Unknown Origin, and for the early detection of non-small cell lung cancer. With clinical colleagues across the UK, Spain and Italy, we launched the CRUK Accelerator UpSMART that will bring digital solutions to the care of patients undergoing early clinical trials.

Intra-tumoural functional heterogeneity in SCLC

Using our CDX biobank, the Preclinical Pharmacology team continue to explore and describe novel elements of phenotypic diversity within small cell lung cancer (SCLC) that were not previously apparent due to the lack of available tissue and patient-relevant models. The expanding panel of CDX models within our biobank (42 models, including 6 pairs) has enabled us to build on our studies into acquired drug resistance, vasculogenic mimicry (VM) and neurogenic transcription factor expression, particularly to understand the novel role of ATOH1 in SCLC.

Intra-tumour SCLC heterogeneity plays a role in metastasis and therapy resistance, with the minority non-neuroendocrine (non-NE) cell subpopulation supporting growth, survival and dissemination of NE cells. We have shown that these non-NE cells are also the population that are VM-competent, whereby an epithelial to endothelial transition occurs to enable vessels with blood transporting capabilities. RNA sequencing (RNAseq) of VM competent non-NE cells revealed a pseudo-hypoxic gene signature, implicating a role for hypoxia-associated genes in VM and highlighting the plasticity of SCLC cells that can adapt and modulate their phenotypic characteristics to support tumour growth. We have also shown that formation of SCLC hollow tubular structures on matrigel (a VM surrogate assay) occurs via active integrin signalling leading to collagen deposition and remodelling (Figure 1). Our continued molecular characterisation of VM seeks biomarkers and/or targets for non-NE SCLC cell targeted therapies.

Biomarkers to inform immunotherapy trials

The Cells and Proteins team (CAP) continue to expand and develop the CBC immune biomarker 'toolkit'. Through a new partnership with ThermoFisher, the IonTorrent platform is now installed in our Tumour Immunology and Inflammation Monitoring Laboratory and validation of TCRseq assays for analysis of clonality, diversity and convergence in blood samples is ongoing. Working in close collaboration with Prof Fiona Blackhall at the Christie Hospital Foundation Trust, the processing and banking of liquid biopsy samples from a cohort of SCLC patients receiving immunotherapy at the Christie is underway allowing CTC, ctDNA and TCRseq analyses aimed at identification of candidate biomarkers that predict and monitor patient responses to immunotherapy. To complement these liquid biopsies, T-cell infiltrates are being evaluated in SCLC tumour samples to develop an understanding of the heterogeneity and mechanisms of immune evasion in advanced metastatic disease. Methods have also been established for ex vivo co-cultures of peripheral blood lymphocytes and disaggregated SCLC CDX cells to enable investigation of anti-tumour T-cell responses and resistance to immunotherapy (Figure 2). A range of MHC1 expression levels were observed across our SCLC CDX panel, highlighting impaired antigen

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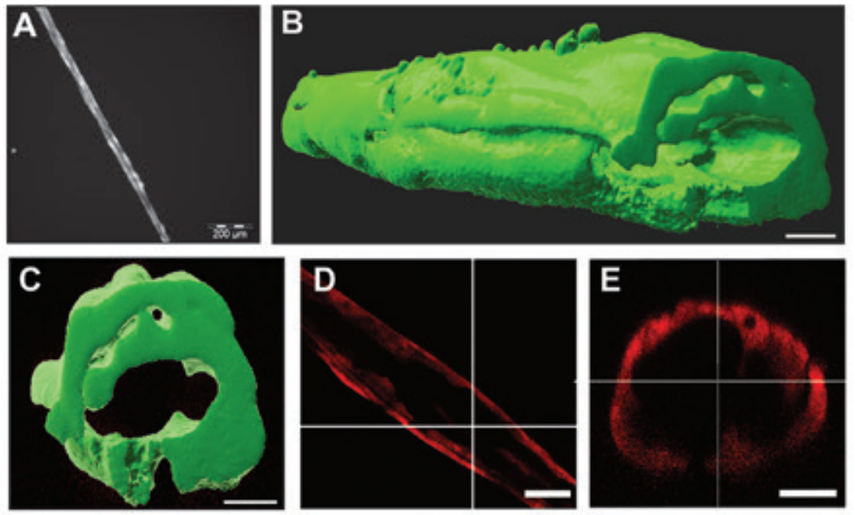

Figure 1.
SCLC CDX non-NE cells form tubules with hollow lumens when cultured on Matrigel.
A, Tubule formation of CDX17 non-NE cells visualised via brightfield (i) and fluorescence microscopy (ii). B,C, Confocal Imaging of CDX17 non-NE cells forming hollow tubules visualised using Imaris software z-stack reconstruction, showing a defined outer tubule wall (Bi), with a hollow lumen (Bii, Biii) and cross sections of CDX17 non-NE cells showing a defined cell containing an outer tubule wall (Ci), and continuous layer of cells forming the hollow centre (Cii and Ciii). All images are representative.

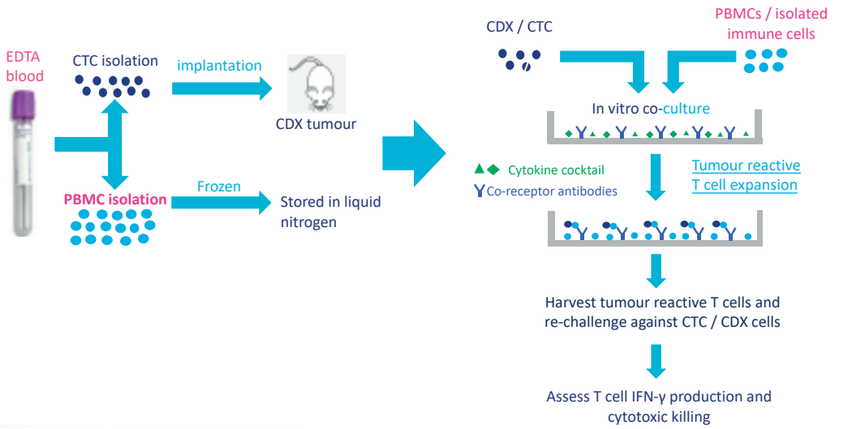

Figure 2.
Circulating tumour cells (CTCs) and peripheral blood mononuclear cells (PBMC) are isolated from a patient blood sample. CTCs can be cultured or implanted into mice to generate a CTC Derived Explant Model (CDX) in mice. CTCs or disaggregated CDX cells are co-cultured with PBMC to determine the extent of tumour reactive T-cell activation. This system is being developed to test the effect of therapeutics on the patient's response to immunotherapy.

presentation as a potential mechanism of immune evasion. Generation of new CDX models with banking of matched PBMCs from SCLC patients on immunotherapy is being prioritised to enable CTC and T-cell co-culture studies. This co-culture capability is being extended to innate immune cell populations, starting with natural killer cells.
As a first step towards testing of a COX-inflammatory signature as a predictive biomarker for immunotherapy in clinical trials, we are working with Dr Santiago Zelenay (supported by a MRC Confidence in Concept Award) to develop a standardised assay on the clinically compatible NanoString platform to measure expression of this signature in baseline patient tumour biopsies. A new ACED funded PhD student is also working jointly between the Zelanay group and CBC to explore the potential for the COX-inflammatory signature to predict relapse in early stage lung cancers and its relationship with the broader immune landscape of these tumours.

We are also working with the digital ECMT team and have demonstrated the feasibility of detecting cytokines by ELISA in as little as 30µl blood collected using a micro-sampler device, with optimisation of workflows for sample processing and analysis underway. This approach will be used in the NOTION study to evaluate home based blood sampling kits that assess cytokines as an early warning system of adverse events and cytokine release syndrome in patients receiving immunotherapy and advanced T-cell therapies. We are also working with Dr Phil Monaghan, to enable transfer of our cytokine and sTie2 ELISAs to the Christie Hospital Diagnostic Biochemistry Laboratory for routine NHS use and deployment in the upcoming

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VALTIVE 1a trial (CI: Prof Gordon Jayson, University of Manchester/Christie NHS Foundation Trust - CFT) to optimise anti-angiogenic therapy respectively.

Nucleic acid-based biomarkers that direct therapy decisions

The Nucleic Acids Biomarkers team has continued to develop and validate molecular profiling and disease monitoring liquid biopsy workflows to support clinical trials and translational projects across the CBC.

Mutational profiling of ctDNA to assist selection of Phase I clinical trials

We profiled ctDNA in the TARGET Trial (Tumour chARacterisation to Guide Experimental Targeted therapy, CI Matt Krebs UoM/CFT) with completion of Part B (patient 520) expected by the end of the year. Mutational analyses across 641 cancer-associated genes as well as copy number aberration (CNA) analysis were routinely presented at the monthly Molecular Tumour Board. Analysis of immune biomarkers from tumour biopsies to assist selection to immunotherapy trials was also added to the CBC TARGET biomarker portfolio over the past year working alongside the CAP and Tissue Biomarkers teams.

ctDNA analysis within the CACTUS and DETECTION trials

Over the last year the NAB team has performed GCP compliant droplet digital PCR (ddPCR) analysis of ctDNA within the CACTUS Trial (CirculAting Tumour DNA gUided therapy Switch, CI Paul Lorigan UoM/CFT) for advanced cutaneous melanoma phase II patients. The ddPCR ctDNA assay measures mutated BRAF levels to instruct treatment switch from targeted to immunotherapy and requires validated results to be returned to the clinic within 7 days of blood draw. Modification of the assay has enabled inclusion of more patients within the trial, with 23 patients screened to date. Validation of a more extensive (11 targeted mutations) and sensitive ddPCR-based primary clinical assay has also been developed within the group for use in the DETECTION trial (Circulating tumour DNA guidEd Therapy for stage IIB/C BRAF or NRAS mutant- positive mElanoma after surgiCal resection, CI: Paul Lorigan). This trial involves ddPCR ctDNA analysis to detect early relapse/micro-metastatic disease and select patients for targeted therapy and will open to recruitment in Q2 2021. NAB has now validated the lower levels of detection for 4 BRAF mutations and 3 NRAS mutations, with validation of 4 additional hTERT mutations on going.

Methylation profiling of ctDNA for the early detection of cancers

We have further refined our genome-wide cfDNA methylation pull-down assay that is capable of high-throughput processing of the large number of samples anticipated in early detection screens. The assay combines a patented in-house NGS library preparation method with a methyl-binding domain protein-based methylation enrichment approach. When compared to other commercially available NGS library preparation kits, including those incorporating UMIs, our method had superior performance in the cfDNA methylation assay, resulting in increased enrichment and reduced background (Figure 3A). We tested our methylation assay on 20 NSCLC tumour samples (10 adenocarcinoma, 10 squamous cell carcinoma) and compared to published TCGA data sets with good concordance seen between data sets confirming the accuracy of the CBC T7 assay. We have also tested the sensitivity of the assays in preliminary studies using 10 ng input spike-in experiments and shown the CBC T7 assay is able to detect tumour specific methylation signal at 0.01 - 0.1% VAF (Figure 3B). Additional studies are currently on going to further test the sensitivity and improve the bioinformatic analysis to enable calling of tumour positive or negative samples.

We have used our methylation assay to generate a SCLC specific methylation signature derived from tumour tissue and cfDNA from the same patient using a panel of 8 CDX tumours and the ctDNA isolated from the patient that gave rise to the CDX model. This comparison found a strong correlation between differentially methylated regions detected in both sample types, confirming the viability of ctDNA as a source of methylation profiling. This signature is now being tested in a cohort of 99 SCLC cfDNA samples to determine the sensitivity and specificity of the SCLC classifier which will then be used to monitor SCLC patients, identify early relapse and potentially give insight into mechanisms of resistance.

The methylation workflow is also being used in a collaboration with Natalie Cook (UoM/CFT) on Cancer of Unknown Primary (CUP) study where tissue-specific methylation profiles will be used to identify the tissue of origin for these difficult to treat patients. To date we have applied the CBC T7 methylation approach to cfDNA analysis of 37 HNVs and 51 cancer patient samples using a pan-cancer signature consisting of 1022 differentially methylated regions (Figure 3C). This found that the pan-cancer signature could separate 49/51 cancer patient samples from HNVs, even in samples where somatic mutation analysis failed to detect a quantifiable VAF (lower level of detection 1%). Further refinement of the methylation workflow and generation of more robust cancer specific methylation profiles are currently on going.

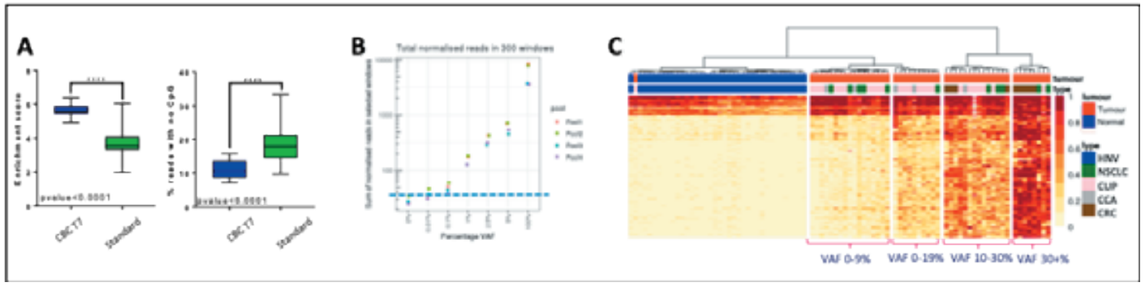

Figure 3.
(A) Improved enrichment and on-target reads with CBC T7 approach. (B) Spike-in experiments showed detection of a cell line specific 300 region DMR signature down to sensitivity levels of 0.1-0.01% VAF (dotted blue line). (C) Using a 1022 DMR pan-cancer signature ctDNA methylation detected 49/51 cancer patients (96% specificity) across 4 tumour types.

Bioinformatics and Biostatistics across the Cancer Biomarker Centre

The Bioinformatics and Biostatistics (BBS) team is active throughout the Cancer Biomarker Centre, integrating bioinformatics and statistical methods for its many and varied projects and providing input into experimental designs, including those developed for the afore-mentioned NOTION trial and the VALTIVE1a trial that seek to qualify our liquid biopsy measuring sTie2 to instruct optimal use of anti-angiogenic therapy in ovarian cancer. A key focus is to enable robust, reproducible workflows to analyse different types of next-generation sequencing data. The TARGET trial (CI: CBC alumnus, Matt Krebs) aims to match patients with a broad range of advanced cancers to early phase clinical trials; analysis of both somatic mutations and copy number alterations across a 641 cancer-associated-gene panel in a single ctDNA assay support this aim. As TARGET is nearing completion, a final update on our data processing workflow will be used to rerun all the ctDNA analyses to provide a rich and internally consistent dataset for exciting retrospective research.

We are developing a custom pipeline and an R software package for analysis of DNA CpG methylation to investigate chemoresistance in patient derived mouse models, to determine SCLC subtypes and tissue-of-origin for Cancer of Unknown Primary (CUP) in cfDNA and within a multi-modal liquid biopsy for early detection of lung cancer.

In a cross CBC team study with dECMT and with clinical colleagues across the UK (CI: Rebecca Lee, UoM/CFT), we assisted the development of a machine learning platform to predict COVID severity for cancer patients (see below).

Digital solutions to support treatment for cancer patients

The digital Experimental Cancer Medicines Team (digital ECMT) 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.

The PROACT Study was delivered, which investigated the feasibility of implementing an app for capturing patient reported outcomes and experiences electronically and remotely. The In-Home study, which assesses the feasibility of Acute Kidney Injury (AKI) detection in the patient's home, was initiated with recent completion of Part A (with 12 Head and Neck cancer patients enrolled). This trial measures renal function based on a pin-prick of blood and creatinine sensor. The NOTION study (a CBC cross team project with the CAP team), has the objective of enabling dry blood spot technology to measure cytokine levels in the home for early detection of immune-related toxicities, has been developed and will receive ethical approval in 2021.

This year a digital ECMT Artificial Intelligence (AI) capability was formally established within our team focussing on development of ethical AI and investigation of novel algorithmic methods to deliver direct patient benefit. Aligned to this was development of the CORONET tool (COVID-19 risk in Oncology Evaluation Tool) by digital ECMT (with assistance from the BBS team) in collaboration with clinicians throughout the UK. The decision support tool supports health care professionals in deciding which COVID-19 cancer patients to admit to hospital. A formal collaboration with University Hospital Southampton to deliver the REACT COVID-19 prospective observational study was also established. As part of this, the REACT tool was repurposed to enable hospital data to be visualised.

The digital ECMT, along with our EU colleagues, including those from Fondazione IRCCS Istituto Nazionale dei Tumori Milano and Instituto de investigación Oncologica de Vall d'Hebron, Barcelona was awarded a CRUK Accelerator Award, UpSMART, to enable SMART Experimental Cancer Medicine Trials. The 5-year UpSMART programme commenced in 2020 with the initial aim of identifying digital healthcare products (DHPs) which are proving valuable to either/both clinical trial data acquisition and data interpretation from the network of (currently 24) participating experimental cancer medicine centres/early drug development units across Europe. In year 1, the programme identified 29 potential DHPs across the network and prioritised 10 of those to develop, using UpSMART Accelerator funding, into open-source accessible products; free of license fees to use.

Publications listed on page 64

CANCER INFLAMMATION AND IMMUNITY

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Immune checkpoint blockade therapy, especially that based on the use of PD(L)-1 targeted monoclonal antibodies, has transformed cancer treatment becoming the standard of care for multiple tumour types. Despite the advent of these transformative treatments, few patients derive profound and long-lasting benefit. Moreover, our ability to predict who will respond is very limited.

Our group at the Cancer Research UK Manchester Institute investigates the signals and pathways that regulate the establishment of tumour environments that favour anti-cancer immunity and response following immunotherapy. Under the central hypothesis that the type of inflammatory response most prevalent in clinically-apparent tumours promotes cancer progression, immune escape and therapy resistance, we combine the use of genetically engineered pre-clinical cancer models with the analysis of patient samples to study the cellular and molecular inflammatory determinants that underpin immunotherapy success.

Since 2006, more than 3500 clinical trials have been started to test PD(L)-1 blockade as a monotherapy or in combination with other agents. Many of these trials are still active and evaluate combination regimens of PD(L)-1 antibodies with other immune checkpoint inhibitor drugs (mainly anti-CTLA4 antibodies), chemotherapy, radiotherapy or targeted therapy. Notwithstanding this central role of PD(L)-1 axis blockade in clinical practice, our current understanding of the basis underlying therapy response, resistance or relapse is limited. Likewise, and very much connected to this central gap in knowledge, there is an urgent need to develop biomarkers to distinguish responders from non-responders. Within this rapidly evolving clinical scenario, our group performs fundamental and translational research to help answer these crucial open questions in the immuno-oncology field. Our working hypothesis is that the inflammatory landscape at the tumour bed, before or shortly on-treatment, determines the outcome of immunotherapy. However, cancer-associated inflammation is a very complex process that manifests in different 'flavours' with dual tumour-promoting and restraining effects. Consistent with this notion, our recent work in preclinical cancer models rendered immunogenic by genetic ablation of the cyclooxygenase (COX)-2 pathway, has uncovered antagonistic inflammatory profiles that anticipate immune-dependent progressive or regressive tumour growth (Bonavita et al, *Immunity* 2020; Figure 1).

Through deep cellular and molecular profiling of tumours formed by COX-2 competent or deficient cancer cells, we identified Natural Killer (NK) cells as central orchestrators of T cell-mediated tumour immunity. While NK cells are well known for their direct cytocidal activity against cancer cells, in our experimental systems we found that early accumulation of NK cell in tumours is essential to drive an inflammatory reprogramming characteristic of the so-called 'hot' T cell-inflamed tumours. Critically, while NK cells themselves inhibited tumour growth, T cells, and in particular cytotoxic T lymphocytes, were indispensable for the full eradication of tumours.

The use of multiple genetically-modified mouse strains allowed us to ascribe IFN-γ as a critical molecular mediator of NK cell activity. We showed that NK cell-derived IFN-γ drives an intratumoural inflammatory reprogramming that attracts and stimulates the differentiation of anti-cancer effector T cells. Accordingly, single cell RNA sequencing analysis of tumour-infiltrating immune cells revealed that the transcriptome of the most abundant leukocyte subsets in tumours, monocytes and tumour-associated macrophages, was enriched in IFN-γ-driven signalling in NK cell competent mice. In contrast, following the acute depletion of NK cells, the transcriptional profile of tumour-infiltrating myeloid cells showed a marked enrichment in signalling pathways commonly associated with malignant tumour growth. In agreement, tumours that spontaneously regressed in wild-type immunocompetent mice, grew progressively in mice lacking NK cells, or deficient in IFN-γ. Lastly, the use of genetically-modified mice in which NK cells are selectively insensitive to prostaglandin E2 (PGE2) effects, the main factor that drives immune escape in our tumour models (Zelenay et al. *Cell* 2015), established NK cells as the main target of cancer cell-derived PGE2.

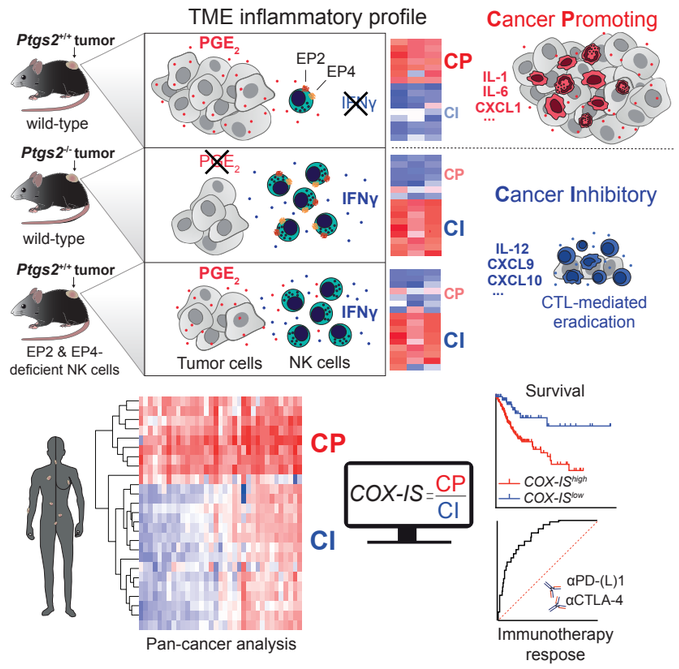

Figure 1.
Graphical abstract of the study published in Immunity by Eduardo Bonavita *et al.* Searching for what makes inflammation 'good' or 'bad', the Cancer Inflammation and Immunity group identified tumour cell-derived PGE2 selectively acting on NK cells as a major regulator of T cell-inflammation in murine models. Based on these findings in murine models, the team developed an approach that improves our ability to predict patient survival and response to immunotherapy in various cancer types.

Mining datasets from The Cancer Genome Atlas, we established that the pro-tumourigenic or anti-tumourigenic inflammatory landscapes driven in murine models by PGE2 or NK cells, respectively, can also be found within human malignancies. Based on this observation, we designed a gene-expression signature that, by integrating cancer-promoting and inhibitory inflammatory mediators in one single indicator, exhibits powerful prognostic utility and predicts response to immunotherapy. This gene signature, termed COX-IS (COX-inflammatory signature), is the subject of a patent application by Cancer Research UK Commercial Partnerships (http://commercial.cancerresearchuk.org/lo-biomarkers-search). The COX-IS showed value for the prediction of responses and survival across multiple datasets from patients that underwent immune checkpoint blockade, independently of the cancer type or immune checkpoint inhibitor drug used. Notably, the COX-IS outperformed other current immune-biomarkers in anticipating outcome even in cancer types in which tumour mutational burden or PD-L1 expression do not, such as in clear cell renal cell carcinoma, the most common type of kidney cancer.

Building on from these in silico analyses, we have embarked on a research project aimed at developing a protocol to measure the COX-IS in patient samples from the Manchester Cancer Research Centre Biobank. Funded by a Medical Research Council *Confidence in Concept* award and in close collaboration with the Cancer Biomarker Centre and various oncologists and pathologists from the NHS Christie Foundation Trust, we are developing a standardised protocol to monitor the COX-IS in patient biopsies using a clinically-compatible platform. Using patient samples from three tumour types, clear cell renal cancer, triple-negative breast cancer and non-small cell lung cancer, we are evaluating the feasibility of an assay to accurately determine the COX-IS in RNA extracted from formalin-fixed paraffin-embedded and validating its prognostic utility. We argue that establishing this protocol is a critical step to prospectively test the COX-IS predictive power and to guide the selection of patients for immune checkpoint blockade therapy alone or in combination with COX-2 inhibition. The latter combinations are currently being planned and evaluated across the globe including in Manchester in two clinical trials led by Dr Anne Armstrong, a Consultant Medical Oncologist from The Christie NHS Foundation Trust. These trials will constitute ideal settings to further examine the value of the COX-IS as relevant immune-biomarker.

In direct connection with the translational and clinical implications of our findings in genetically-modified cancer models, we have made significant progress in various other lines of investigation. This includes expanding our search for putative, common cancer cell-intrinsic immune evasive mechanisms or advancing our understanding of the basis for the synergistic effect of combining immunotherapy with COX-2 inhibitors. Likewise, we have further extended and deepened our examination of tumour human samples through bioinformatic analysis of large publicly available datasets. This analysis provided further compelling evidence for the intimate link between the 'flavour' of inflammation at the tumour bed and patient outcome and response to treatment.

All these studies have in turn opened new research lines aimed at shedding light onto questions such as 'how other regularly used cancer treatments alter the inflammatory response at the tumour site', or 'how they influence other aspects of aggressive tumours, like recurrence or metastatic spread'. Equally, our in-depth profiling of mice and human tumours has identified candidate immune cell subsets with rather unique features. Despite being rare components of the whole leukocyte infiltrate, we speculate that their presence and/or activation status determine the magnitude and quality of the local immune response. As such, we argue these poorly characterised cell-types define the success of cancer treatments that rely on the cancer suppressing function of immunity.

Publications listed on page 65

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The inappropriate proliferation of cancer cells can arise from unchecked cell division, a failure to engage cell death pathways, or simultaneous changes in both. Understanding how the diverse cues are integrated to co-ordinate cell division and death is therefore key to understanding the biology of cancer.

The DNA damaging approaches of chemotherapy and irradiation owe much of their success to the checkpoint pathways that ensure that 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 study the 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 decision point of commitment to the cell cycle in G1 phase that is known as the "Restriction point" (Figure 1), and the transition from G2 into M. Passage through these key transitions is driven by the activation of distinct CDK-Cyclin protein kinase complexes.

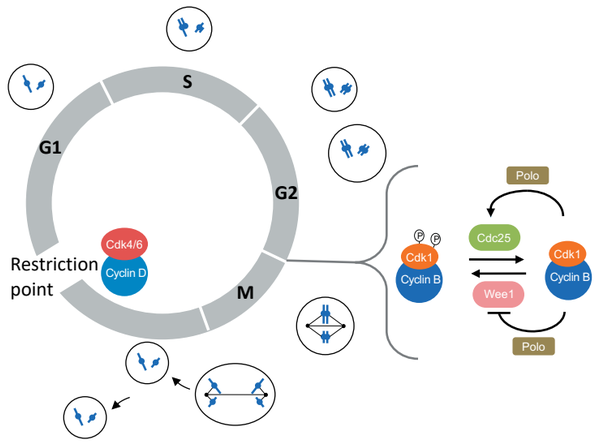


Figure 1.
The human cell cycle with Cdk1-Cyclin B control of the G2/M transition
Cdk4/6-Cyclin D activities drive cells through the restriction point to commit to the cell cycle in G1 phase. DNA replication in S is separated from mitosis by a second gap phase, G2. Cdk1-Cyclin B is held in check in interphase 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 enhance Cdc25 and inhibit Wee1 activities to make this transition a bi-stable switch between two distinct states.

The G2/M transition is a critical safeguard of genome integrity; incomplete DNA replication or DNA damage triggers checkpoint pathways that block entrance into mitosis, to ensure that chromosomes are not segregated when DNA is 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 employs Polo kinase to boost Cdc25 and inhibit Wee1 activities to ensure that mitotic commitment is a rapid and irreversible switch from one state (interphase) into another (division) (Figure 1). The checkpoint pathways that block mitotic commitment when DNA is damaged or replication is incomplete do so because they boost Wee1 and inhibit Cdc25 activities. Once the damage is repaired, or the replication completed, the block to mitotic commitment is relieved and cells divide.

Centrosomes nucleate all the microtubules in the cell to generate the interphase cytoskeleton and the bipolar mitotic spindle that physically segregates the chromosomes. However, centrosomes may organise more than just the microtubule spindle. The initial appearance of active Cdk1-Cyclin B on human centrosomes, before propagation throughout the cell, suggests that this organelle provides a specific microenvironment to trigger the G2/M transition. Our studies of the fission yeast centrosome equivalent, the spindle pole body (SPB), provide

Figure 2.
The fission yeast spindle pole body triggers mitotic commitment
Recruitment of PP1 to Cut12 at the spindle pole body determines the level of Polo kinase activity throughout the cell and so sets the threshold for the feedback loops that convert sparks of Cdk1-Cyclin B activity into a mitotic commitment wave driving cells through division.

Figure 3.
Synchronising cell cycle progression throughout a population with Palbociclib
Cell cycle progression of a population of hTERT-RPE1 cells was arrested at the restriction point by the addition of 150 nM Palbociclib for 24 hours (0 h) before the medium was replaced with fresh, drug free, medium at the start of hourly sampling to monitor DNA content by FACs analysis following propidium iodide staining. While asynchronous populations (asyn) have cells with unreplicated 2N DNA alongside those that have duplicated their genomes (4N) (top plot), 24 h treatment with the Cdk4/6 inhibitor arrests cells at the restriction point with 2N DNA (0h). This DNA is duplicated between 10 and 12 h after drug removal to generate 4N DNA profiles, after which cells divide to return to display 2N profiles 25 hours after release from the arrest.

Figure 2

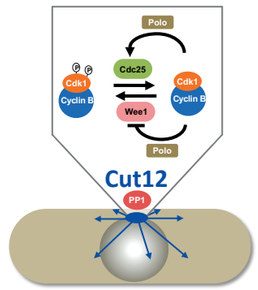
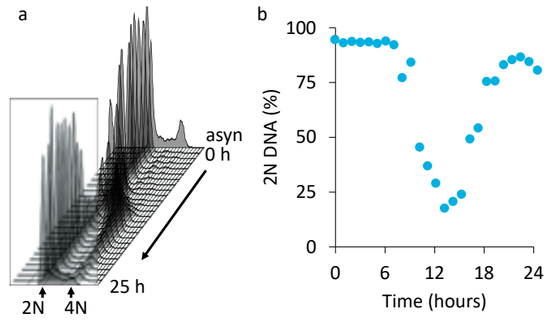


Figure 3



molecular insight into how and why this switch may operate (Figure 2). 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. We are pursuing the hypothesis that Polo activity overcomes the need for Cdc25 because it boosts Polo's ability to inhibit Wee1 to such a degree that it completely silences Wee1. In this scenario, the absence of the kinase that places the phosphate onto Cdk1, removes the need for the phosphatase that normally reverses the missing phosphorylation event.

We are now using these lessons from the fission yeast system to guide the interrogation of analogous controls in human cells. One of our first steps in the study of the human cell division cycle has been to develop a new approach with which to synchronise progression of a population of human cells through the cell division cycle. Synchronisation approaches are very powerful because the biochemical changes that accompany the progression of a synchronised population through the cell division cycle are a direct reflection of the changes that accompany division within each individual cell within that population.

The most widely applied approach to synchronisation is the "double thymidine block", developed in the 1960s, in which inhibition of DNA replication blocks commitment to mitosis for the equivalent of one doubling time, before release of the block supports the synchronised completion of DNA replication and cell division. There are challenges with this approach as the degree of synchrony is not great, so that two consecutive block/release cycles are required to generate good synchrony and the extended DNA replication arrest generates significant DNA damage. Importantly for our goal, inappropriate accumulation of proteins that are regulated by cell cycle dependent destruction may obscure the normal controls that govern the G2/M transition.

Commitment to the cell division cycle at the restriction point is driven by Cdk4-Cyclin D and Cdk6-Cyclin D (Figure 1). Remarkably, although these kinases play a key role in regulating commitment to the cell cycle when active, mice from which the gene encoding either kinase has been deleted, still develop normally. Thus, normal tissues are often able to use an alternative Cdk-Cyclin complex, Cdk2-Cyclin E, to regulate mitotic commitment when Cdk4/6 are inhibited. This flexibility contrasts with tumours that generally rely upon the inappropriate activation of Cdk4/6 to proliferate. This disparity prompted the development of the Cdk4/6 inhibitors palbociclib, abemaciclib and ribociclib that are proving highly effective in the clinic. We therefore asked whether transient arrest at the natural pause point in the cell cycle, the restriction point, would support cell cycle synchronisation. Our characterisation of a non-transformed h-TERT-RPE1 cell line was extremely encouraging. Release from a single round of cell cycle arrest was sufficient to generate good levels of synchronisation with minimal DNA damage (Figure 3). One useful feature of this approach is that synchrony was preserved when the duration of the arrest was extended from one to three days. This means that it will be possible to remove, replace or induce molecules during the arrest while cells are out of the cycle, before releasing the cell cycle arrest to assess the impact of the manipulation on cell cycle progression. As a third of a panel of transformed lines exhibited useable levels of synchronisation, we are hopeful that this approach may be widely adopted as it supports the study of aspects of transcription, DNA replication, repair and chromatin biology that are obscured by the damage incurred during a thymidine block. We now exploit this synchronisation approach to refine our studies of G2/M control in human cell lines.

Publications listed on page 66

www.cruk.manchester.ac.uk/Our-Research/Cell-Plasticity-and-Epigenetics

CELL PLASTICITY & EPIGENETICS

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Collective evidence from all areas of biology currently suggests that multiple levels of biological information, distinct from that encoded into DNA, act in concert to determine observable phenotypes. In striking contrast to genetic information, non-genetically encoded information can take many forms, such as highly dynamic chromatin states, alternative gene expression profiles, among a multitude of others. The ability of a single genotype to produce many discrete and sometimes dramatically different phenotypes clearly plays a key role during cancer development and resistance to therapy, as the aforementioned highly dynamic information re-arrangements grant cancer cells with the ability to rapidly adapt when challenged by environmental fluctuations. 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 biological cues, such as oncogene mediated transformation, experimentally induced epithelial-to-mesenchymal transition and/or the challenge with therapeutic agents. Ultimately, we would like to address the intricacies of genetic and non-genetic networks underlying cancer evolutionary models to build a framework where both core biological information frameworks are considered non-negligible and equally fundamental.

Research Highlights

It is becoming increasingly apparent that individual cells within a clonal population show significant heterogeneity, particularly in their response to biological cues. Strikingly, the observed phenotypic heterogeneity is present despite there being no genetic variability in the population, thus leading us to hypothesise that the observed differences rely instead on non-genetic information. Interestingly, recent technical developments from our lab allowed us to trace in a multimodal manner, and at single-cell resolution, the gene expression programmes of clonal populations of cells and their evolution in time throughout its lineage. By tracing hundreds of individual cells and their progeny, we have uncovered that $HRAS^{G12V}$ transformed cells display 10-11 metastable states that are readily inherited in a robust manner. Strikingly, cells found in each of the identified states are continuously interconverting following defined trajectories, suggesting that molecular barriers and/or active mechanisms constrain the plasticity displayed by the overall population. Moreover, by following protein markers that identify a subset of states, we were able to demonstrate that the observed gene expression profiles correlate with the phenotypic variability shown in their capacity to grow in 3D cultures or in the resistance that these cells display when faced with therapeutic agents.

Given our data, our initial hypotheses contemplated the possibility that the existence of gene expression states would be restricted to the transformed state or, conversely, that the transformed state may display enhanced plasticity. Notably, we observed that non-cancerous cells and cells undergoing an epithelial to mesenchymal transition also display dynamic gene expression plasticity in the form of numerous metastable states, thus suggesting that this feature is a universal attribute of mammalian cells rather than simply an oddity of the malignant state. Importantly, our recent data supports that the observed phenotypic states, defined as gene expression programmes, are not linked to active transcriptional events. Indeed, experiments in which RNA PolII transcription was blocked showed no impact in the re-establishment or inheritance of each state, suggesting that the information encoding the total number of observed states and their identity is hidden elsewhere other than the transcriptional machinery.

In light of these results, we reasoned that the answer to our queries can only be extracted from biological systems by means of multimodal single cell analysis. Therefore, we endeavour to build an experimental and bioinformatics framework of single cell tools that will provide an in depth understanding of the underpinnings of cell plasticity during cancer onset and progression. To this end, we have built a toolkit of single cell experimental tools to explore several aspects of genome control and architecture such as histone modifications, DNA-RNA interactions, transcription factor binding, among other features. We expect that in the near future our results will shed light on the molecular details underpinning cell plasticity in cancer models and beyond.

The main goals of our lab in the upcoming years are to identify the key molecular players and mechanisms orchestrating the establishment, maintenance and propagation of stable phenotypic states within isogenic populations of cells and investigate their role in the emergence of resistance to therapeutic agents. In that regard, we have already accumulated strong evidence suggesting that the biological response to a given biological cue (TRAIL induced apoptosis or growth on 3D matrices) is restricted to individual states and does not necessarily imply cooperativity in the overall response of the population as a whole. Indeed, whilst certain states are fully primed to grow aggressively in 3D settings, others fail to grow altogether, suggesting that in clonal populations phenotypic diversity is encoded at different levels other than the "genetic" and highlights the importance of non-genetic mechanisms of adaptation to environmental cues.

Collectively, our research will develop our understanding of cellular heterogeneity and plasticity, whilst expanding the concept of non-genetic information in order to address the intricacies of genetic and non-genetic networks underlying current cancer evolutionary models.

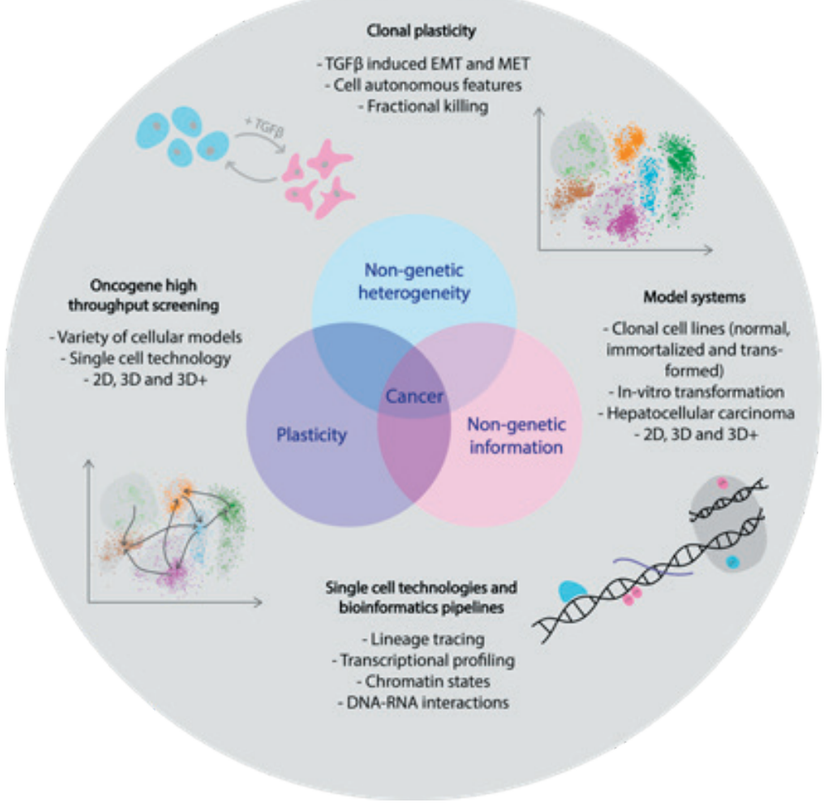

Figure 1.
Figure depicts the current research axis of our lab and their interconnection. Understanding the interrelation between non-genetic information, cell plasticity and non-genetic heterogeneity is at the core of our research and we are convinced that the intersection of those concepts will provide fundamental understanding of cancer biology in the near future. Several aspects related to model systems and technology development from our lab are also depicted.

CELL SIGNALLING

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The main focus of the Cell Signalling group is the identification of therapeutic targets in lung cancer. Lung cancer is the most commonly diagnosed cancer and the most common cause of cancer related deaths worldwide, with non-small cell lung cancer (NSCLC) being the major histological subtype. Despite growing knowledge of the molecular mechanisms driving lung cancer, the overall 5-year survival rate of lung cancer patients remains less than 15%. The most common histological subtype of NSCLC is adenocarcinoma, of which the most common driver mutation is KRAS. Presently, no approved targeted therapies exist for KRAS mutant NSCLC. Current drug development efforts focus on KRAS itself or its downstream targets. One such downstream target under investigation in our laboratory is the small GTPase RAC1.

RAC is a member of the RHO-like family of GTPases and cycles between a GDP- and a GTP-bound state. When GTP-bound, it interacts with various effector molecules that regulate several cellular processes, including proliferation and migration. Multiple mechanisms control RAC activity, including control of nucleotide binding and hydrolysis by guanine nucleotide exchange factors (GEFs) and GTPase Activating Proteins (GAPs) respectively, regulation of subcellular localisation, and modulation of RAC protein levels (reviewed in Porter et al. *Small GTPases* 2017). Several studies using recombinant RAC and RAC GEF mice have shown that RAC is required for the formation and growth of tumours. In particular, it has been shown that RAC is required for the formation of KRAS-induced lung tumours in mice. Moreover, the RAC GEF TIAM1 has been shown to be required for the formation and growth of HRAS-induced skin tumours (Malliri et al. *Nature* 2002). Interestingly, TIAM1 and its homologue STEF/TIAM2, both contain a RAS-binding domain and are considered effectors of RAS.

Although RAC seems always to promote tumour formation and growth, it may promote or antagonise malignant progression. There are cases where deletion of RAC GEFs leads to more invasive tumours and there are reports suggesting that reduced RAC activity levels correlate with more aggressive tumours (Porter et al. *Small GTPases* 2017). In vitro data have shown that activation of RAC may lead to opposing migratory phenotypes, raising the possibility that targeting RAC in a clinical setting could exacerbate tumour progression. For these reasons, it is important to identify the factors that influence whether RAC activation will promote or inhibit migration. One such factor that we have identified is the GEFs that activate RAC. RAC GEFs are often multi-domain proteins with many binding partners. We showed that TIAM1 and another RAC GEF, P-REX1, have diametrically opposite effects on cell migration through RAC: TIAM1 promotes cell-cell adhesions to oppose cell migration, while P-REX1 promotes migration. They perform these contrasting roles in cell migration by selecting RAC effectors (Marei et al. *Nat Comm* 2016). Over-expression of specific GEFs, which occurs commonly in many cancers, can therefore drive different oncogenic signalling pathways.

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

Even though, as mentioned above, TIAM1 knockout mice were resistant to the formation of RAS-induced tumours, the few tumours which did form were more aggressive (Malliri et al. *Nature* 2002). This highlights two distinct roles for TIAM1/RAC signalling: stimulating tumour formation and suppressing malignant progression. Early work on TIAM1's role in suppressing migration and invasion focused on its role in strengthening cell-cell junctions, associated with anti-migratory effects (Malliri et al. *J Biol Chem* 2004). It was also shown that cell-cell adhesion disassembly and scattering of epithelial cells requires depletion of TIAM1 from cell-cell adhesions. Specifically, we found that during dispersal of epithelial cells either via activation of the oncoprotein SRC or via hepatocyte growth factor treatment, TIAM1 present at cell-cell junctions is degraded (Woodcock et al. *Mol Cell* 2009; Vaughn et al. *Cell Rep* 2015).

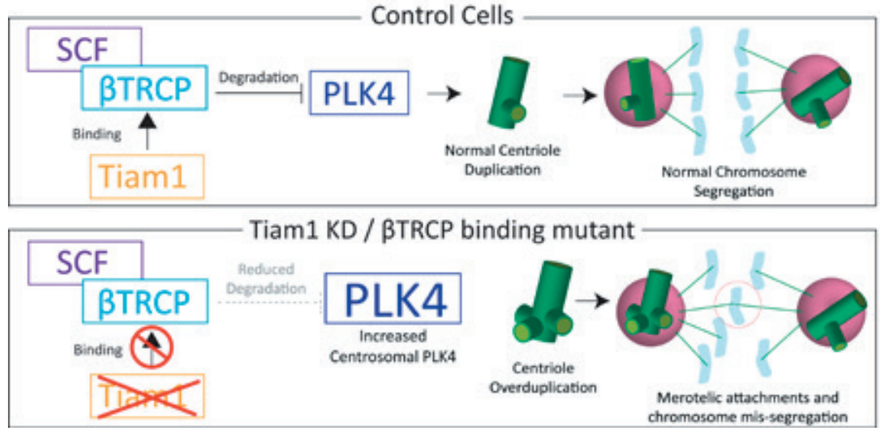

Figure 1.
Model of how the TIAM1-βTRCP interaction affects PLK4 protein levels, centriole duplication and chromosome segregation.

But besides these studies showing that TIAM1 inhibits migration by promoting cell-cell adhesion, we have also identified another mechanism by which TIAM1 hinders migration. We demonstrated that TIAM1 localises in the nucleus of several colorectal cancer cell lines and that nuclear TIAM1 inhibits their migration via suppressing the interaction of the transcriptional co-activator TAZ with its cognate transcription factor TEAD. Suppression of this interaction by TIAM1 inhibited expression of TAZ/YAP target genes implicated in epithelial-mesenchymal transition and cell migration. Consistent with these in vitro data, we showed by staining a microarray of colorectal cancer biopsies that TIAM1 localised to the nuclei of tumour cells. Moreover, nuclear staining intensity significantly decreased with advancing Dukes stage and patients with high nuclear TIAM1 had significantly better survival than those with low nuclear TIAM1 (Diamantopoulou et al. *Cancer Cell* 2017).

More recently, we have uncovered a new role for TIAM1 in regulating the duplication of centrioles, structures at the core of centrosomes found at the poles of the mitotic spindle. Cells normally duplicate centrioles only once per cell cycle. Centriole overduplication is common in many cancers, however, promoting aneuploidy but also increasing invasiveness of tumour cells. We found that TIAM1 localises to centrosomes and showed that TIAM1 depletion leads to an increase in centrosomal PLK4, the master regulator of centriole duplication, and to centriole overduplication. Ultimately, TIAM1 depletion leads to lagging chromosomes at anaphase and aneuploidy, potential drivers of malignant progression. The effects of TIAM1 depletion on centrosomal PLK4 levels and centriole overduplication can be rescued by re-expression of both wild-type TIAM1 and catalytically inactive (GEF*) TIAM1, but not by TIAM1 mutants unable to bind to the F-box protein βTRCP, implying that TIAM1 regulates PLK4 levels through promoting its βTRCP-mediated degradation independently of RAC1 activation.

Role of RAC and its regulators in promoting cell migration

As mentioned above, the RAC activator P-REX1 promotes cell migration and invasion. Apart from P-REX1, we have previously shown that activation of RAC by STEF/TIAM2 promotes cell migration (Rooney et al. *EMBO* Rep 2010).

More recent data from our lab also demonstrated a role for perinuclear RAC activity in the regulation of a subset of the actin cytoskeleton known as the perinuclear actin cap – thick actin bundles which 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 (Woroniuk et al. *Nat Comm* 2018).

We are currently investigating the role of TIAM1 and STEF/TIAM2 in lung cancer formation and progression using in vitro and in vivo models.

Publications listed on page 66

DRUG DISCOVERY

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Jennifer Miles[5]
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The last year has been heavily impacted by the coronavirus pandemic, leading to closure of our chemistry and biology laboratories for a significant period of time. Despite this disruption, research has continued and the laboratory work resumed smoothly in a COVID-safe manner.

This research led to exciting new in vivo biological data with our lysyl oxidase inhibitors and our suicide gene therapy approach. We have fostered further collaborations with our colleagues in the CRUK Manchester Institute, and working closely with Iain Hagan and Claus Jorgensen we have advanced drug discovery efforts against two exciting new targets involved in cancer cell cycle and in tumour stroma regulation. New interactions were also initiated with Caroline Dive on biomarkers and with Stephen Taylor on PARG. The integrated medicinal chemistry, computational chemistry, biochemistry, cellular biology and in vivo biology work of the DDU scientists led to the discovery and biological assessment of new potent and selective inhibitors of several cancer targets. Across our projects, wherever possible, we work to ensure that our DDU projects are integrated with Caroline Dive's biomarker discovery programme, so that all nominated targets have selection and predictive biomarkers. On all our late-stage projects, we are also delighted to work closely with the excellent committed clinicians at the Christie NHS Foundation Trust.

Kinase inhibitors can achieve excellent responses in cancer patients when matched to specific driver mutations. However, cancer cell signalling is highly dysregulated, so cancer cells can recruit parallel and/or feedback pathways to bypass single kinase inhibitors and cause acquired or intrinsic resistance. In KRAS-driven tumours such as pancreatic ductal adenocarcinoma (PDAC), colorectal carcinoma (CRC) and non-small cell lung cancer (NSCLC), proliferation requires both RAF and SRC signalling. Using this knowledge, we discovered CCT3833, a panRAF inhibitor that – uniquely for this class of drug – also inhibits SRC. CCT3833 is effective in *KRAS*-driven pre-clinical models of PDAC, CRC and NSCLC, with biomarker evidence of effective inhibition of both pathways. CCT3833 is an orally bioavailable, well-tolerated panRAF/SRC inhibitor, developed in collaboration with Richard Marais and is designed to treat mutant RAS cancers and mutant BRAF melanoma resistant to current RAF pathway inhibitors. In a Phase I clinical trial (NCT02437227) at the Christie and Royal Marsden NHS Foundation Trusts, CCT3833 significantly prolonged progression-free survival in a patient with a KRAS-driven spindle cell sarcoma who did not respond to the third-generation kinase inhibitor ponatinib (which targets SRC, but not RAF), and therefore had limited treatment options. The preclinical and clinical research data for CCT3833 were recently published in *Annals of Oncology.*

RET is an oncogenic kinase driver activated in multiple cancers including non-small cell lung cancer and medullary thyroid cancer. Our RET pre-clinical candidate development programme was licenced to Stemline Therapeutics in 2019. Following acquisition of Stemline by Menarini in 2020, IND-enabling studies are ongoing and we anticipate that our RET inhibitor SL-1001 will enter clinical studies in 2021.

Tumour metastases are responsible for >90% cancer-associated deaths. Lysyl oxidases (LOX/LOXL1-4) are enzymes that increase the tensile strength of the extracellular matrix (ECM) by crosslinking collagens and elastin, promoting primary tumour growth and metastatic spread in breast, PDAC and CRC. Uncontrolled ECM deposition is also the key characteristic of fibrosis, pathological wound scarring that can be caused by infections including COVID19. Notably, LOX plays a key role in establishing fibrotic lesions, so LOX is both an anti-cancer and an anti-fibrotic therapeutic target. In collaboration with Richard Marais, we have discovered LOX and LOX family inhibitors with good pharmacokinetic properties and have demonstrated therapeutic activity in different primary tumour models of CRC, PDAC and breast cancer as well as anti-metastatic efficacy in preclinical models. Exciting preliminary data indicate that our LOX inhibitors also demonstrate biomarker inhibition in models

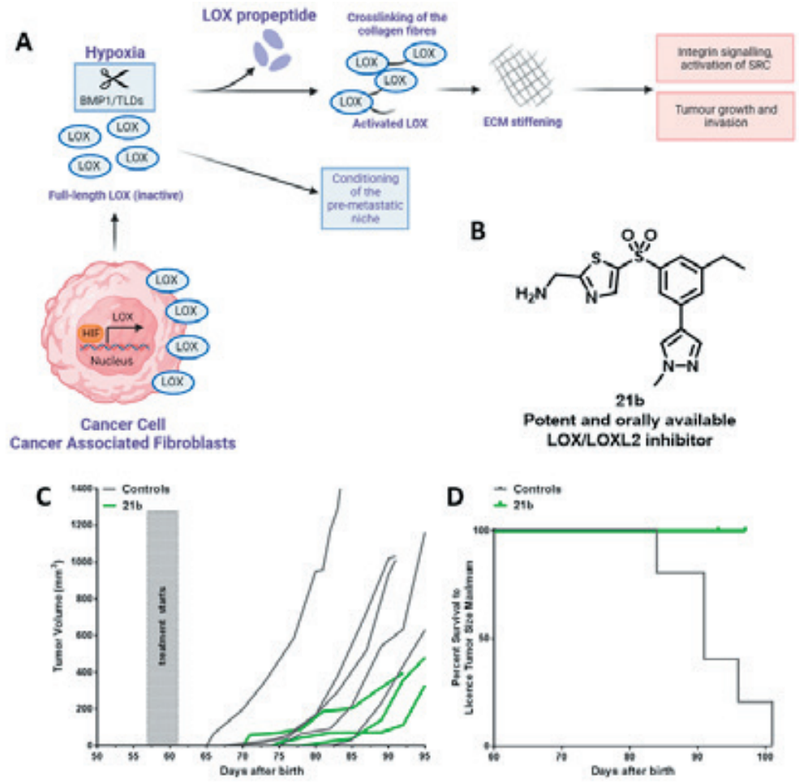

Figure 1.
A. Role of LOX in cancer growth and metastasis. B. LOX and LOX family inhibitor discovered by DDU. C. Anti-tumour efficacy of our LOX inhibitor in a model of breast cancer. D. Improved survival upon treatment with LOX inhibitor.

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[3]Joint with Cancer Biomarker Centre
[4]Joint with Stephen Taylor (Division of Cancer Sciences, University of Manchester)
[5]Joint with Richard Bayliss (University of Leeds)
[6]Joint with Systems Oncology

of lung fibrosis. 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 have identified a target that is highly overexpressed in CSCs in a number of cancers and has an important role in their stemness potential and drug resistance. We have discovered potent, selective inhibitors of our CSC target, with excellent physicochemical properties, in vitro ADME and safety profile and in vivo pharmacokinetics. We are collaborating with Richard Marais and breast cancer expert Robert Clarke (Division of Cancer Sciences, University of Manchester), in elucidating the biology of this target, and we will progress our most advanced inhibitors to in vivo evaluation imminently.

We engineered novel vaccinia virus vectors for our suicide gene therapy programme, in collaboration with Richard Marais and John Bell (Ontario University), to target tumours selectively and produce a unique bacterial enzyme (carboxypepidase G2, CPG2) in the cancer cells, which is able to convert subsequently administered prodrugs to cytotoxic drugs locally thus attacking the tumour. We have shown highly selective tumour targeting with our engineered oncolytic virus, and also specific and persistent CPG2 transgene expression in tumours from a single systemic administration. When we combine this oncolytic viral targeting with the prodrug, we elicit tumour generated cytotoxic drug leading to long term regressions in vivo in human tumour xenograft models. This approach

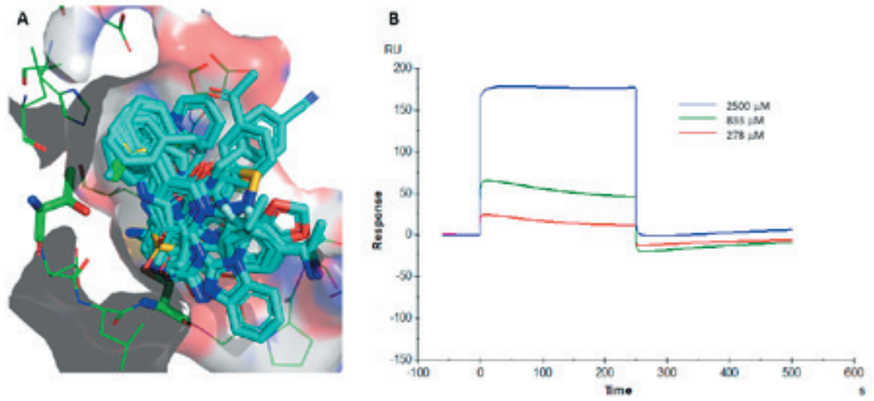


Figure 2.
A. X-ray crystallography of multiple fragments binding within the active site of our cell cycle target. B. Biophysical validation of binding between a fragment and protein.

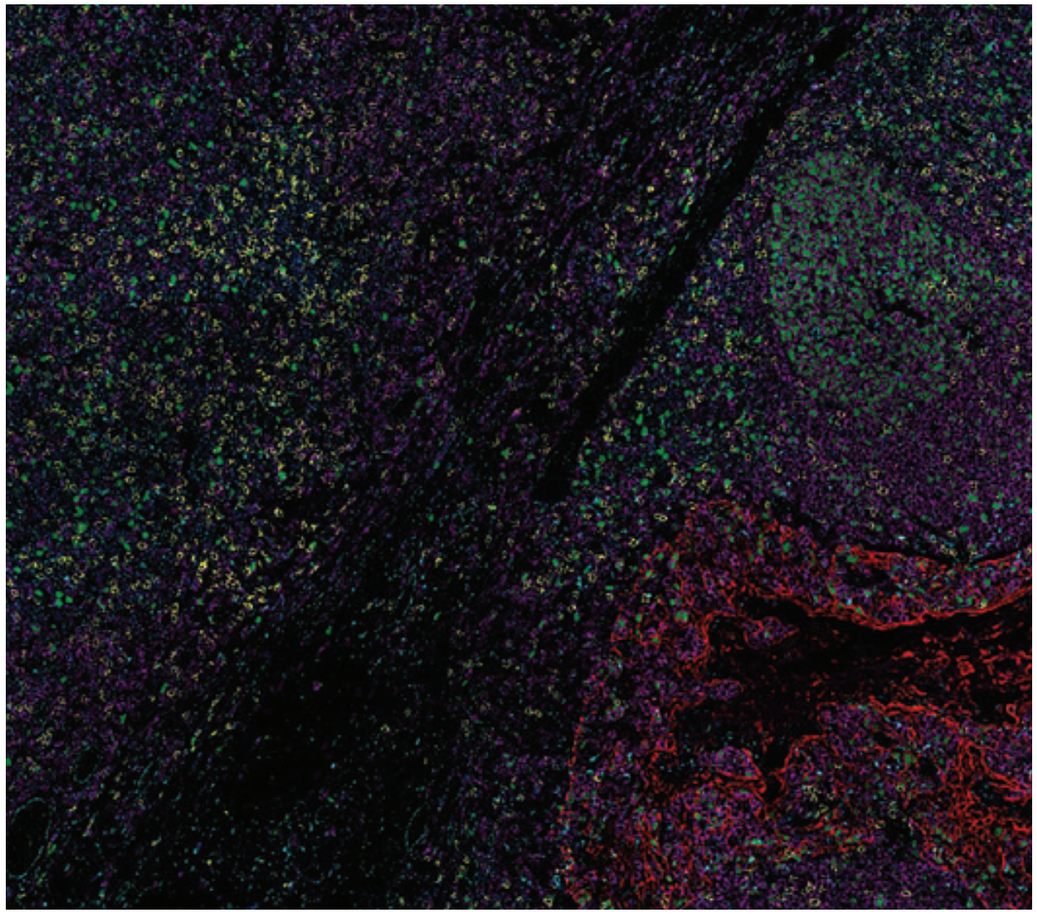
is likely to be effective in multiple solid tumour types including CRC, head and neck, and lung.

Our partnership with IDEAYA Bioscience on our Poly(ADP-ribose) glycohydrolase (PARG) programme continues to progress. In this collaboration, we have optimised the properties of PARG inhibitors to obtain compounds suitable for demonstration of in vivo activity. Biological studies to better understand disease linkage are advancing and through a collaboration with Stephen Taylor at The University of Manchester, in vivo efficacy studies at the DDU will be progressing shortly

We are working closely with scientists from the CRUK MI to apply their cutting-edge biology research and exciting new targets to discovery of new anticancer drugs. Due to genomic instability, tumours tolerate less DNA damage from chemotherapy and radiation than normal tissues. DNA damage triggers activation of proteins that stop the cell cycle until damage is repaired. Abolition of this blockage could lead to progression of cancerous cells to division with damaged chromosomes, ultimately resulting in mitotic catastrophe and cell death. In collaboration with Iain Hagan, we initiated drug discovery on a key cell cycle controlling target that will enhance the sensitivity of tumour cells to current DNA-damaging therapeutics while sparing normal cells. Our medicinal chemistry has been greatly supported by crystallography and fragment screening through a fruitful collaboration with Richard Bayliss at the University of Leeds, leading to detailed mapping of the active site of our target (Figure 2) and the discovery of potent selective inhibitors. This programme is progressing through lead identification.

Tumour microenvironment plays an essential but complex role in tumour progression in highly desmoplastic cancers, in particular in PDAC. In collaboration with Claus Jørgensen, we have started a drug discovery programme against a target discovered in his lab that plays a key role in cancer cell mediated fibroblast activation. Inhibition can lead to the deactivation and normalisation of these fibroblasts, reshaping the tumour stroma and rendering the cancer vulnerable to chemotherapeutic and immunotherapeutic agents. We have discovered very potent inhibitors of this stromal target and are excited to assess their biological effect in Dr Jørgensen's complex pancreatic cancer models. This project synergises very well with our LOX programme that also targets tumour stroma by a different mechanism.

Publications listed on page 66



CODEX presents the ability to sequentially label up to 40 fluorescent markers on tissue sections and immobilised cells. This is a new technology that was implemented within the Institute so to allow complex relationships to be examined. As a feasibility study this image of a human tonsil tissue section was generated which was labelled with CD3e (blue), CD107a (teal), CD20 (purple), ki67 (green) and panCK (red).

Image supplied by Steve Bagley (Visualisation, Irradiation & Analysis)

LEUKAEMIA BIOLOGY

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[1]Left in 2020

2020 was a uniquely challenging year for our team with a COVID-mandated complete closure of the laboratory for nearly 15 weeks from mid-March, followed by significantly restricted working for six weeks thereafter. All of us were happy to resume more normal laboratory working in a COVID-secure environment as autumn ensued and, despite the disruption, we published four significant studies in the calendar year, and submitted a further two for peer review. Two of the published studies were described in detail in our annual report from 2019 (Williams et al., 2020, *Journal of Clinical Investigation*; and Deb, Wingelhofer et al., 2020, *Leukemia*) and will not be discussed further here. However, I am delighted to report that Mark Williams in a single day in late January 2020 was awarded his PhD, the Institute's Dexter Prize and The University of Manchester's Presidential Fellowship. Not to be outdone, Bettina Wingelhofer was shortly thereafter awarded a John Goldman Fellowship from Leukaemia UK. Another piece of great news was the award of a PhD to one of our MCRC/CRUK clinical fellows, John Chadwick, as the year drew to a close.

A core goal of our group is to identify candidate therapeutic targets and disease mechanisms in acute myeloid leukaemia (AML) and bring them through to the clinic for evaluation and future patient benefit. This year marked something of a milestone for our work on a histone demethylase enzyme called LSD1. When I established the lab in 2008, I suggested to one of my first PhD students William Harris that he should investigate whether a number of genes I had identified in microarray analysis of a mouse model of human acute myeloid leukaemia (AML) (Somervaille et al., 2009; *Cell Stem Cell*) might regulate leukaemia stem cell activity. He discovered that when he knocked down LSD1, the mouse leukaemia cells began to differentiate and undergo apoptosis, whereas normal haematopoietic stem and progenitor cells were relatively unaffected. In an extension of the work, he found similar results in human AML cell lines and also leukaemia cells from patients being treated at The Christie NHS Foundation Trust. Working with the Institute's Drug Discovery Unit, we synthesised small molecule inhibitors of LSD1 which had recently been patented by a Spanish Biotechnology company called Oryzon Genomics. We discovered that these tranylcypromine-derivative inhibitors of LSD1 induced differentiation of leukaemia cells in vitro and in vivo. These preclinical studies, published in *Cancer Cell* in 2012, set the scene for an early phase clinical trial of LSD1 inhibition as a novel therapeutic approach in myeloid leukaemia.

We established a direct collaboration with Tamara Maes and Carlos Buena at Oryzon Genomics to evaluate their advanced lead compound ORY-1001, a novel, highly potent and selective inhibitor of LSD1, in the treatment of human relapsed or refractory AML, and prepared an early phase clinical trial protocol. The trial was funded through a commercial-academic (Barcelona/Manchester) European Union funding scheme called EUROSTARS. With centres open in the UK, Spain and France, we recruited the first UK patient to the study in 2014 at The Christie. The trial completed its recruitment in late 2016, with follow up and evaluation of the results over the subsequent two years. In 2020 we were delighted to be able to publish the results of the study in the *Journal of Clinical Oncology*. ORY-1001, now called iadademstat, shows a good safety profile and was well-tolerated. Adverse events were as expected and included myelosuppression, infections, asthenia, mucositis, and diarrhea.

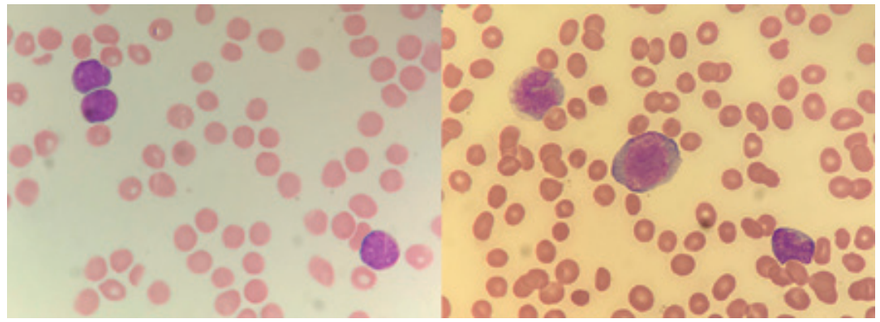

Figure 1.
Iadademstat induces differentiation of leukaemia blasts in vivo.
Left panel: blast cells in peripheral blood with few features of differentiation in a patient with AML before the start of treatment with oral iadademstat. Right panel: 21 days after the start of treatment blood blasts now show features of morphological differentiation along the monocytic lineage.

Importantly, tantalising signs of efficacy were observed including reductions in blood and bone marrow blast percentages, and induction of blast cell differentiation which were observed, in particular, in patients with MLL translocations. Indeed, two patients developed a differentiation syndrome, a particularly effusive type of differentiation which is both encouraging from a drug efficacy point of view, but also requiring additional medical intervention. One complete remission with incomplete count recovery was also observed. This turns out to be a noteworthy example of concordance between pre-clinical laboratory and subsequent clinical trial findings. These encouraging data have led on to a phase II trial in Spain, called ALICE, of iadademstat in combination with azacitidine as first line therapy in older patients with acute myeloid leukaemia. The first patient was recruited to this protocol just over two years ago, and there are really exciting signs of preliminary efficacy with this combinatorial regimen.

We also reported in *BMC Cancer*, with Mark Williams as lead author, and in collaboration with Stephen Taylor's group in the Division of Cancer Sciences at The University of Manchester, a novel technique called targeted nanopore long-read sequencing. We developed this to identify translocations of the drug efflux pump gene *ABCB1* in cancer. Resistance to chemotherapy is the most common cause of treatment failure in AML and the drug efflux pump ABCB1 is a critical mediator. ABCB1 pumps standard-of-care chemotherapy drugs such as daunorubicin and cytarabine out of cancer and leukaemia cells to facilitate chemoresistance. Its high-level expression is one of the strongest predictors for treatment failure in AML. In view of recent studies which had identified promoter translocations as common drivers of high ABCB1 expression in recurrent, chemotherapy-treated high-grade serous ovarian cancer and breast cancer, a question arose as to whether a similar mechanism might be operational in AML. The promoter translocations found in ovarian cancer place *ABCB1* under the control of a strong promoter while leaving its open reading frame intact.

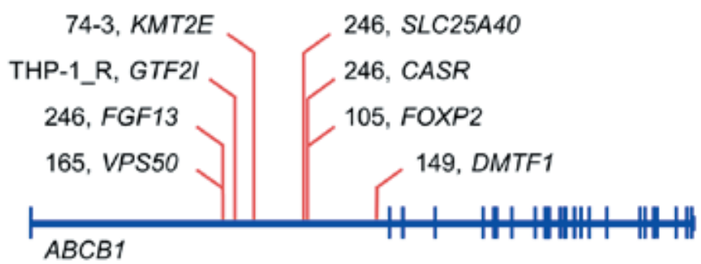

Figure 2.
***ABCB1* promoter translocations identified by long read nanopore sequencing.**
ABCB1 intron 1 breakpoints identified in high grade serous ovarian carcinoma samples & THP-1 AML cells are indicated, together with the partner gene.

Previous techniques had been quite laborious, but with the development of nanopore sequencing technology, which allows for much longer sequencing reads spanning DNA translocation breakpoints, Mark hypothesised that it could be adapted to the question in hand. Using both an in vitro model leukaemia cell system as well as primary patient ovarian cancer samples, he was able to demonstrate that his sequencing approach could readily identify ABCB1 structural variants in cancer; in doing so he identified a number of novel promoter translocation variants. In contrast, activating *ABCB1* promoter translocations were not identified in any of the *ABCB1*^{high} primary patient AML samples from patients with relapsed disease. In combination with Mark's work on ABCB1 published in *The Journal of Clinical Investigation* this year, this tells us that the high cellular expression of *ABCB1* in human AML arises through epigenetic mechanisms such as enhancer activation and other forms of endogenous regulation rather than through genetic rearrangement.

As we look forward to better times in 2021, we continue working hard in a number of our core areas which we will highlight in future reports. In particular, we have new and exciting data on the mechanism by which FOXC1 confers a differentiation block in AML, and also the mechanism by which its expression is inappropriately up regulated in a lineage-inappropriate manner. Our studies, translational and clinical, and in collaboration with CellCentric, studying the mechanism by which a first-in-class histone acetyltransferase inhibitor affects leukaemia cell function, are also yielding intriguing results.

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MOLECULAR ONCOLOGY

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We study melanoma biology and strive to use the knowledge we generate for public health benefit. Our ties to the clinic ensure close interactions between discovery scientists and clinicians. Those ties also provide access to patient tissues, allowing us to develop real-time monitoring techniques for patient responses to treatment. In parallel, we use cell and animal models to characterise the different subtypes of melanoma to determine how melanoma develops and identify new treatment strategies for patients. Thus, we aim understand melanoma to address the clinical needs of patients, and to provide public health information and education on how melanoma can be prevented.

Targeted and immunotherapies have transformed melanoma care over the last decade, driving impressive improvements in survival for some patients. However, metastatic melanoma patients still face significant mortality risk due to acquired or intrinsic resistance to targeted therapies and our limited understanding of who will respond to the various immunotherapies now approved for this disease. Additionally, immunotherapies carry a high risk of severe toxicity, in many cases without clinical benefit. To use these drugs more effectively, we therefore need better understanding of their impact on cancer cells, the tumour microenvironment and the patient. This is particularly important for patients with metastatic brain disease, as exemplified by our in-depth analysis of an individual patient.

The patient presented with acral melanoma and, despite a very low mutation burden, initially responded well to immunotherapy, but then developed an immunotherapy-resistant brain lesion. The tumour harboured a *BRAF* mutation, so the patient received a BRAF inhibitor and initially responded well to this second-line therapy, but once more relapsed with a brain lesion. We retrieved sequential samples from the patient to characterise the molecular changes that took place during therapy. This revealed that immunotherapy resistance coincided with distinct changes in tumour cytokines, and targeted therapy resistance coincided with brain-specific cell signalling adaptions (Figure 1). This case study shows that acral melanoma patients should not be excluded from immunotherapy. The results show that tumour cell interactions with the tumour microenvironment are complex and heterogenous, that changes in the microenvironment can mediate resistance to therapy, and that understanding resistance at a molecular level could guide salvage therapy for individual patients. As described in the report from the Drug Discovery Unit, we continue to develop new agents for individualised cancer patient care.

Epidemiological studies in humans link melanoma to exposure to ultraviolet radiation (UVR) from sunlight and sunbeds. Complimentary experimental studies in animals confirm that UVR causes melanoma. UVR is therefore an accepted environmental carcinogen for cutaneous melanoma, the most common form of melanoma which arises on the skin. However, the molecular details of how UVR causes melanoma remain unclear, so we continue to investigate this question using mouse models where we express melanoma oncogenes in mouse melanocytes (the pigment producing cells that become melanoma) and then expose the animals to UVR. In the last year, we reported that over 50% of our mouse *BRAF/* UVR-driven melanomas acquire mutations in a gene called *Map3k1*. The mutations target the protein's so-called RING domain, which is known to suppresses signalling downstream of BRAF. We genetically depleted *Map3k1* in mouse melanocytes and confirmed that this made *BRAF/*UVR-driven tumours more prevalent. Moreover, we showed that low expression of *MAP3K1* is associated with reduced survival in melanoma patients. Our study suggests that MAP3K1 antagonises melanomagenesis and so its loss is permissive of melanoma progression,

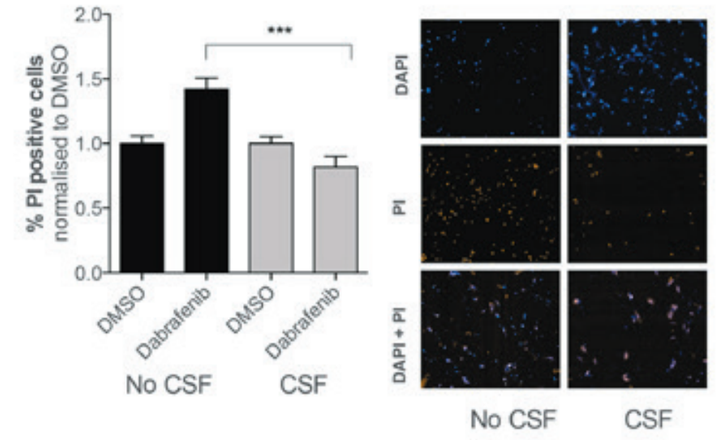

Figure 1.
Cerebrospinal fluid overcomes dabrafenib-mediated inhibition of cell growth.
Left panel.
Graph showing propidium iodide (PI)-positive cells as a relative measure of cell death. In the absence of cerebrospinal fluid (No CSF), the BRAF inhibitor dabrafenib induces a 1.5-fold increase in death of cells derived from an acral melanoma that harboured a *BRAF* mutation (black columns). However, the addition of CSF blocked the dabrafenib-induced cell death (grey columns).
Right panel. Images from dabrafenib-treated cells without (No CSF) or with CSF stained with the nuclear dye DAPI (blue) and the dye propidium iodide (PI, orange; indicates dying cells).

demonstrating how powerful and relevant mouse models can provide insight into human melanoma biology.

Although UVR can cause melanoma, it is not the only cause. In particular, rare melanomas, including those on the eyes (uveal and conjunctival melanoma) and mucosa (the moist membranes lining the bodies cavities, including the gastrointestinal and genitourinary tracts and, again, the conjunctiva) often arise on sun-protected sites and do not show evidence of UVR having played a role in their aetiology. Specifically, whereas most common cutaneous melanoma present genomes with a high burden of UVR-induced mutations, rare melanoma genomes present large-scale genome gains and losses that are not thought to be UVR-driven. Conversely, in 2018, we reported that about 15% of common cutaneous melanomas do not appear to be UVR-driven. These data suggest that melanomas can develop along distinct aetiologies and critically, patients with rare melanomas still have limited treatment options. To investigate if UVR can drive mucosal melanoma, we performed whole genome sequencing on 10 conjunctival melanomas because the conjunctiva is a UVR-exposed mucosal membrane. We compared our conjunctival melanoma genomes to those of mucosal melanomas from other sites and to common cutaneous melanoma genomes. Our conjunctival melanoma genomes presented the large gains and losses common to mucosal melanoma but additionally, 9 of the 10 samples also presented high mutation burdens (Figure 2), large numbers of UVR-signature mutations, large numbers of mutations in RAS-RAF signalling pathway genes, and large numbers of mutations in other genes frequently mutated in cutaneous melanoma.

Thus, conjunctival melanoma genomes present features common to both mucosal and cutaneous melanomas. We posit therefore that conjunctival melanomas are driven by two distinct pathways. One pathway causes large structural genomic changes and appears to be driven by the mucosal microenvironment. Layered over that is a UVR component that causes mutations in the genes that drive common cutaneous melanoma. Our study has important implications for public health. First, it provides a molecular explanation to underpin prevention campaigns highlighting the importance of protecting our eyes from UVR. Second, it suggests that conjunctival melanoma patients could benefit from targeted and immunotherapies that are approved for cutaneous melanoma. However, since UVR cannot be deemed to have played a role in individual conjunctival melanomas based solely on the site of the eye on which the tumour arose, we propose an assay that can determine UVR involvement based on sequencing of 10 genes, and which therefore is simpler and cheaper than whole genome sequencing.

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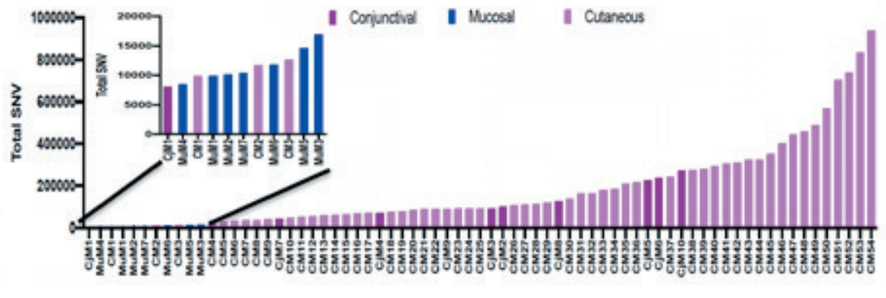

Figure 2.
Mutation burden in conjunctival melanoma is comparable to that in common cutaneous melanoma.
We compared the number of mutations (Total SNV) in 10 conjunctival melanomas (CjM1-10, maroon) to those in 7 non-conjunctival mucosal melanomas (MuM1-7, blue) and 54 common cutaneous melanomas (CM1-54, pink). Note that 9 of the conjunctival melanomas segregate with the common cutaneous melanomas and away from the non-conjunctival mucosal melanomas, but 1 conjunctival melanoma (CjM1) segregated with the non-conjunctival mucosal melanomas.

PROSTATE ONCOBIOLOGY

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[1]Joined in 2020
[2]Left in 2020

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 to understand the onset of aggressive prostate tumours at their early, curable stages by identifying and characterising cells-of-PCa-origin to develop better therapies.

Research highlights

The initiation of aggressive tumours involves the existence of so-called cancer-initiating cells, with the ability to self-renew, to survive anti-tumour 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 for cell of origin and their reprogrammed niche for disease progression.

Our studies identified a subpopulation of luminal progenitors characterised by LY6D expression and intrinsic castration resistance, as cell of origin of aggressive prostate cancer. This marker allows us for the first time to isolate and functionally characterise castration-resistant cells, even before the clinical appearance of castration resistant prostate cancer (CRPC). Importantly, these studies provide evidence that human homologous LY6D can serve as a prognostic maker for advanced prostate cancer, which allows to further stratify risk profiles for PCa patients and to tailor more specific therapies. Our follow up analysis focused on characterising the transcriptional programme driving castration resistance in these prostate progenitors. We have identified a novel signalling pathway activated uniquely in the LY6D progenitors in early tumour stages. Functional characterisation by organoid-culture and in situ lineage-tracing analysis in mouse models have further shown the potential of targeting this pathway to abrogate tumour cells growth. To pursue further studies on the dependencies of LY6D$^+$ cells, we have developed new mouse models enabling the study of PCa progenitor cells in vivo.

LY6D is a gene with as of 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.

Furthermore, our collaboration with Georges Lacaud's lab contributed to the identification of a distinct subset of castration-resistant luminal cells from early stages of prostate embryonic development: RUNX1 expressing luminal cells localise at the base of the prostate in adult animals, and they do not contribute to rebuilding the prostate after castration. Our studies provide new insights into the lineage relationship of the prostate epithelium, and highlight the presence of co-existent progenitors with unique location within the prostate, suggesting a role of progenitor niches for prostate cancer initiation and treatment response.

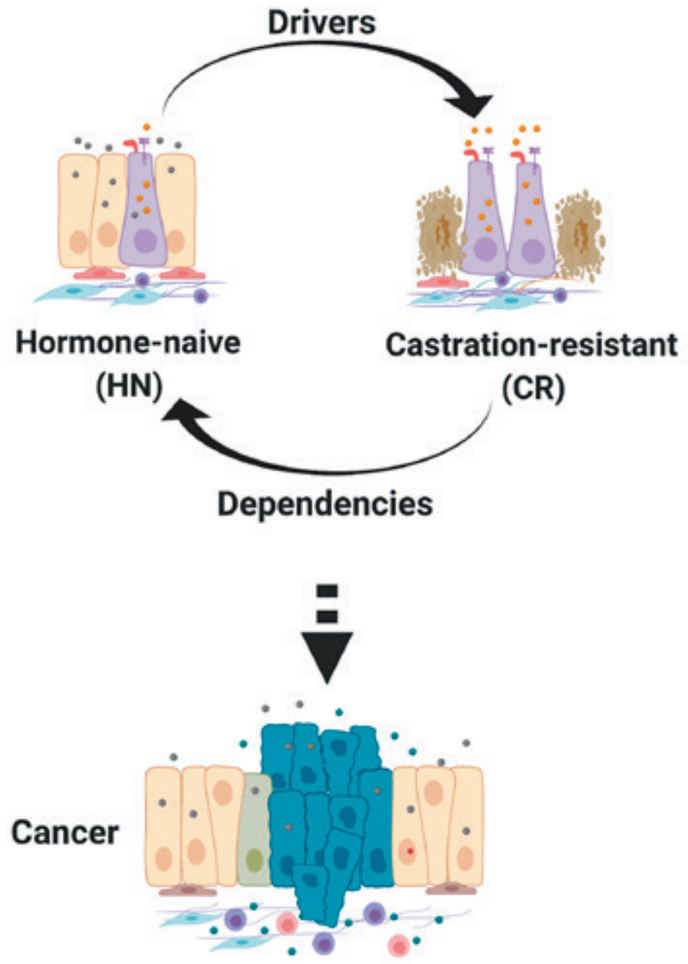

Figure 1.
The inherent resistance to androgen-deprivation therapy (aka. castration-resistance) in the prostate epithelium favours the onset of aggressive prostate cancer.

In a complimentary study, we are characterising localised high-risk prostate cancer patients (due to the current pandemic and its effects on elective diagnostic procedures, sampling has been limited). We have focused on further optimisation of our novel sampling method, established in Manchester in the previous year. We have built up a retrospective collection of specimens from patients with multisite lesions, for which matching clinical parameters are available. For the prospective collection of samples, patients are selected based on team discussions with clinicians and pathologists, and strictly considering study inclusion criteria. Our results so far show the importance of implementing a more accurate sampling strategy in PCa to address the challenges imposed by the clinical heterogeneity, in particular the spatial distribution of the tumours. Following up on our multifocal PCa studies, we have broadened our collaboration with the clinical oncology team and established a new clinical study for the collection of clinical samples before and after androgen-deprivation treatment. Currently the Prostate Oncology team is collecting samples and performing single-cell RNAseq and multiplex histology analysis to understand the role of cellular distribution of PCa cells for disease onset.

Our studies thereby advance patient stratification and establish a pipeline to develop novel therapeutics. Further studies are warranted in the coming year to determine the cellular composition of tumours during progression and their association with mpMRI visibility. In addition, the precise role of LY6D in prostate epithelial heterogeneity, PCa initiation and progression to adenocarcinoma will be assessed to validate its utility as a novel prognostic marker for patient stratification. Ultimately, we aim to develop therapies to specifically target CR LY6D$^+$ cells as a novel approach to prevent the development of CR-PCa.

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SKIN CANCER AND AGEING

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[1]Joined in 2020

Cancer is more common in elderly patients and aged patients are the primary subset of the population who is diagnosed with melanoma. Additionally, most patients who die with melanoma are older than 60, and melanoma specific death is increasing in the elderly. Older patients are more likely to suffer from comorbidities, other cancers and other melanocytic and non-melanocytic skin cancers. The overall survival for patients with aggressive early stage primaries (Stage IIB-IIC) at five years is 60% and 45%, respectively, despite being localised to the skin and non-metastatic at diagnosis. Age leads to an additional, unexplained decrease in survival with increasing decades of life, with an almost 20% decrease from ages 60 to 69, to ages greater than 80 years. This decrease in survival is despite adjusting for the main prognostic factors, which shows that age is the strongest independent adverse prognostic factor together with tumour thickness.

This year the we have progressed our understanding of the changes in aged skin that promote melanoma and the biology of aggressive disease affecting the elderly. Tim Budden has worked on how the long-term effects of UV damage to the dermis modifiy how melanoma cells behave. We found that as the skin ages, there is a net loss of collagen in the dermis due to UV light exposure, which destroys collagen. Once melanoma arises, at the early localised tumour stages, the loss of collagen in the dermis delays melanoma cell invasion through the dermis. However, in some cases fibroblasts can synthesise new collagen that is visible at the invasive front of the tumour, and this strongly correlates to poor outcome.

We are also working on how other components of the aged skin may affect melanoma. We are specifically looking at how subcutaneous adipocytes, which are the main cellular component of the deepest layer of the skin, vary their function and lipid content with age, and how this then affects melanoma progression. We find that specific components that are secreted by the adipocyte decrease with age, contributing differently to melanoma cell behaviour.

Aged people who are at high risk of melanoma skin cancer are also at high risk of non-melanoma skin cancers, which are the most common cancers to affect humans. Within the non-melanocytic skin tumours, cutaneous squamous cell carcinoma is the most common cancer to affect immunosuppressed people, and is much more prevalent and aggressive in men. The assumption is that the sex bias is due to behaviour, as men are more exposed to sunlight than women. However, we have discovered and published that male and female animals exposed to the same dose of carcinogen have a different course of disease. Males have more aggressive variants and more frequently metastasize, whereas females strongly upregulate immune responses and recruit CD8+ T cells to the skin to delay cancer progression. We find that immunocompetent women also have less aggressive disease than men; however immunosuppressed women have more aggressive squamous cell carcinoma, similar to men. We are keen to continue investigating how the different immune response to carcinogens by sex influences cancer onset, progression and therapy responses.

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

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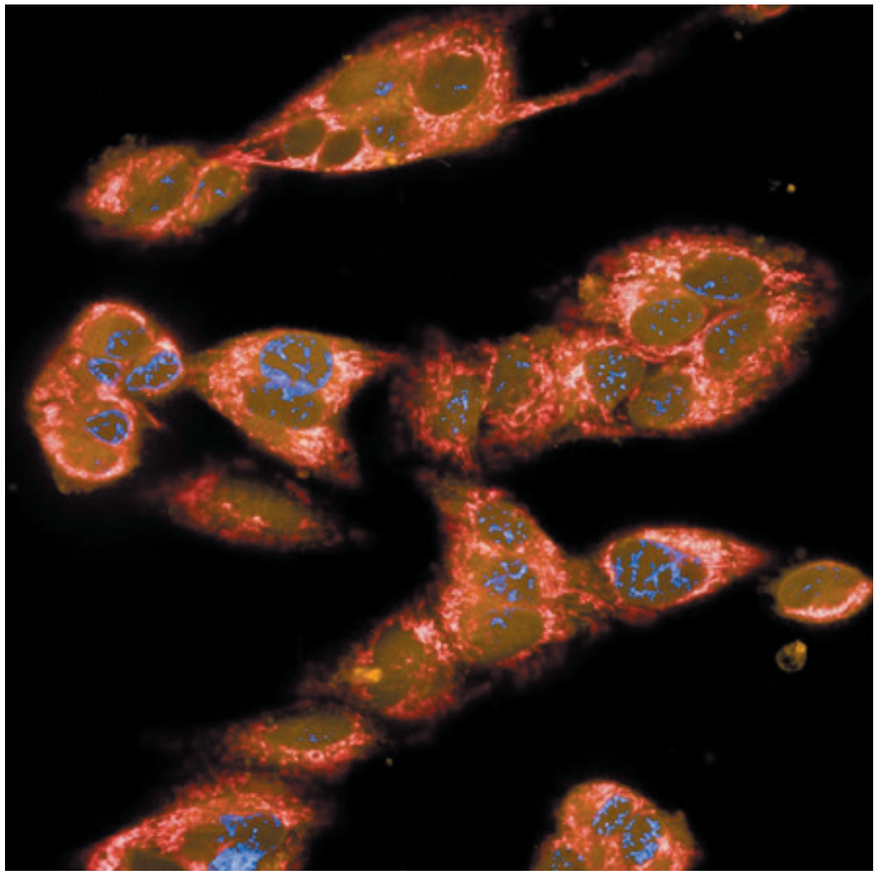

Figure 1.
Image of mitochondria activated in a melanoma cell.
Melanoma cells have been exposed to adipocyte secretome and stained for nucleus (blue), active mitochondria (red) and cytoplasm (orange) at 63x using the Opera Phenix.

Supplied by Shilpa Gurung (Skin Cancer and Ageing).

STEM CELL BIOLOGY

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[1]Funded by Blood Cancer UK
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Genes encoding the AML1/RUNX1 transcription factor and its cofactor CBFβ are frequently rearranged or mutated in human leukaemia, such as acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL). Consistent with its implication in leukaemia, RUNX1 has also been shown to be critical for haematopoietic development. Similarly, the transcriptional co-activator MOZ is involved in independent myeloid chromosomal translocations fusing *MOZ* to the partner genes *CBP*, *P300* or *TIF2* in human leukaemia.

Our group studies RUNX1 and MOZ's function in haematopoietic development and maintenance to better understand how alterations of these functions might lead to leukaemogenesis. Besides these transcription factors and transcriptional activators, long noncoding RNAs (lncRNAs) have also emerged as important regulators of gene expression. In this context, we more recently started the investigation of lncRNAs essential for leukaemia.

Investigation of long noncoding RNAs in acute myeloid leukaemia

The haematopoietic system homeostasis is primarily controlled by transcription factors and epigenetic factors (writers, erasers or readers) that regulate self-renewal and differentiation. Alterations of transcription factors and epigenetic factors are critical molecular events leading to leukaemia and other malignancies. Recent studies have revealed that long noncoding RNAs (lncRNAs) could also be implicated in regulating gene expression. LncRNAs represent a large fraction of the human genome (Figure 1). Through acting as tethers for the epigenetic machinery, or participating in chromatin looping, lncRNAs participate in gene regulation. Therefore, lncRNAs could be essential factors in Acute Myeloid Leukaemia (AML) development and represent a potential novel therapeutic avenue. However, our understanding of lncRNAs, and their functions in AML, is currently limited.

To identify lncRNAs important in the proliferation of leukaemic cells, we employed a CRISPR interference (CRISPRi) screening approach to induce sequence-specific repression of lncRNA expression. We selected the THP-1 cell line, a human AML cell line with an MLL-AF9 translocation, to express dCas9-KRAB (dead Cas9 fused to Krüppel associated box (KRAB) domain) and repress transcription of targeted lncRNAs. In addition to the THP-1 cell line, we also generated other MLL-rearranged AML cell lines (MOLM-13 and MV4-11) and the non-MLL-rearranged cell line Kasumi1, giving us a panel of leukaemic cell lines stably expressing dCas9-KRAB. These cell lines were validated for CRISPRi performance using previously published control single guide RNAs (sgRNAs). The dCas9-KRAB expressing THP-1 cell line was transduced with a library of sgRNAs targeting 3,882 lncRNAs (10 guides for each lncRNA). Transduced cells were selected and then grown in culture for 20 cell doublings. Of the 3882 lncRNAs screened, a total of 19 were identified as significantly influencing cell proliferation. Within these 19 hits was the mir17HG, which encodes for the miR17-92a-1 cluster. The microRNAs within this cluster have been identified as essential factors in MLL-rearranged leukaemia, validating our screen's performance in identifying lncRNAs important in leukaemic maintenance. We selected the top 6 hits and confirmed their screen phenotype individually, experimentally using an internally controlled growth assay. Of these six hits, five including Lnc79 (Figure 2A) showed a significant change in proliferation in the THP-1 cell line. We also evaluated their effect on the other leukaemic cell lines we developed. Three of these lncRNAs showed a significant change in proliferation in all cell lines tested. Two of our hits showed near-complete localisation to the chromatin and nucleoplasm while the others were present in all three cellular fractions. To prioritise clinically important lncRNAs, we also examined the expression of our hits in available patient datasets (TCGA, GTEx, Blueprint consortium).

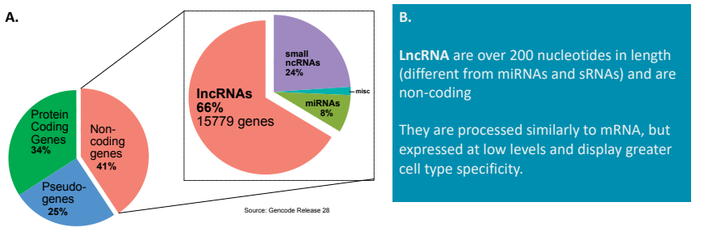

Figure 1.
LncRNAs represent an important fraction of the human genome.
A. Representation of LncRNA compared to other RNAs. B. General features of LncRNAs.

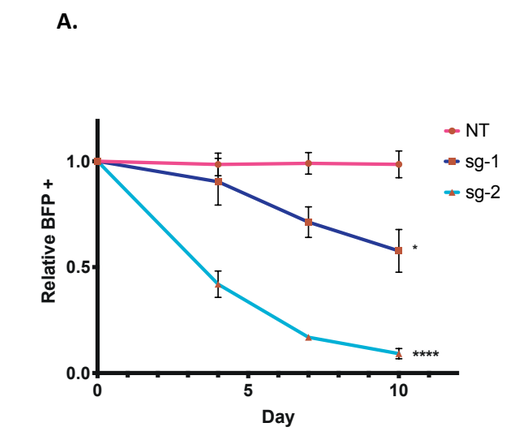

Figure 2.
Lnc79 is upregulated in AMLs.
A. Downregulation of Lnc79 by CRISPRi approach impairs the proliferation of THP-1 human AML cells (representation of cells expressing Lnc79 sgRNA is quantified thought detection of blue fluorescent protein (BFP)). B. Lnc79 expression is higher in AML patient samples (LAML, red) than in healthy samples (green). C. Higher expression of Lnc79 is associated with poorer overall survival.

We observed that our top hit, lnc79, is upregulated in AML patient samples when compared to normal bone marrow (Figure 2B). AML patients show the highest median expression, across all samples, including both cancer and healthy samples.

Furthermore, higher expression of lnc79 correlates with lower overall survival in patients (Figure 2C). Compared to healthy haematopoietic stem and progenitor cells, comparison of this lncRNA expression in AML patients shows a similar expression of lnc79 in AML patients and the haematopoietic stem cells and higher expression than in the other progenitor cell populations.
We are currently investigating whether knockdown of these LncRNA influences differentiation, apoptosis or proliferation and determining changes in gene expression by RNAseq. We are also investigating the critical molecular mechanisms they regulate. Finally, we are validating these hits further, and potentially identifying new ones, by performing a similar full CRISPRi screen in vivo.
For the final in vitro validation, we are now using antisense oligonucleotides (ASO) complementary to our target lncRNA to specifically downregulate our lncRNA transcript without modifying adjacent chromatin as with CRISPRi. To start identifying their molecular functions, we are currently determining which proteins are associated with our lncRNA, using RNA affinity purification followed by mass spectrometry (RAP-MS). Any interactions of interest will be confirmed by RNA immunoprecipitation (RIP) qPCR. Knowledge of the lncRNAs protein partners will help direct further experiments, such as analysing changes in epigenetic modifications upon lncRNA knockdown, using chromatin immunoprecipitation (ChIP).

In addition to identifying associated proteins, we are identifying regions in the genome where our lncRNA binds, using chromatin isolation by RNA precipitation (ChIRP) as well as measuring the changes in gene expression upon lncRNA knockdown using RNA-seq. By overlapping these datasets, we will be able to identify the direct targets of our lncRNA and understand how the lncRNA affects their expression. Altogether these studies will identify LncRNAs critical for leukaemia maintenance and define the molecular mechanisms regulated by them.

Besides this approach, we have identified several lncRNAs differentially expressed in murine models of MLL rearranged, and MOZ rearranged AMLs. Using progenitor cell expression in mouse and humans has allowed us to identify lncRNAs that display conserved expression patterns in both species and may play a conserved role in human/mouse normal and malignant haematopoiesis. We will next functionally evaluate the role of these LncRNAs in the maintenance of leukaemia.
The successful identification, and characterisation, of lncRNAs essential in AML could offer possible new avenues for therapeutics. Use of antisense oligonucleotides has been developed as a potential clinical approach for reducing gene expression. Antisense therapy targeting a lncRNA essential for AML might therefore improve patient survival.

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SYSTEMS ONCOLOGY

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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%. Thus, while PDA is only the 11th most common occurring cancer in the UK, it is currently the 4th largest contributor to cancer related deaths. 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 interstitial pressure, which results in decreased therapeutic delivery. Moreover, the microenvironment contains an abundant fibroblast and myeloid cell infiltrate, which reduces immune surveillance and confers resistance to therapy.

Genetic heterogeneity of tumours is associated with aggressive behaviour and rapid onset 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. To interrogate such interactions, we recently studied the interactions between single cell-derived clones of pancreatic cancer cells and stromal fibroblasts. Curiously, we observed that individual tumour cell clones instigate diverse stromal behaviour. Whereas some tumour cell clones induce fibroblasts to increase extracellular matrix deposition and remodelling, other clones induce the expression of immune regulatory genes in the fibroblasts, suggesting that diverse tumour cell populations drive distinct stromal phenotypes. Notably, the signalling response of tumour cells to stromal interactions was context dependent; whereas Ras and MAPK signalling were equalised by fibroblast interactions, activation of the AKT signalling pathway was further diversified across the tumour cell clones. Importantly, differences in expression of receptors across tumour cells creates cell-autonomous differences in their response to stromal reciprocal signals. 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 unanticipated effects 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. The 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

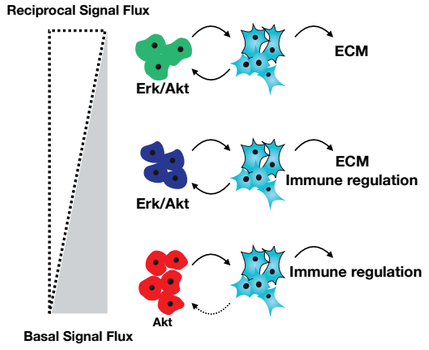

Figure 1.
Model of heterotypic interactions across heterogeneous tumour cells and stromal fibroblasts. Clonal tumour cell populations drive diverse phenotypic responses in stromal fibroblasts, which in turn differentially engage tumour cell signalling to normalise output.

pathways regulating tumour cell resistance. In parallel, we are identifying targetable pathways in the tumour stroma and optimising their use for combination therapy.

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 collaboration with clinicians 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). In an effort to further improve these patient-derived organoid models we have optimised use of fully synthetic hydrogel scaffolds. Importantly, these matrices can be tuned to replicate the rigidity of human tumours and also enable inclusion of stromal cells, to mimic the complex microenvironment observed in PDA. By modelling the specific cellular and biophysical constraints of the patient tumour, these models may be used to define optimal combination of therapies for tumour and stromal cells.

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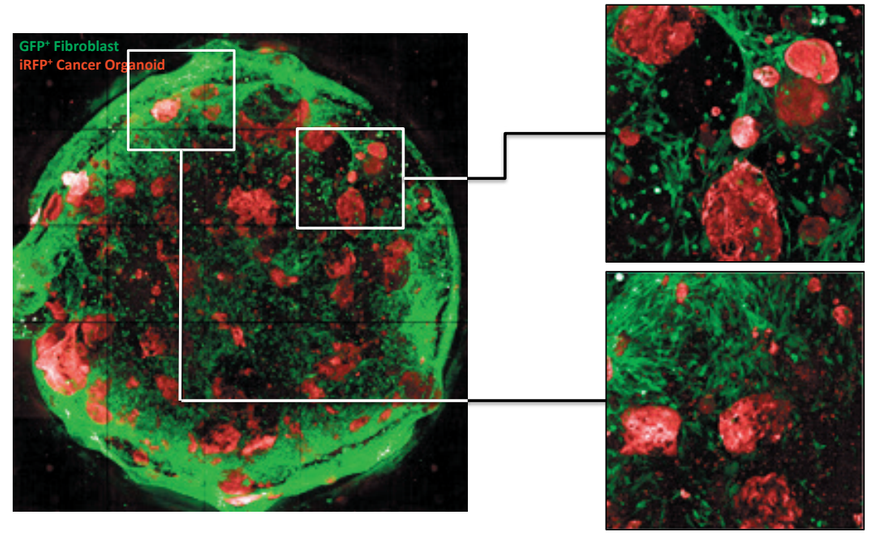

Figure 2.
Pancreatic cancer organoids and stromal cells.

TRANSCRIPTIONAL NETWORKS IN LUNG CANCER

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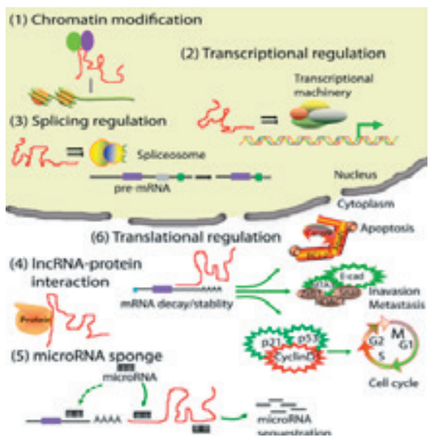
A KRAS-responsive lncRNA controls microRNA processing

The KRAS oncogene regulates gene expression through multiple molecular mechanisms and its dysregulation culminates in tumour initiation and progression. Wild-type *KRAS (KRASWT)* amplification has been shown to be a secondary means of KRAS activation in cancer and associated with poor survival. Nevertheless, the precise role of *KRASWT* overexpression in lung cancer progression is largely unexplored.

LncRNAs are non-protein coding transcripts longer than 200 nucleotides that have been considered for many years as spurious transcriptional noise. It is now clear, that they play a major role in cancer initiation and progression. LncRNAs affect gene expression through interaction with DNA, RNA or protein (Figure 1). They can mediate epigenetic changes by recruiting chromatin remodelling complexes to specific genomic loci. A large number of lncRNAs act as scaffolds or allosteric activators/inhibitors through direct interaction with proteins or protein complexes. Other lncRNAs have been found to act as competing endogenous RNAs (ceRNAs) by binding miRNAs ("sponging") and reducing their inhibitory effect on gene targets (Figure 1) (5). Thus, lncRNAs, via their ability to modulate all these processes are major players in tumorigenesis, impacting not only cell proliferation and survival but also cell motility, invasion and metastasis. We identified and characterised a KRAS-responsive lncRNA, *KIMAT1* (ENSG00000228709) and showed that it correlates with KRAS levels both in cell lines and in lung cancer specimens (Figure 2).

Figure 1.
LncRNAs regulate gene expression by multiple mechanisms. They guide chromatin-remodeling complexes to the correct chromosomal locations controlling the balance between transcriptionally active euchromatin and silent heterochromatin (1). LncRNAs control the transcription of target genes by inhibiting or facilitating the recruitment of RNA pol II and transcription factors to gene promoters (2) and regulate alternative splicing of pre-mRNAs (3). Their flexible scaffold nature enables lncRNAs to bind proteins affecting their function (4). LncRNAs can compete for miRNA binding, thereby influencing the expression of miRNA target gene expression (5). They affect the stability and translation of mRNA by base pairing with mRNA molecules (6).

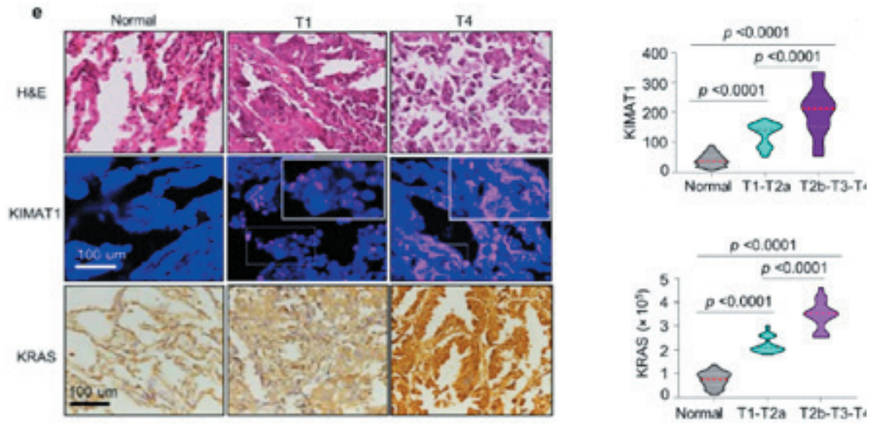


Mechanistically, *KIMAT1* is a MYC target and drives lung tumorigenesis by promoting the processing of oncogenic microRNAs (miRNAs) through DHX9 and NPM1 stabilisation while halting the biogenesis of miRNAs with tumour suppressor function via MYC-dependent silencing of p21, a novel component of the Microprocessor Complex (MC). *KIMAT1* knockdown suppresses not only KRAS expression but also KRAS downstream signalling, thereby arresting lung cancer growth in vitro and in vivo. Therefore, we have uncovered a role for *KIMAT1* in maintaining a positive feedback loop that sustains KRAS signaling during lung cancer progression and provides a proof of principle that interfering with *KIMAT1* could be a strategy to hamper KRAS-induced tumorigenesis (Figure 3), Shi et al. *Nature Communications*, in press.

ALK-EML4 lung tumours

In non-small cell lung cancer (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 2.
(Left) Representative images of KIMAT1 and KRAS in matched FFPE normal-tumour adenocarcinoma lesions at stage 1 (T1) and stage 4 (T4) stained with DAPI (blue), KIMAT1 (pink) (smFISH) or KRAS (brown). Scale bar, 100uM. (Right) Quantification of KIMAT1 and KRAS expression by smFISH in tumour and normal lung tissues. Spots were counted using the online JAVA StarSearch.



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 (Paliouras et al. *EMBO Mol Med* 2020).

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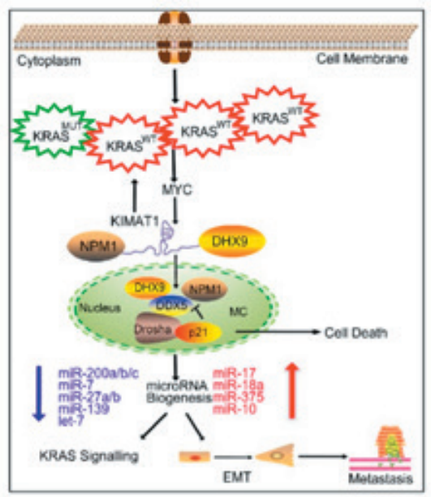
Figure 3.
Schematics depicting *KIMAT1's* mechanism of action.
KRAS amplification activates *KIMAT1* via MYC-mediated transcription. *KIMAT1* binds to and stabilises DHX9 and NPM1, which promote the processing of oncogenic miRNAs sustaining the KRAS signalling in a positive feedback loop. p21 antagonises the binding between DDX5 and DHX9 and between DDX5 and NPM1 fostering the processing of tumour suppressor miRNAs which halt KRAS signalling and EMT.



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

TRANSLATIONAL ONCOGENOMICS

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[1]Joined in 2020
[2]Left in 2020

Almost 50,000 men living in the UK will be diagnosed with prostate cancer every year, and since the 1990s the trend has been one of increasing incidence. This is explained by the adoption of a simple PSA blood test to diagnose disease that hitherto would have gone undetected.

In the era of widespread PSA testing, the majority of men now present with disease that is localised to the prostate and is potentially curable. However, localised prostate cancer represents a broad spectrum of disease, and in this scenario the challenge faced by clinicians is to accurately assess the risks for each patient so that the best treatment option may be offered. Low risk, indolent tumours with favourable pathology may never progress, and in such cases active surveillance may be suitable. Intermediate risk cancers that remain confined to the prostate are potentially curable with a localised treatment.

On the other hand, high risk cancers have an increased probability of producing incurable metastases which may go undetected at the point of diagnosis, and here aggressive treatment may be required. 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 this regard, there is growing interest in the potential of genes involved in maintenance of genome fidelity to form the basis of new biomarkers. In addition, tumour-associated hypoxia represents an endogenous stress that is associated with genome instability, and understanding the mechanistic basis for this correlation is a key aim of our research.

Over recent years there has been a growing appreciation of the role of DNA repair genes in the biology of prostate cancer. In depth analyses of the prostate cancer genome have shown that somatic mutations in DNA repair genes are relatively frequent and are more common in incurable, castrate-resistant disease (mCRPC) than in primary cancers. Concordantly, it has been shown that men carrying germline mutations in such genes are at a higher risk of developing prostate cancers that progress to become metastatic. Widespread chromosomal instability is a hallmark of end-stage prostate cancer, and it seems likely that impaired DNA repair gene function caused by mutation could drive genome instability, contributing to an aggressive disease phenotype. Since the presence of DNA repair gene mutations is correlated with the incidence and severity of prostate cancer, there is a compelling argument for the incorporation of genomic testing into new classifiers that better stratify patients in terms of risk and also guide their treatment. This approach holds much promise. Indeed, it is already known that tumours harbouring BRCA2 mutations for example, are more likely to respond to PARP inhibitors or to Cisplatin compared with non-BRCA mutated tumours. On the other hand tumours deficient in mismatch repair genes, in addition to showing great sensitivity to androgen blockade, may also be targetable by immune checkpoint inhibitors. Furthermore, enhanced screening of patients harbouring germline mutations could lead to early diagnosis of cancers suitable for surgery. Understandably, there is great excitement in the potential clinical utility of genetic testing in prostate cancer and the current challenge is to consolidate prior studies with further evidence, both to support biomarker development, and to provide a mechanistic framework for clinical observations. Our aim is to comprehensively characterise clinical material sampled from patients with germline DNA repair defects, and to develop matching pre-clinical models that allow experimental approaches.

Several DNA repair genes have been implicated in prostate cancer, but their germline mutation occurs relatively infrequently, and the risk of cancer development varies gene by gene. However, the most frequently observed DNA repair defect is mutation of the breast cancer susceptibility-2 (BRCA2) gene which confers an 8-9 fold increased risk of developing prostate cancer with subsequent failure during standard of care treatment. Overall, the cancer-specific survival is 5-8 years post diagnosis for 50% of

Figure 1

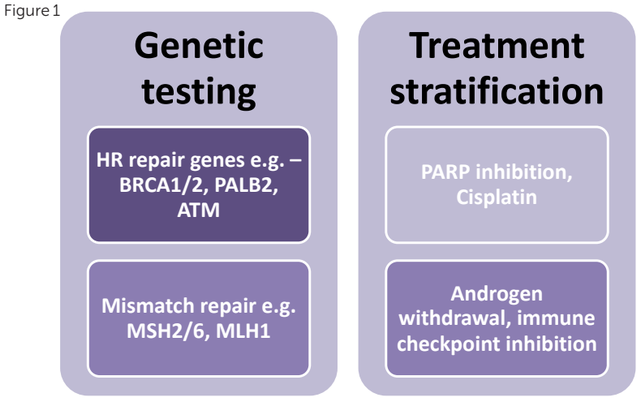

Figure 2

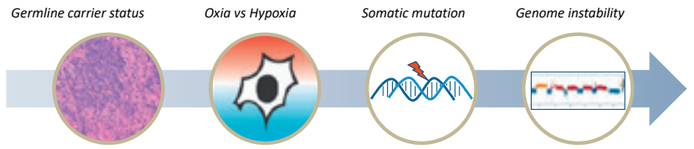

Figure 1.
An outline of patient management utilising information gained by genetic testing. The presence of germline mutations involving DNA repair genes could aid early diagnosis of localised disease that requires only active surveillance. On the other hand, higher risk tumours may be prioritised for specific treatments according to the affected genes. More research is required to build reliable classifiers based on these principles.

Figure 2.
Our research involves detailed characterisation of tumours with features associated with high risk disease. Tumours bearing germline mutation of DNA repair genes, or identified as hypoxic using tracer molecules, will be interrogated to identify drivers of aggressive phenotypes. This information will be important for the development of biomarkers intended to predict risk and responses to treatment.

BRCA2 carriers, compared with >90% 10-year survival for stage-matched non-carriers. Tumours from these patients often exhibit adverse pathological features such as a cribriform architecture and poor differentiation, and our group has shown that the patterns of chromosomal instability normally associated with mCRPC are already present in BRCA2 mutant tumours at diagnosis. Clearly, tumours from patients carrying germline BRCA2 mutation represent an excellent example of high-risk cancer driven by an impairment in DNA repair. Indeed, the examination of such tumours could shed light on the relationship between genome instability and disease progression. But, thus far, the approach has been hampered by the scarcity of biopsy material from prostate cancers with germline BRCA2 deficiencies. However, by collaborating with groups from around the world, we have supplemented a set of fixed tumour samples collected here in Manchester to assemble a unique collection of BRCA2 specimens for further study. We will now carry out a detailed interrogation of these samples including next-generation sequencing studies allied to a detailed pathological examination to define the relationship between specific genomic rearrangements, patterns of gene expression and risk status. We hope to better define the role of BRCA2 deficiency in driving aggressive disease and to uncover new possibilities for personalised therapy for this group of patients.

Germline mutations in the mismatch repair pathway (MMR) are the cause of Lynch syndrome – a hereditary condition predisposing carriers to the development of solid tumours, most commonly colorectal and endometrial cancers. Defects in this pathway lead to erroneous DNA replication, which can be detected most frequently in short repetitive regions known as microsatellites. Thus, microsatellite instability (MSI) is an important feature of tumours found in Lynch syndrome patients. Recent evidence suggests that prostate cancer should also be included in the spectrum of cancers associated with this syndrome. Although mutations in MMR genes are rare in PCa, the presence of mutations in one of the MMR genes, (MSH2, MSH6, EPCAM, MLH1 or PMS2), has been correlated with MSI and adverse pathology in PCa – and overall, patients with Lynch syndrome are at two-fold higher risk of developing prostate cancer. Furthermore, given that the use of PD-1 inhibitors has been approved for treatment of gastro-intestinal tumours with MSI, the detection of MMR-deficient prostate cancers could have therapeutic implications, and there is great interest in developing new biomarkers that assess mismatch repair along with other metrics of genome stability. However, due to the rarity of samples, MMR-deficient tumours have not been comprehensively characterised. To address this we have collated a cohort of PCa samples from patients with a Lynch syndrome diagnosis. We will now carry out next-generation sequencing studies to characterise the drivers of disease unique to these patients and to better inform biomarker development.

Germline mutations in BRCA2 and MMR are cell intrinsic deficiencies placing genome fidelity at risk, but the tumour microenvironment also has a strong influence on the growth and progression of cancers. For instance, it is characterised by dynamic gradients of oxygen diffusion and consumption, leading to sub-regions of hypoxia in about half of all solid tumours. The presence of hypoxia in PCa is correlated with a poor prognosis and several factors may contribute to this observation, including resistance to radiotherapy leading to failure of local control, impaired DNA repair, and adaptive responses that promote metastasis. In addition, we have shown that hypoxia is tightly correlated with levels of genome instability across a range of cancer types. Further work is now required to provide a mechanistic framework for these observations. To address this we are initiating clinical investigations wherein a small molecule marker of hypoxia (Pimonidazole) is administered to prostate cancer patients prior to their treatment. This will allow assessment of hypoxia in biopsy or radical prostatectomy specimens collected as part of their standard treatment. Detailed analysis will clarify the relationships between levels of oxygenation and DNA repair, genome instability and metastatic spread. Such studies will allow us to better understand the potential use of hypoxia as a biomarker to predict prognosis and to guide improved treatment strategies in prostate cancer.

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TUMOUR SUPPRESSORS

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[1]Joined in 2020

p53 is a transcription factor and tumour suppressor regulating the decision between cell death and cell survival upon stress. If stresses are too much, p53 will initiate apoptosis. If stresses are mild, p53 will cause cell cycle arrest and allow for DNA repair. This is extremely important in preventing tumour growth and it is therefore not surprising that p53 is found mutated in more than half of all cancers. Mutations in p53 are predominantly located around the DNA binding domain, but can occur on almost any amino acid in p53. In the majority of cases, these mutations lead to the expression of a mutant p53 protein. These proteins lose some or all of wildtype function, but importantly also gain novel functions in promoting tumour formation, cell migration, invasion and chemoresistance. Many of these mutants are not correctly folded. Even the wildtype p53 can unfold when exposed to hypoxia or metals such as copper. Most interestingly, this unfolded wildtype molecule seems to behave like an oncogenic mutant. Work in the Tumour Suppressors group this year focussed on the interplay between copper and p53 function and the oncogenic function of p53 mutant proteins in chemoresistance.

Previously, we discovered that mutant p53 interacts with p63 to promote RCP (Rab-Coupling protein)-dependent recycling of integrins and growth factor receptors and in this way enhances cell invasion. In a screen to detect novel RCP-interaction proteins, we detected P-glycoprotein. As mutant p53 is known to promote chemoresistance and P-gp (P-glycoprotein) is one of the best studied proteins involved in chemotoxic drug efflux, we decided to validate these findings. In various cell lines we could detect this interaction endogenously. Using CRISPR knockouts we determined that mutant p53 A431 cells were dependent on RCP and mutant p53 expression to promote resistance to cisplatin and etoposide. This resistance was also dependent on P-gp as loss of P-gp expression or inhibition with the third-generation P-gp inhibitor tariquidar restored sensitivity to chemotherapy. Loss of mutant p53 or RCP expression coincided with an increased expression of cleaved caspase 3 cells. In xenografts, loss of RCP in mutant p53 cells slowed down tumour growth and also showed an increase in cleaved caspase 3 when mice were challenged with cisplatin.

Interestingly, restoration of RCP expression in RCP knockout cells restored resistance to cisplatin and etoposide, but expression of RCP in p53 KO cells did not. These data suggest that mutant p53 regulates RCP function, but not RCP expression to promote chemoresistance. We therefore decided to look at the location of P-gp in response to chemotherapeutic challenge. In mutant p53 cells, P-gp was rapidly detected on the plasma membrane in response to cisplatin, where it co-localised with RCP. Loss of RCP or loss of mutant p53 greatly reduced P-gp plasma membrane expression in response to cisplatin (Figure 1). Finally, we looked at drug efflux function and used two different reporter assays. Calcein AM and Efflux gold dye are both substrates of P-glycoprotein and can be detected by fluorescent accumulation of these drugs in the cells. Inhibition of P-gp with tariquidar or loss of p53 or RCP expression caused substrate accumulation in A431 cells. Together these data uncover a novel role for RCP in chemoresistance. Our data support a model in which RCP and P-gp are localised in the same intracellular vesicles in mutant p53 cells that can rapidly be moved to the plasma membrane to increase P-gp membrane expression in

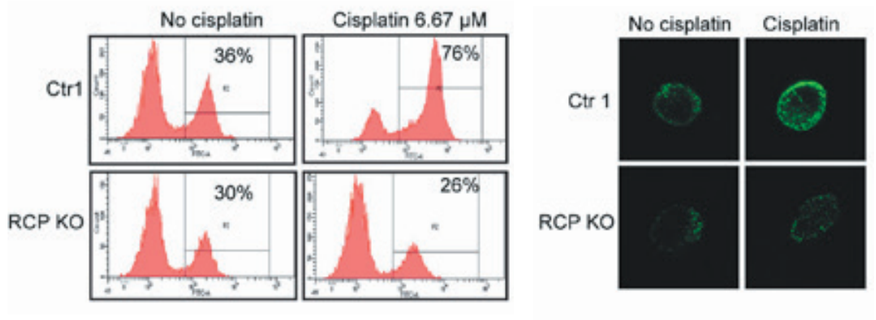

Figure 1.
Plasma membrane expression of P-glycoprotein in A431 ctr or A431 KO cells in the presence or absence of cisplatin (1hr), measured by flow cytometry (left) and fluorescent microscopy (right). It is clear that the amount of plasma membrane in mutant p53 A431 ctr cells increases more than 2-fold upon cisplatin treatment (left). Loss of RCP prevents this relocalisation.

response to chemotherapeutic challenge. Big questions that remain to be answered in the future are: How does mutant p53 regulate RCP function? Does RCP regulate integrins, growth factor receptors and P-gp at the same time by interacting with them at the same time? Are they localised on the same vesicles? And how does chemotherapeutic challenge influences RCP-dependent invasion?

Although we predominantly investigated the R273H mutant p53 protein in RCP/P-gp chemoresistance, some other mutant proteins were found to enhance this pathway as well. Thousands of different mutant p53 proteins are present in cancers and it is clear that not all mutants behave in a similar manner. However, it remains unclear what the difference between these mutants is. What is known is that some are folded, whereas others seem to be unfolded. Hundreds of mutant p53 proteins have been analysed in vitro or in yeast for their folding state and are listed in the p53 database www.p53.iarc.fr.

Many researchers have tried correlating folding status to phenotype, but it seems difficult to find a consensus. This research is hampered by the fact that folding state cannot easily be detected in tissues. Folding is affected by freezing and by fixation, making it hard to analyse this.

Additionally, folding is not a fixed state. Even the wildtype molecule is known to unfold in response to hypoxia or certain metals. Previous work had shown that unfolding of the wildtype molecule caused wildtype p53 to promote invasion and cell migration. In order to see if wildtype unfolding is similar to the invasion seen in mutant p53 expressing cells, we looked at mutant p53 specific signalling pathways. We could detect a specific interaction between wildtype p53 and mutant p53 specific interaction partners Ago2, p63 and p73 in the presence of copper, but not in the absence of copper.

Importantly, copper induced invasion of p53 wildtype cells (Figure 2) and this was dependent on wildtype p53 expression. In order to determine how copper unfolded p53, we looked at a possible direct binding and we could indeed identify a direct binding of copper to p53. Interestingly, copper binding impaired p53 in its ability to bind zinc, which is needed for binding to DNA. Together these data show that the copper status in tumours can influence how well p53 functions. Future work will focus on whether mutants proteins of p53 are more affected by copper and to what extent copper mediated unfolding of p53 plays a role in metastasis in vivo.

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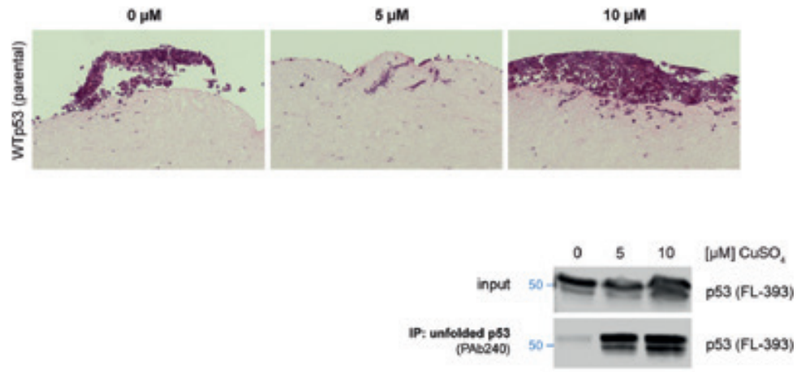


Figure 2.
WT p53 HEK293T cells were incubated with increasing amounts of copper. Even a low dose such as 5-10 uM is able to promote invasion into organotypic plugs (top), which coincided with an increased expression of unfolded p53 as determined by immunoprecipitation with the 240 antibody and p53 detection in western blot (bottom).

CANCER RESEARCH UK MANCHESTER INSTITUTE

RESEARCH SERVICES

Multiplex fluorescent image of the skin of mice in which keratinocytes (green) can be observed in the epidermis and dermis of the skin and melanocytes can be found in deep dermis of the skin (magenta) with their nuclei in blue.

Image supplied by Candelaria Bracalente and Valeria Pavet (Molecular Oncology)

RESEARCH SERVICES

Chief Laboratory Officer
Stuart Pepper

2020 was an unusual year by any standard. It is impossible to ignore the fact that COVID-19 has had an impact on all aspects of life and work and it is evident in the following articles that there have been some new challenges to operating core facilities over the last year.

Chief Laboratory Officer
Stuart Pepper

What is also apparent is how effectively core facilities have adapted to maintain largely uninterrupted provision of service. This has required a mix of teams working shifts or extended options for remote access to software to facilitate working from home. The real success is that by autumn many services were operating close to normal throughput and providing broad support for research groups. Recruitment has also continued over the last year, and we have welcomed new staff members in both Scientific Computing and the FACS team.

A common theme that appears in these reports is the continuation of a trend whereby the core facilities collaborate to provide seamless workflows that span across the traditional core facility areas. The rapidly emerging field of spatial genomics is a good example of this where collaboration between Molecular Biology Core, Histology, Visualisation, Irradiation & Analysis and Sci Com has enabled development of new workflows.

Another continuous theme over the last few years has been the expansion of multiplex analysis approaches. The Helios platform is now well established as an Institute service, offering a far higher multiplex approach than traditional FACS analysis; over the last year the introduction of the CODEX platform has provided another highly multiplexed approach, this time for staining of sections. As with spatial genomics, CODEX has been the result of a collaborative approach involving Histology and VIA. Another new multiplex workflow is a 16 channel TMT quantitation approach that has been introduced in Biological Mass Spectrometry, which will facilitate a range of new studies.

Scientific Computing have adapted very effectively to predominantly off site working and have had a highly productive year. A major upgrade to the storage system was completed and numerous collaborations with other groups have been developed. In contrast, the in vivo facilities do not have the same flexibility when it comes to off site working and have adapted in different ways. One opportunity has been to dedicate extra time to staff training, and the introduction of non-aversive handling is a good example of this. The Transgenic Production Facility took the opportunity early in the year to complete a major project, cryopreserving many of the Institute's mouse lines.

Aside from service provision described above, a considerable amount of time has been spent on continuing design work for the new building project. The schedule for the new building shows completion at the end of 2022 and so a lot of focus this year was on completing design work ahead of construction starting as the year ended. Detailed design work for the core facilities was completed on schedule, with each manager ensuring that all the appropriate services are available for each piece of equipment. The next major piece of work will come when we start to plan the relocation programme!

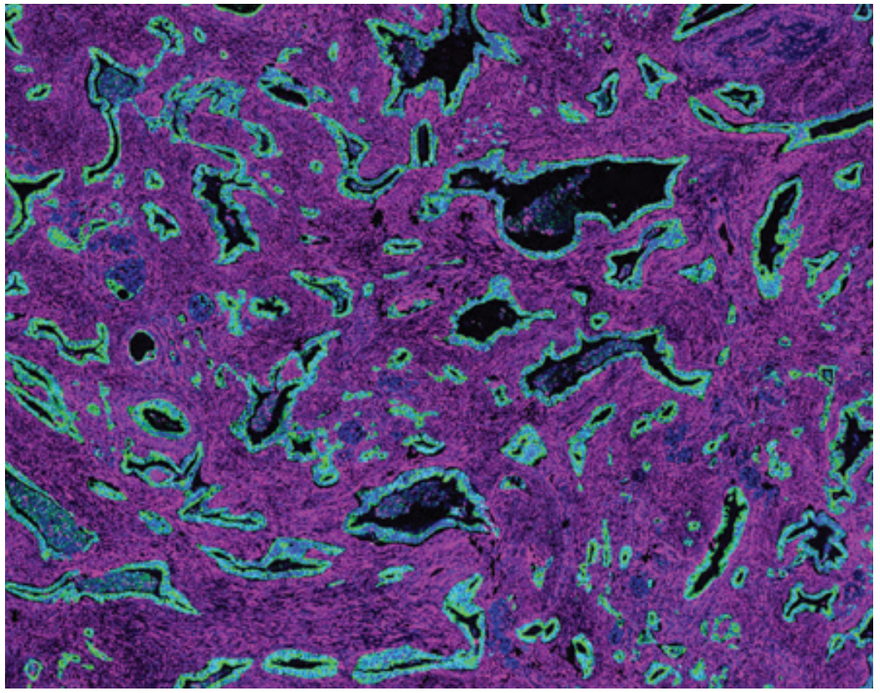
Biological Mass Spectrometry

Duncan Smith, Yvonne Connolly

Following the announcement of laboratory closure due to the COVID-19 pandemic, a planned and controlled instrument shutdown was disrupted. Fortunately, virtual access into the mass spectrometry lab directed successfully the shutdown sequences. The service eventually resumed in June, after an absence of 12 weeks, during which data analysis continued and also provided the time to work on the lab design of the laboratory for the new building.
During this year, the priority was to clear a backlog of analyses and facilitate our research

Human resected Pancreatic ductal adenocarcinoma. Purple: Vimentin, Green: Pan Cytokeratin.

Image supplied by Colin Hutton (Systems Oncology)



group's recovery by providing research services to interrupted projects. In addition, we have developed and optimised a bespoke workflow to extend our Tandem Mass Tagging quantitative proteomic capabilities to 16 channels utilising the TMTpro chemistry and the Orbitrap Lumos. Essentially, this workflow allows up to 16 samples to be processed, pooled and run in the same LC-MS/MS experiment. Allowing the quantitative analysis of up to 16 different samples simultaneously is perfect for many time course and drug experiments previously intractable with other approaches.

Biological Resources Unit
Transgenic Breeding

Team Leader: Jennifer Hughes
Dan Bennett, Tim Bloor, Carl Conway, Ali Jammoul[2], Howard Kendrick[1, 2], Edyta Kijak[1], Wesley Moore, Kerry O'shea, Vicky Preston[1], Rose Storey, Lauren Street[2], Natalie Varley[3]

[1]Joined in 2020
[2]Left in 2020
[3]Maternity leave

The BRU Transgenic Breeding Team breeds mice for CRUK MI researchers under a central breeding project license. The team provides husbandry, pairs mice for breeding, monitors timed matings, records and weans litters, takes ear biopsies for genotyping and identification purposes, manages the outsourced genotyping service, translates and transfers genotyping results, and monitors tumour-prone lines for onset of symptoms. 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 maintained free from common mouse pathogens. In order to protect this status, new transgenic lines coming from external sources have to be transferred in as either embryos or sperm and are thoroughly health screened in order to ensure that the resulting offspring are specific pathogen free. At present, mice required by researchers are transferred in weekly shipments to the BRU Experimental Team at Alderley Park upon request, after transfer a minimum of one-week acclimatisation is required before mice can be enrolled onto experiments.

Ten staff members currently provide day-to-day care for 98 different transgenic mouse lines spread across approximately 1200 cages in a facility located within the main university campus. Towards the end of 2020 changes were approved that allow our holding rooms to be covered directly by the CRUK MI Establishment License, giving us greater autonomy and the ability to work in a way that is more consistent with the BRU Experimental Team. In the last year 33 new breeding lines have been started and 72 breeding lines have been closed. The new breeding lines include

some that are new to the unit, having been either rederived in or having been produced by TPF, and some that have been generated by crossing existing lines.

We have now had our first full calendar year of using the tick@lab system for securely recording all of our breeding and stock details, as well as providing an option for delivery of simple instructions from users. This continues to work well and as information accumulates over time it becomes an ever more useful tool for tracking historic details and allowing analysis of data. There are also major benefits of this system in relation to searchability of actions and results. We are working on increasing the range of functionalities that we use within tick@lab and we provide training to new researchers and team members as needed. We are proud of being the first UK based organisation to have implemented semi-automated transfer of genotyping results from our external genotyping provider to the breeding records system. Although not simple to set up, investing time and effort in this has had the benefit of reducing errors and allowing personnel with less comprehensive background knowledge to transfer results successfully.

In this particularly challenging year the team has provided continuous day-to-day care for the mice within our facility, responding in an agile manner to rapidly changing demands, reducing numbers quickly when necessary and making use of the time when experimental requirements were diminished in order to introduce less aversive handling methods and to work on other improvements.

Experimental Services

Team Leader: Joanna Roberts

2020 has been a busy year despite the disruption we have all experienced due to the COVID-19 pandemic. The BRU Experimental Facility has continued throughout the Institute closure and lockdowns to deliver high animal welfare and experimental support to the Institute. In order to ensure safe working practices and achieve social distancing, the amount of experimental work initially had to be reduced, however this increased towards the latter half of the year.

The quieter times during lockdown provided an opportunity to roll out the use of non-aversive handling across the facility. This process involves mice now being picked up using a plastic tunnel or being cupped in the hand rather than picked up by the tail, which has proven to greatly reduce stress in mice. It is important that we continue to review and improve our procedures and animal welfare practices – tail handling has been the gold standard for handling laboratory mice for many years yet recent evidence shows that this is not the best handling method for the welfare of the mice.

Despite the reduction in new work, we have been able to train many of our technicians in the use of different imaging equipment, such as the micro-CT, whilst monitoring some of our long-running experiments. We have further developed our technical capabilities and have been able to utilise our ultrasound system for a small experiment which involved image-guided injections of tumour cells into the liver rather than having to rely on surgery. The procedure showed great promise as it is quick and much less invasive than surgery.

We have continued to share our experience of model development and monitoring refinements with other institutes by attending online meetings and giving presentations via Zoom. Online presentations have also given us the opportunity to learn from institutes in other countries without the need to travel.

Flow Cytometry

Jeff Barry, Antonia Banyard, Yosra Elagili, Michael Rennie[1]

[1]Joined in 2020

The facility's remit is to provide cutting edge technology and expert application support, facilitating the research goals of the Institute. The facility supports both fundamental and translational research through the provision of advanced and innovative cytometry platforms. The Flow Cytometry team also provides training, support and application advice to the Institute. We operate across two sites, offering cell sorting and analysis services at the main site in Alderley Park, Cheshire and at the Oglesby Cancer Research Building, Manchester.

Over the last few years, the Flow Cytometry facility has evolved from service provision to a facility that proactively collaborates with our scientists. The recent recruitment of scientific officers, who are looking to make cytometry a career, has strengthened our ability to engage with researchers resulting in a creative, enthusiastic and dynamic workplace in which science can thrive.
More recently, Toni Banyard the facility Senior Scientific Officer, has laid the foundation of a mass cytometry service, centred on the latest generation of CyTOF mass cytometer. This technique enables the measurement of up to 50 different markers on a single cell by mass spectrometry. This is achieved by using antibodies that are conjugated to metal isotopes as the reporter, which enables the multiplexing on a single cell. The service has developed the expertise to create bespoke antibody panels in parallel with sample preparation and now routinely runs large complex panels for murine and human studies. The power of the technique is that activation markers and cytokines that can be simultaneously measured, giving a much clearer and more detailed picture of the tumour micro-environment or changes in the peripheral blood. At present this technique is being used for human trial samples to determine the effects of different therapies but also to characterise various murine disease models, which is essential for all forms of cellular research.

The cell sorting service offers the unique ability to select specific cells and to sort them individually as single cells or as highly enriched population. We have worked closely with Stem Cell Biology on their investigation of the role of RUNX1 in haematopoiesis, providing the group with highly purified populations of cells needed for downstream genomics, which is key to locking the molecular mechanism behind this. Recently we have sorted modified fibroblasts that have been reprogrammed via a specific set of transcription factors, which instruct the cells to form specific blood cell types. The reprogrammed cells are identified, sorted, cultured and their cell fate determined; such research could ultimately find applications in patient therapy.

On another front, we have supported Systems Oncology in their study of the tumour micro-environment in pancreatic ductal adenocarcinoma and its effect on altering the polarity and function of cancer associated fibroblasts (CAF). CAFs have been linked to tumour promoting effects such as tumour growth, immunosuppression and the promotion of metastasis. The influence of the tumour microenvironment can thus affect disease progression and therapeutic response. This involved the identification and subsequent sorting of specific CAFs, allowing the investigation of the underlying molecular mechanisms associate with these changes.

The facility's flow analysers are invaluable for probing the immune profiles of infiltrating immune cells, an area of interest for several groups. Access to the facility's high end analysers facilitated this type of research, for instance data generated from the Novocyte Flow analyser and CyTOF has helped Cancer Inflammation and Immunity's study of the action of inflammatory immune sub-types and their influence on tumour progression.

The ability of our analysers to rapidly perform cell cycle analysis has aided Cell Division's pursuit of developing Cdk4/6 inhibitors as agents to synchronise cells without perturbing crucial cellular regulatory processes and without damaging DNA. This technique promises to be an invaluable research tool in the field of cell division.

Finally, we were pleased to see Molecular Oncology's recently published paper, entitled "Immune-awakening revealed by peripheral T cell dynamics after one cycle of immunotherapy", appear in the prestigious *Nature Cancer* journal. This study looked at changes to T cell populations in metastatic melanoma patients who received checkpoint inhibitors and found changes that were prognostic of treatment response. This raises the potential to monitor patient response via minimally invasive biopsies. The facility helped to identify, quantify and sort T cell populations used in this study.

Histology

Garry Ashton, Caron Abbey, Marta Madureira da Graca[1], Usman Mahmood, Emma Watson[1], Katherine Lally, Deepti Wilks[2], David Millard

[1]Left in 2020
[2]Haematological Malignancy Biobank

Whilst 2020 was a challenging year, the range and complexity of the services offered has continued to grow, allowing the unit to continue to develop sophisticated labelling techniques and incorporate them into routine practice. In addition, routine service production has experienced heavy demand, processing both human and mouse tissues in addition to organotypic assays, spheroids, agar plugs and cell pellets. Special stains such as Masson Trichrome, PAS and Sirius red, together with the use of the vibratome and allowing for the use of ex vivo tumour cultures in three dimensional studies have both seen increased demand.

The services offered by the core facility are used by both basic and translational research groups within the CRUK MI, allowing for the continued development of tissue-based experimental approaches. Both the Leica and Roche IHC platforms enable access to high throughput routine immunohistochemistry for all groups. In addition, RNAscope and multiplex immunohistochemistry are offered as standard services and together in combination. The unit

continues to be used routinely for phenotyping of CDX models on our automated platforms ensuring consistency, reproducibility and standardisation.

In collaboration with both the Molecular Biology and Visualisation, Irradiation & Analysis Core Facilities, high number multiplex immunohistochemistry and spatial transcriptomic technologies are being evaluated and developed. These techniques will allow for spatial profiling of tissue from multiple angles. The results from these are looking promising and it is hoped these techniques will be rolled out throughout the coming months.

Research projects involving the use of biobank material processed through the facility continues to increase. Laser capture microdissection followed by the downstream extraction of both RNA and DNA, giving sufficient quantity and quality for NGS from relatively small amounts of material, is now routine and continues to prove popular. High quality representative TMA's from a number of disease groups have been constructed and used by several groups. In addition to FFPE and frozen tissue samples, the number of blood, bone marrow and plasma samples collected from haematological malignancy patients continues to increase.

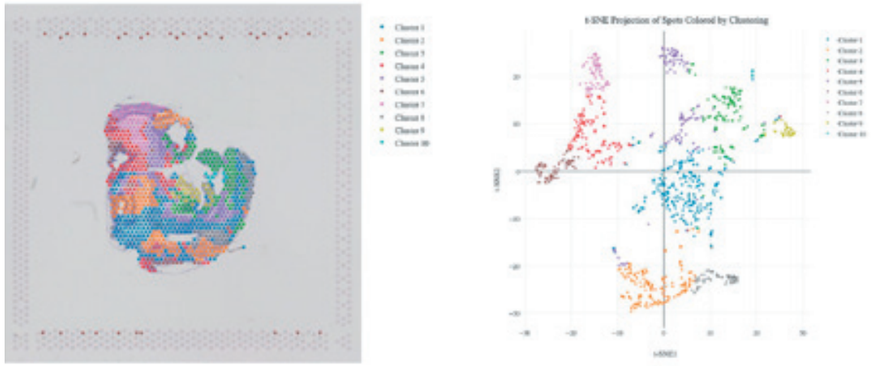
In collaboration with the Targeted Therapy Group within the Division of Cancer Sciences at The University of Manchester, the unit has helped develop and validate a number of mouse and human immune markers for both single plex and multiplex analysis. As with other groups, the development of multiplex panels has been key in this study. In addition, the facility has also been instrumental in the validation and extraction of RNA and DNA from both FFPE mouse and human tumours, and frozen samples for Nanostring and RNA sequencing.

The facility has been able to construct a tissue microarray from triple-negative breast cancer (TNBC) core needle biopsies. In a collaborative project with the Cancer Inflammation and Immunity group this was used for spatial transcriptomics as part of a Manchester BRC Pump Priming project. From this work, a particular target of interest was identified and two multiplex immunofluorescence panels have been optimised and applied to a new TNBC cohort as validation of the original findings.

The facility has also aided in the extraction of RNA and DNA from renal cancer samples. Gene expression profiling from this RNA now serves as pilot data for the MRC Confidence-in-Concept study that is due to commence shortly.

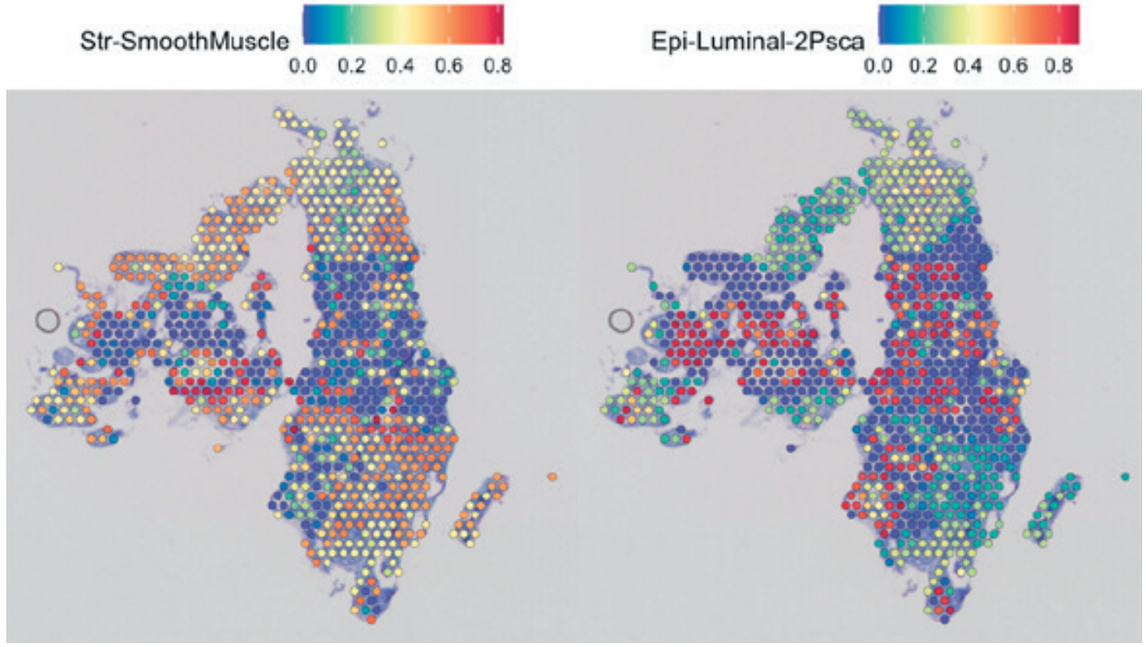
Furthermore, the facility has played a role in a project to elucidate the mechanism through which UVR promotes melanoma with the Molecular Oncology group. The project has relied heavily on routine services, DNA extraction and multiplex immunohistochemistry to assess DNA damage response and other UVR-related effects in skin. In addition, RNAscope has now been optimised and used on tumours from BRAF GEMM cohort for validation of the experimental model.

In addition, the unit has been assisting the Systems Oncology group. Single and dual immunofluorescence was used to evaluate novel 3D synthetic hydrogels and study the vast interactions between ECM molecules and their concomitant integrin receptors. This work forms part of a current submission for publication.



Single Cell RNA-Seq analysis: Effect of resolution on cluster stability (silhouette width) according to different numbers of highly variable genes (HVG).

Image supplied by Wolfgang Breitwiester (Molecular Biology Core) and Garry Ashton (Histology)



Validation of 10x Genomics Visium Spatial Transcriptomics technology.

Image supplied by Wolfgang Breitwiester (Molecular Biology Core) and Garry Ashton (Histology)

Finally, in collaboration with the Translational Oncogenomics group, development of a multiplex IF stain for RAD51, GLUT1 and Geminin has been used to investigate the relationship between hypoxia and DNA repair in high risk localised prostate cancer patient cohorts. In addition, RNAscope will be employed to investigate BRCA2 RNA expression in germline BRCA2 carrier prostate cancer cases and to also correlate this to areas of intra-tumoural hypoxia.

Molecular Biology Core and Computational Biology Support

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

Last year saw the introduction of the Illumina NovaSeq 6000 to the Molecular Biology Core, replacing the HiSeq 2500 as the high throughput platform for Next Generation Sequencing at the Institute. The NovaSeq is Illumina's most powerful instrument with the highest system specifications on the market, capable of simultaneously generating up to 20 billion sequencing reads on two flow cells running side by side. Benefiting from greater sequencing efficiency, as well as improved scalability at reduced costs and significantly diminished run times, the NovaSeq has since been used for the majority of our genomics and transcriptomics applications, including high coverage whole genome sequencing as well as single cell transcriptome sequencing.

Among a number of technical innovations, the NovaSeq features patterned flow cells, promoting increased sequence cluster formation and improved read accuracy. However, these new features also required us to introduce and validate a number adaptations in sequence library preparation protocols, including the introduction of Unique Dual Indexing (UDI). Further developments in MBC's library preparations included the validation of Unique Molecular Identifiers (UMIs) incorporated into exome and targeted sequencing protocols. The benefits of UMIs lie in the discrimination between prep artefacts, introduced for example by PCR amplification, and true sequence variants. As shown by our bioinformatics validation, these innovations demonstrate clear benefits when incorporated into variant analysis workflows.

Over the last year we also had the opportunity to validate Illumina's DRAGEN Bio-IT platform. DRAGEN uses Field-Programmable Gate Array (FPGA) hardware featuring optimised algorithms for mapping, aligning, sorting, duplicate marking, and variant calling. This technology is aimed at delivering fast secondary genomic analysis of sequencing data.

Another highlight was the introduction and validation of a novel methodology for human cell line authentication (HCLA) by clonal sequencing. Using Verogen technology, the HCLA service has been transferred from Sanger Sequencing to NGS-based analysis of short

tandem repeat (STR) profiling run on MBC's MiSeq instrument, thus providing refinement in analysis as well as adding information on single nucleotide polymorphisms.

Expanding on the latest developments in the field, single cell analyses feature increasingly strongly in the MBC's genomics and transcriptomics applications. Thus, the Molecular Biology Core now supports a range of methodologies including single cell transcriptome analysis of cultured cells as well as primary tissue samples, such as tumour biopsies, patient blood and bone marrow cells. We have also proceeded with applications for single cell ATAC-Seq as well as single cell immune profiling.

As well as performing validation work on the MBC's novel technology applications, the Computational Biology Support team provides a plethora of bioinformatics support across the Institute. In the last year one particular emphasis was on establishing and benchmarking analysis workflows for single cell sequencing projects. Using a range of available analysis tools such as Seurat, we also undertook a variety of multi-omics analyses, e.g. for CITE-Seq, enabling the simultaneous analysis of transcriptome and lineage marker proteins in single cells. This proves to be a powerful tool for annotation and refinement of cell types in complex tissues.

A further validation project was undertaken for Spatial Transcriptomics. This highly innovative technology integrates histological information of biological tissues into their gene expression profiles. In collaboration with Histology and VIA, we carried out a study using the 10x Genomics Visium technology to capture and analyse cellular transcriptomes of tumour sections in a spatial context. This, together with other technology platforms such as the NanoString GeoMx, will be the focus of further method validation and refinement in spatial gene expression profiling.

In addition to providing comprehensive analysis support, the CBS team is continuously active in training Institute researchers in bioinformatics analysis as well as offering individual advice on projects. To aid this we held a series of training sessions on Partek Flow Software. This analysis suite, which is licensed to the Institute, is used for bulk and single cell RNASeq analysis and is aimed at bench scientists undertaking their own bioinformatics analysis.

Phosphorylation is a post-translational modification that is involved in regulation of many crucial biological processes. Correct identification of the phosphosite position and subsequent quantification of phosphorylation isomers is a challenging problem in proteomics-based mass spectrometry, notably because of the need for 'site-determining ions' (unambiguous fragmentation ions corresponding to specific isomers). The Institute's MS Core team has applied a targeted approach known as parallel reaction monitoring (PRM), a sensitive and precise method that produces high-quality fragmentation information towards the phosphorylation challenge. CBS has assisted in the development of isomer identification and quantification.

Initiated in the previous year, the Histology and Molecular Biology Core teams have this year invested a significant amount of time and effort to implement a Laboratory Information Management System (LIMS). This is designed to track all samples that are submitted for processing in the core services, and will capture all processing steps, and storage location of samples. While the system testing is currently ongoing, the aim is to expand its role out across the Institute over the coming year.

Scientific Computing

Marek Dynowski, John Campion[1], Kevin Doyle, Nadeem Baig[2], Stephen Kitcatt, ZhiCheng Wang

[1]Joined in 2020
[2]Left in in 2020

2020 was a great year for introducing new services and performing some major improvements to the Scientific Computing (SciCom) infrastructure and data management processes. We are therefore very pleased to welcome our new Lead Data Architect John Campion to our team. John will work with the CRUK MI scientists and other core facilities to manage and store the massive amounts of data produced by our users more efficiently and effectively. Expansion of the monitoring and reporting capabilities of the SciCom infrastructure also helps us proactively identify and respond to potential issues before users are affected.

The stability and availability of SciCom services and software that runs on the oVirt virtual server platform was increased by introducing the Vinchin software for the automatic backup creation and recovery of virtual servers. The management of those servers was simplified and further automated by the introduction of the Ansible provisioning and configuration management software. Both changes allow SciCom to deploy virtual servers and workstations faster and more reliably. The parallel storage system for storing scratch data produced by the Phoenix High Performance Compute (HPC) system became end-of-life in 2020. Despite the challenges this year, SciCom managed to replace the old Lustre based high performance storage with a new Panasas parallel storage system. The capacity was doubled to 1 Petabyte and the aggregated I/O band increased from 7GB/s per second to 15GB/s. It also offers new possibilities regarding data management and data transfer. With this new parallel storage system, CRUK MI is well-prepared for future challenges, such as increased data capacity and I/O requirements due to new AI applications.

SciCom's Shiny Web app hosting service is in high demand. This demand caused some problems due to increasing incompatible cross-dependencies between different Shiny apps hosted on the same server, which made the management and deployment of Shiny apps more difficult. To solve this problem, the Shiny proxy server was introduced. It allows the deployment of Shiny apps using Docker Linux containers to give the developer more control over the app-specific R environment and to prevent cross dependencies.

A Cytofkit2 service for integrated mass cytometry data analysis was introduced in close cooperation with the Flow Cytometry core facility and the Systems Oncology group. It allows the high-throughput analysis of mass cytometry data produced by the core facility.

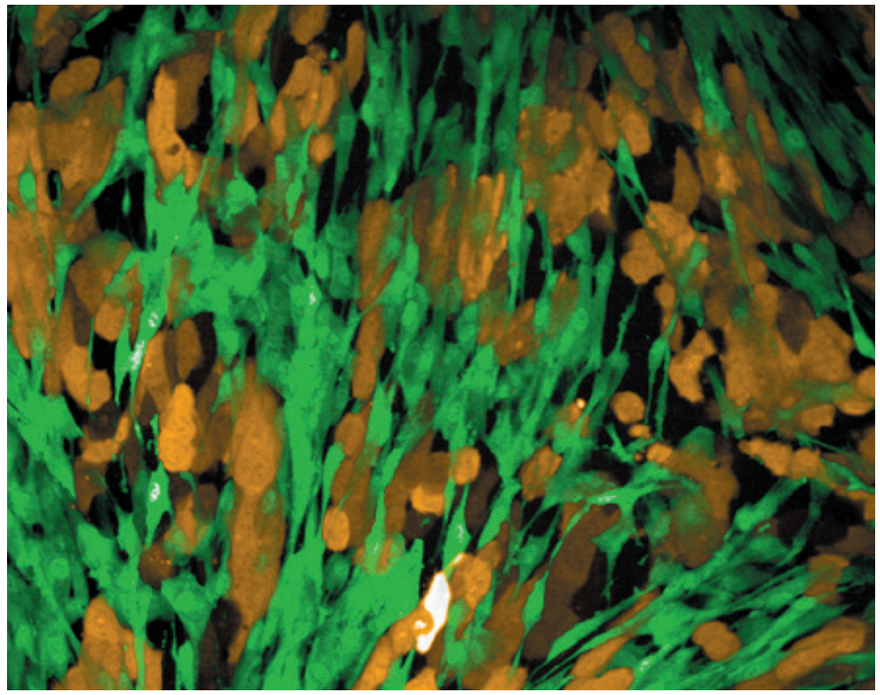
Together with the Visualisation, Irradiation and Analysis core facility, a virtual Windows workstation was deployed to allow users the location independent remote image analysis using the Olympus Slide Scanning software. The VIA core facility and SciCom are also involved in a project to establish an integrated data analysis pipeline on Phoenix for mapping spatial gene expression of complex tissues samples for the Molecular Biology Core facility.

Working with the Cancer Biomarker Centre and collaborators at the University of Southern California, SciCom has added HPC functionality to the Okular pipeline used by both institutes for the analysis of High Definition Single Cell analysis. The software was adapted to work with Phoenix's MOAB/Torque based batch system, and Docker Linux containers are now used for providing the software components of the pipeline. This ensures that the pipeline can be used on public cloud-platforms as well as on-prem HPC systems such as Phoenix.

SciCom had to make substantial changes to its pre-processing platform for sequencing data (Octopus) following the MI's purchase of the new NovaSeq sequencer. It can now automatically handle the parallel processing of several projects at the same time, which is necessary due to the increased capacity of the

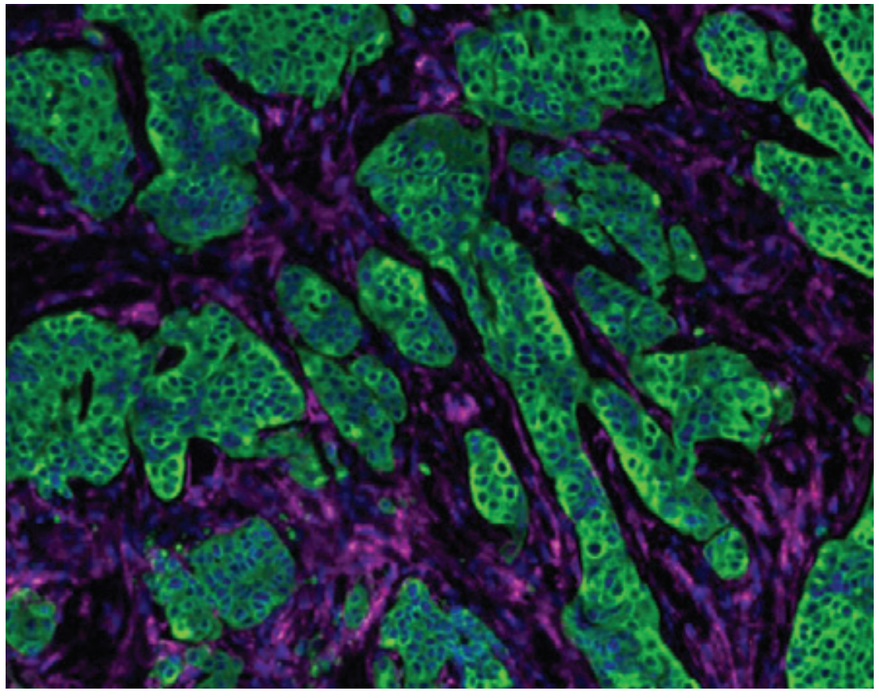
Fibroblasts (green) were co-cultured with mutant p53 cancer cells (red) in 3D. A431 mutp53 cells were seeded on NFG layer.

Image supplied by Lobsang Dolma and Patricia Muller (Tumour Suppressors)



Multiplex immunofluorescence was carried out using triple negative breast cancer FFPE tumour samples. Nuclei are labelled in blue, cancer cells in green, and two different stromal cell markers in purple and yellow.

Image supplied by Christopher Bromley (Cancer Inflammation and Immunity)



new sequencer. This functionality will also simplify the planned connection of Octopus to new LIMS system currently implemented in the Molecular Biology core facility. Much work has been done to ensure that preprocessing and certain types of analysis can be fully automated using the Octopus backend once the LIMS is up and running.

One of our most exciting projects is a deep learning-based solution for a standardisation approach for automated gating of mass cytometry data. The DeepCyTOF framework serves as the basis for automatically assigning individual cells into discreate groups of cell types. SciCom improved the framework's code base and developed a Shiny app that allows users to easily access Phoenix's GPU resources to train and use models for high-throughput automated gating of cells. The close collaboration between the Visualisation, Irradiation and Analysis core facility, the Flow Cytometry core facility and the Systems Oncology group enabled us to test the DeepCyTOF approach intensively on real data. This made it possible to develop a user-friendly interface for a highly efficient gating framework and to hide the complexity of the calculations in the background. The app and code will be released shortly and made available to other researchers.

Transgenic Production Facility

Natalia Moncaut, Athina Papaemmanouil, Lauren Street[1] and Satish Arcot-Jayaram[2]

[1]Joined in 2020
[2]Left in 2020

The Transgenic Production Facility (TPF) is an advanced and efficient technology platform responsible for providing the generation of new genetically engineered mouse models (GEMMs). We work closely with the researchers providing strategic advice on the generation of the best cancer mouse model that will allow the study of initiation and progression of the disease. By using CRISPR-mediated gene targeting, we are able to modify the mouse genome and introduce precise genetic modifications, recapitulating the changes found in different human cancer types.

This year TPF had the urgency to focus our activity on our most recent service: archiving and assisted reproduction, including embryo and sperm cryopreservation and in vitro fertilisation. Since the Covid-19 national lockdown imposed the possible closure of the animal facility and diminution of staff, we decided to reduce significantly the breeding activity. Under this scenario we were able to safely archive all mouse lines present at the Institute. Around 120 genetically modified mouse strains were cryopreserved and quality-control tested using in vitro fertilisation. We have also securely duplicated our local storage of cryopreserved mouse strains between two different sites.

We are also participating in several NC3Rs initiatives about challenges related to the management and archiving of genetically modified mouse colonies. We generated an online resource that provides guidance for researchers and colony managers about critical aspects of breeding, colony management and archiving of mouse strains.

Visualisation, Irradiation & Analysis

Steve Bagley, Alex Baker, Jianhua Tang, Kang Zeng

The facility, like all laboratories, has had a challenging year, and has operated in such a way to enable home working for the research groups via remote access to both the instruments and software. The equipment was set up so to enable control from the home office, monitoring of equipment to enable remote support and new forms of operating within the laboratory to enable social distancing. Access to IT systems was set up to permit data analysis of microscopy data and with the support of Scientific Computing, access to the raw data space permitted researchers to assess all of their experimental data. During the first COVID lockdown, equipment was powered up, calibrated and standardised so that as soon the laboratories could reopen, the equipment would be able to collect data immediately. This year has enabled the facility and the researchers to explore new ways of operating, enabling remote data processing and allowing researchers to maintain productivity wherever their location.

Despite time lost from the laboratory, workflows were altered to ensure productivity whilst maintaining social distancing. Just over 12,000 histology slides were visualised, high content screening was utilised for a total of 1,500 hours, microscopy for 29,000 hours and use of image analysis software via remote working exceeded 4,000 hours. With social distancing measures in place, over 300 hours of instrument and software training was performed.

A collaboration between facilities (VIA, Histology, Molecular Biology and Computational Biology Support) was initiated this year to assess spatial transcriptomics technology, in order to spatially resolve RNA-seq data by mapping onto the tissue imaging data. This promises to be an exciting new research tool, which is hoped will be used routinely in the new year.

This year has seen the introduction of CO-Detection by indEXing (CODEX), a contemporary method of labelling single cells and tissues with up to 40 fluorescent labels. When using a camera or spectrophotometer for detection of fluorescence signals on tissues, there is a limit of eight fluorescent signals; beyond this point elucidation of separate signals becomes challenging. Within the facility over the last five years, there has been a growing requirement to be able to interrogate tissues with a larger array of fluorescent labels to study the complex relationships across tissues. Working in collaboration with Garry Ashton in the Histology facility, workflows are being developed so that research groups can visualise and numerate tissue-wide and niche relationships.

CANCER RESEARCH UK MANCHESTER INSTITUTE

PUBLICATIONS AND ADMINISTRATION

RESEARCH PUBLICATIONS

Cancer Biomarker Centre

(page 16)
Caroline Dive

Refereed research publications

Barriuso J, Nagaraju RT, Belgamwar S, Chakrabarty B, Burghel GJ, Schlecht H, Foster L, Kilgour E, Wallace AJ, Braun M, Dive C, Evans DG, Bristow RG, Saunders MP, O'Dwyer ST, Aziz O.
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Nature Medicine 26(7):1054-1062.

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Cell Reports 31(5):107550.

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Nature Genetics 52(3):283-293.

Morgan RD, Banerjee S, Hall M, Clamp AR, Zhou C, Hasan J, Orbegoso C, Taylor S, Tugwood J, Lyon AR, Dive C, Rustin GJS, Jayson GC. (2020)
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Gynecologic Oncology 156(3):545-551.

Other publications

Poirier JT, George J, Owonikoko TK, Berns A, Brambilla E, Byers LA, Carbone D, Chen HJ, Christensen CL, Dive C, Farago AF, Govindan R, Hann C, Hellmann MD, Horn L, Johnson JE, Ju YS, Kang S, Krasnow M, Lee J, Lee SH, Lehman J, Lok B, Lovly C, MacPherson D, McFadden D, Minna J, Oser M, Park K, Park KS, Pommier Y, Quaranta V, Ready N, Sage J, Scagliotti G, Sos ML, Sutherland KD, Travis WD, Vakoc CR, Wait SJ, Wistuba I, Wong KK, Zhang H, Daigneault J, Wiens J, Rudin CM, Oliver TG. (2020)
New approaches to SCLC therapy: from the laboratory to the clinic.
Journal of Thoracic Oncology 15(4):520-540.

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Liquid biopsy-based biomarkers of treatment response and resistance.
Cancer Cell 37(4):485-495.

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Circulating tumour cells in lung cancer.
Recent Results in Cancer Research 215:105-125.

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Impact of lineage plasticity to and from a neuroendocrine phenotype on progression and response in prostate and lung cancers.
Molecular Cell 80(4):562-577.

Berns A, Ringborg U, Celis JE, Heitor M, Aaronson NK, Abou-Zeid N, Adami HO, Apostolidis K, Baumann M, Bardelli A, Bernards R, Brandberg Y, Caldas C, Calvo F, Dive C, Eggert A, Eggermont A, Espina C, Falkenburg F, Foucaud J, Hanahan D, Helbig U, Jönsson B, Kalager M, Karjalainen S, Kásler M, Kearns P, Kärre K, Lacombe D, de Lorenzo F, Meunier F, Nettekoven G, Oberst S, Nagy P, Philip T, Price R, Schüz J, Solary E, Strang P, Tabernero J, Voest E. (2020)
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Molecular Oncology 14(8):1589-1615.

Cancer Inflammation and Immunity

(page 20)
Santiago Zelenay

Refereed research publications

Bonavita E, Bromley CP, Jonsson G, Pelly VS, Sahoo S, Walwyn-Brown K, Mensurado S, Moeini A, Flanagan E, Bell CR, Chiang SC, Chikkanna-Gowda CP, Rogers N, Silva-Santos B, Jaillon S, Mantovani A, Reis E Sousa C, Guerra N, Davis DM, Zelenay S. (2020)
Antagonistic inflammatory phenotypes dictate

Inflammation can fuel or limit cancer growth and the response to therapy. Bonavita et al. revealed an intimate link between the COX-2/PGE2 pathway, NK cells and cytotoxic immunity that defines the intratumoral inflammatory composition and anticipates tumor fate. The illustration depicts the complex Yin and Yang interaction between pro- (red) and anti-tumorigenic (green) inflammatory responses within a tumor. By in-depth profiling of murine cancer models and bioinformatics analysis of cancer patient datasets, the researchers devised a gene-expression signature that predicts overall patient survival and response to immunotherapy across multiple cancer types (in the background) with varying degrees of opposing inflammatory milieus. Cover illustration by Sam Falconer.

Image supplied by Santiago Zelenay (Cancer Inflammation and Immunity)

tumor fate and response to immune checkpoint blockade.
Immunity 53(6):1215-1229.e8.

Cell Division
(page 22)
Iain Hagan

Refereed research publications
Trotter EW, Hagan IM. (2020)
Release from cell cycle arrest with Cdk4/6 inhibitors generates highly synchronized cell cycle progression in human cell culture.
Open Biology 10(10):200200.

Cell Signalling
(page 26)
Angeliki Malliri

Other publications
Dunnett-Kane V, Burkitt-Wright E, Blackhall FH, Malliri A, Evans DG, Lindsay CR. (2020)
Germline and sporadic cancers driven by the RAS pathway: parallels and contrasts.
Annals of Oncology 31(7):873-883.

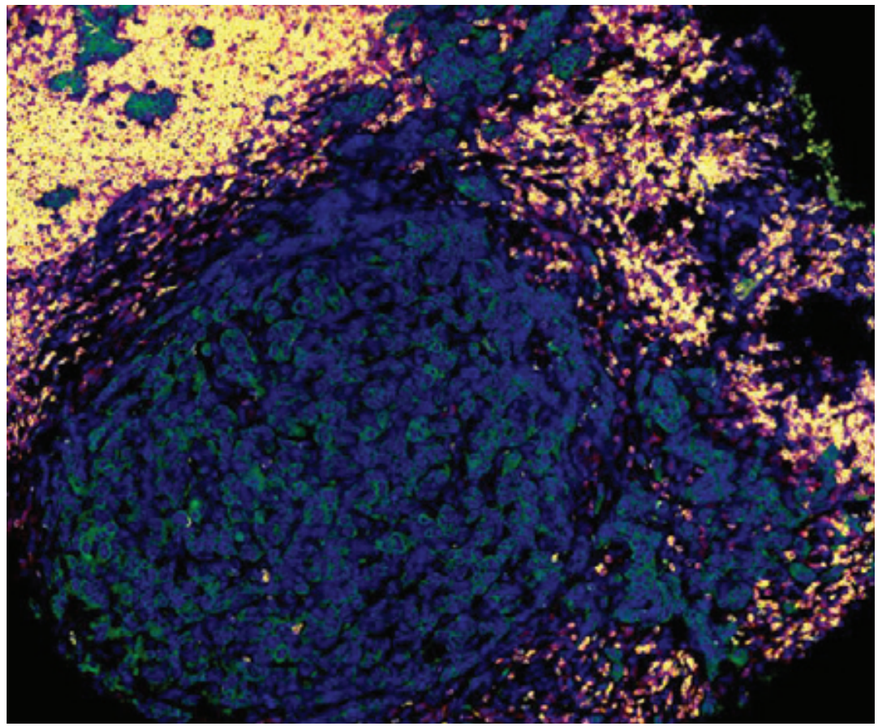
Maltas J, Reed H, Porter A, Malliri A.
Mechanisms and consequences of dysregulation of the Tiam family of Rac activators in disease.
Biochemical Society Transactions [Epub 16 November 2020]

Drug Discovery
(page 28)
Caroline Springer

Refereed research publications
Saturno G, Lopes F, Niculescu-Duvaz I, Niculescu-Duvaz D, Zambon A, Davies L, Johnson L, Preece N, Lee R, Viros A, Holovanchuk D, Pedersen M, McLeary R, Lorigan P, Dhomen N, Fisher C, Banerji U, Dean E, Krebs MG, Gore M, Larkin J, Marais R, Springer C.
The paradox-breaking panRAF plus SRC family kinase inhibitor, CCT3833, is effective in mutant KRAS-driven cancers.
Annals of Oncology [Epub 29 October 2020]

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 Christopher Bromley (Cancer Inflammation and Immunity)



Leukaemia Biology
(page 32)
Tim Somervaille

Refereed research publications
Williams MS, Basma NJ, Amaral FMR, Williams G, Weightman JP, Breitwieser W, Nelson L, Taylor SS, Wiseman DH, Somervaille TCP. (2020)
Targeted nanopore sequencing for the identification of ABCB1 promoter translocations in cancer.
BMC Cancer 20(1):1075.

Paredes R, Kelly JR, Geary B, Almarzouq B, Schneider M, Pearson S, Narayanan P, Williamson A, Lovell SC, Wiseman DH, Chadwick JA, Jones NJ, Kustikova O, Schambach A, Garner T, Amaral FMR, Pierce A, Stevens A, Somervaille TCP, Whetton AD, Meyer S. (2020)
EVI1 phosphorylation at S436 regulates interactions with CtBP1 and DNMT3A and promotes self-renewal.
Cell Death and Disease 11(10):878.

Salamero O, Montesinos P, Willekens C, Pérez-Simón JA, Pigneux A, Récher C, Popat R, Carpio C, Molinero C, Mascaró C, Vila J, Arévalo MI, Maes T, Buesa C, Bosch F, Somervaille TCP. (2020)
First-in-Human Phase I study of Iadademstat (ORY-1001): A first-in-class lysine-specific histone demethylase 1a inhibitor, in relapsed or refractory acute myeloid leukemia.
Journal of Clinical Oncology 38(36):4260-4273.

Paredes R, Schneider M, Pearson S, Teng HY, Kelly JR, Pierce A, Somervaille TCP, Whetton AD, Meyer S. (2020)
EVI1 oncoprotein expression and CtBP1-association oscillate through the cell cycle.
Molecular Biology Reports 47(10):8293-8300.

Wiseman DH, Baker SM, Dongre AV, Gurashi K, Storer JA, Somervaille TC, Batta K. (2020)
Chronic myelomonocytic leukaemia stem cell transcriptomes anticipate disease morphology and outcome.
EBioMedicine 58:102904.

Schwaab J, Naumann N, Luebke J, Jawhar M, Somervaille TCP, Williams MS, Frewin R, Jost PJ, Lichtenegger FS, La Rosée P, Storch N, Haferlach T, Horny HP, Fabarius A, Haferlach C, Burchert A, Hofmann WK, Cross NCP, Hochhaus A, Reiter A, Metzgeroth G. (2020)
Response to tyrosine kinase inhibitors in myeloid neoplasms associated with PCM1-JAK2, BCR-JAK2 and ETV6-ABL1 fusion genes.
American Journal of Hematology 95(7):824-833.

Williams MS, Somervaille TCP. (2020)
Dynamic induction of drug resistance through a stress-responsive enhancer in acute myeloid leukemia.
Molecular & Cellular Oncology 7(2):1705730.

Molecular Oncology
(page 34)
Richard Marais

Refereed research publications
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The paradox-breaking panRAF plus SRC family kinase inhibitor, CCT3833, is effective in mutant KRAS-driven cancers.
Annals of Oncology [Epub 29 October 2020]

Lee RJ, Khandelwal G, Baenke F, Cannistraci A, Macleod K, Mundra P, Ashton G, Mandal A, Viros A, Gremel G, Galvani E, Smith M, Carragher N, Dhomen N, Miller C, Lorigan P, Marais R. (2020)
Brain microenvironment-driven resistance to immune and targeted therapies in acral melanoma.
ESMO Open 5(4):e000707.

Trucco LD, Mundra PA, García-Martínez P, Hogan K, Baenke F, Dhomen N, Pavet V, Marais R.
Map3k1 loss cooperates with BrafV600E to drive melanomagenesis.
Journal of Investigative Dermatology [Epub 22 May 2020]

Valpione S, Galvani E, Tweedy J, Mundra PA, Banyard A, Middlehurst P, Barry J, Mills S, Salih Z, Weightman J, Gupta A, Gremel G, Baenke F, Dhomen N, Lorigan PC, Marais R. (2020)
Immune awakening revealed by peripheral T cell dynamics after one cycle of immunotherapy.
Nature Cancer 1(2):210-221.

Galvani E, Mundra PA, Valpione S, Garcia-Martinez P, Smith M, Greenall J, Thakur R, Helmink B, Andrews MC, Boon L, Chester C, Gremel G, Hogan K, Mandal A, Zeng K, Banyard A, Ashton G, Cook M, Lorigan P, Wargo JA, Dhomen N, Marais R. (2020)
Stroma remodeling and reduced cell division define durable response to PD-1 blockade in melanoma.
Nature Communications 11(1):853.

Litchfield K, Stanislaw S, Spain L, Gallegos LL, Rowan A, Schnidrig D, Rosenbaum H, Harle A, Au L, Hill SM, Tippu Z, Thomas J, Thompson L, Xu H, Horswell S, Barhoumi A, Jones C, Leith KF, Burgess DL, Watkins TBK, Lim E, Birkbak NJ, Lamy P, Nordentoft I, Dyrskjøt L, Pickering L, Hazell S, Jamal-Hanjani M; PEACE Consortium, Larkin J, Swanton C, Alexander NR, Turajlic S. (2020)
Representative sequencing: unbiased sampling of solid tumor tissue.
Cell Reports 31(5):107550.

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Stahel RA, Lacombe D, Cardoso F, Casali PG, Negrouk A, Marais R, Hiltbrunner A, Vyas M; Clinical Academic Cancer Research Forum (CAREFOR). (2020)
Current models, challenges and best practices for work conducted between European academic cooperative groups and industry.
ESMO Open 5(2):e000628.

Prostate Oncobiology

(page 36)
Esther Baena

Refereed research publications

Murphy RG, Roddy AC, Srivastava S, Baena E, Waugh DJ, M O'Sullivan J, McArt DG, Jain S, LaBonte MJ. (2020)
Prostate cancer heterogeneity assessment with multi-regional sampling and alignment-free methods.
NAR Genomics & Bioinformatics 2(3):lqaa062.

Mevel R, Steiner I, Mason S, Galbraith LC, Patel R, Fadlullah MZ, Ahmad I, Leung HY, Oliveira P, Blyth K, Baena E, Lacaud G. (2020)
RUNX1 marks a luminal castration-resistant lineage established at the onset of prostate development.
Elife 9:e60225.

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Lie-A-Ling M, Mevel R, Patel R, Blyth K, Baena E, Kouskoff V, Lacaud G. (2020)
RUNX1 dosage in development and cancer.
Molecules and Cells 43(2):126-138.

Skin Cancer and Ageing

(page 38)
Amaya Virós

Refereed research publications

Saturno G, Lopes F, Niculescu-Duvaz I, Niculescu-Duvaz D, Zambon A, Davies L, Johnson L, Preece N, Lee R, Viros A, Holovanchuk D, Pedersen M, McLeary R, Lorigan P, Dhomen N, Fisher C, Banerji U, Dean E, Krebs MG, Gore M, Larkin J, Marais R, Springer C.
The paradox-breaking panRAF plus SRC family kinase inhibitor, CCT3833, is effective in mutant KRAS-driven cancers.
Annals of Oncology [Epub 29 October 2020]

Lee RJ, Khandelwal G, Baenke F, Cannistraci A, Macleod K, Mundra P, Ashton G, Mandal A, Viros A, Gremel G, Galvani E, Smith M, Carragher N, Dhomen N, Miller C, Lorigan P, Marais R. (2020)
Brain microenvironment-driven resistance to immune and targeted therapies in acral melanoma.
ESMO Open 5(4):e000707.

Lotz M, Budden T, Furney SJ, Virós A.
Molecular subtype, biological sex and age shape melanoma tumour evolution.
British Journal of Dermatology [Epub 8 June 2020]

Other publications

Craig S, Virós A. (2020)
New biomarkers improve stratification of patients with melanoma.
British Journal of Dermatology 182(1):5-6.

Stem Cell Biology

(page 40)
Georges Lacaud

Refereed research publications

Mevel R, Steiner I, Mason S, Galbraith LC, Patel R, Fadlullah MZ, Ahmad I, Leung HY, Oliveira P, Blyth K, Baena E, Lacaud G. (2020)
RUNX1 marks a luminal castration-resistant lineage established at the onset of prostate development.
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Maqbool MA, Pioger L, El Aabidine AZ, Karasu N, Molitor AM, Dao LTM, Charbonnier G, van Laethem F, Fenouil R, Koch F, Lacaud G, Gut I, Gut M, Amigorena S, Joffre O, Sexton T, Spicuglia S, Andrau JC. (2020)
Alternative enhancer usage and targeted polycomb marking hallmark promoter choice during T cell differentiation.
Cell Reports 32(7):108048.

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Lacaud G. (2020)
Decoding hematopoietic stem cells' birth.
Blood 136(7):775-776.

Lie-A-Ling M, Mevel R, Patel R, Blyth K, Baena E, Kouskoff V, Lacaud G. (2020)
RUNX1 dosage in development and cancer.
Molecules and Cells 43(2):126-138.

Systems Oncology

(page 42)
Claus Jørgensen

Refereed research publications

Chastney MR, Lawless C, Humphries JD, Warwood S, Jones MC, Knight D, Jorgensen C, Humphries MJ. (2020)
Topological features of integrin adhesion complexes revealed by multiplexed proximity biotinylation.
Journal of Cell Biology 219(8):e202003038.
Kershaw S, Morgan DJ, Boyd J, Spiller DG, Kitchen G, Zindy E, Iqbal M, Rattray M, Sanderson CM, Brass A, Jorgensen C, Hussell T, Matthews LC, Ray DW. (2020)
Glucocorticoids rapidly inhibit cell migration through a novel, non-transcriptional HDAC6 pathway.
Journal of Cell Science 133(11):jcs242842.

Wagner MJ, Hsiung MS, Gish GD, Bagshaw RD, Doodnauth SA, Soliman MA, Jørgensen C, Tucholska M, Rottapel R.J. (2020)
The Shb scaffold binds the Nck adaptor protein, p120 RasGAP, and Chimaerins and thereby facilitates heterotypic cell segregation by the receptor EphB2.
The Journal of Biological Chemistry 295(12):3932-3944.

Other publications

Sahai E, Astsaturov I, Cukierman E, DeNardo DG, Egeblad M, Evans RM, Fearon D, Greten FR, Hingorani SR, Hunter T, Hynes RO, Jain RK, Janowitz T, Jorgensen C, Kimmelman AC, Kolonin MG, Maki RG, Powers RS, Puré E, Ramirez DC, Scherz-Shouval R, Sherman MH, Stewart S, Tlsty TD, Tuveson DA, Watt FM, Weaver V, Weeraratna AT, Werb Z. (2020)
A framework for advancing our understanding of cancer-associated fibroblasts.
Nature Reviews Cancer 20(3):174-186.

Transcriptional Networks in Lung Cancer

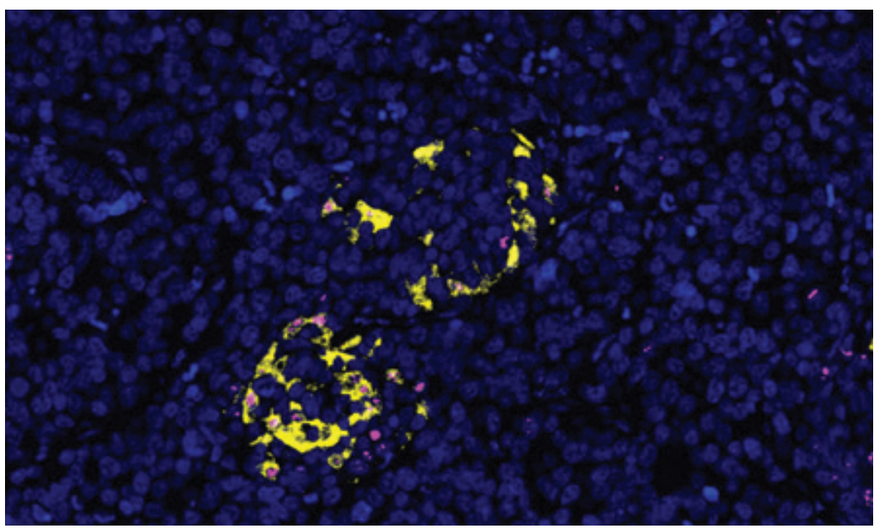
(page 44)
Michela Garofalo

Refereed research publications

Paliouras AR, Buzzetti M, Shi L, Donaldson IJ, Magee P, Sahoo S, Leong HS, Fassan M, Carter M, Di Leva G, Krebs MG, Blackhall F, Lovly CM, Garofalo M. (2020)
Vulnerability of drug-resistant EML4-ALK rearranged lung cancer to transcriptional inhibition.
EMBO Molecular Medicine 12(7):e11099.

SCLC CDX tissue, YAP1 staining in yellow and REST staining in pink, with DAPI blue.

Image supplied by Sarah Pearsall (Cancer Biomarker Centre)



Other publications

Ginn L, Shi L, Montagna M, Garofalo M. (2020)
LncRNAs in non-small-cell lung cancer.
Non-coding RNA. 6(3):25.

La Montagna M, Ginn L, Garofalo M.
Mechanisms of drug resistance mediated by long non-coding RNAs in non-small-cell lung cancer.
Cancer Gene Therapy [Epub 25 August 2020]

Translational Oncogenomics

(page 46)
Rob Bristow

Other publications

Rubin MA, Bristow RG, Thienger PD, Dive C, Imielinski M. (2020)
Impact of lineage plasticity to and from a neuroendocrine phenotype on progression and response in prostate and lung cancers.
Molecular Cell 80(4):562-577.

Ashton J, Bristow R. (2020)
Bad neighbours: hypoxia and genomic instability in prostate cancer.
British Journal of Radiology 93(1115):20200087.

Rebello RJ, Bristow RG. (2020)
Intermediate risk prostate cancer: disease heterogeneity linked to measurable biological features.
Clinical Oncology (Royal College of Radiologists) 32(5):298-302.

Tumour Supressors

(page 48)
Patricia Muller

Refereed research publications

O'Regan L, Barone G, Adib R, Woo CG, Jeong HJ, Richardson EL, Richards MW, Muller PAJ, Collis SJ, Fennell DA, Choi J, Bayliss R, Fry AM. (2020)
EML4-ALK V3 oncogenic fusion proteins promote microtubule stabilization and accelerated migration through NEK9 and NEK7.
Journal of Cell Science 133(9):jcs241505.

Select additional publications

Johnson SM, Banyard A, Smith C, Mironov A, McCabe MG. (2020)
Large extracellular vesicles can be characterised by multiplex labelling using imaging flow cytometry.
International Journal of Molecular Sciences 21(22):8723.

Xu H, Medina-Sánchez M, Zhang W, Seaton MPH, Brison DR, Edmondson RJ, Taylor SS, Nelson L, Zeng K, Bagley S, Ribeiro C, Restrepo LP, Lucena E, Schmidt CK, Schmidt OG. (2020)
Human spermbots for patient-representative 3D ovarian cancer cell treatment.
Nanoscale 12(39):20467-20481.

Bagnall J, Rowe W, Alachkar N, Roberts J, England H, Clark C, Platt M, Jackson DA, Muldoon M, Paszek P. (2020)
Gene-specific linear trends constrain transcriptional variability of the toll-like receptor signaling.
Cell Systems 11(3):300-314.e8.

McHugh DJ, Lipowska-Bhalla G, Babur M, Watson Y, Peset I, Mistry HB, Hubbard Cristinacce PL, Naish JH, Honeychurch J, Williams KJ, O'Connor JPB, Parker GJM. (2020)
Diffusion model comparison identifies distinct tumor sub-regions and tracks treatment response.
Magnetic Resonance in Medicine 84(3):1250-1263.

Gilmartin AG, Groy A, Gore ER, Atkins C, Long ER 3rd, Montoute MN, Wu Z, Halsey W, McNulty DE, Ennulat D, Rueda L, Pappalardi M, Kruger RG, McCabe MT, Raoof A, Butlin R, Stowell A, Cockerill M, Waddell I, Ogilvie D, Luengo J, Jordan A, Benowitz AB.
In vitro and in vivo induction of fetal hemoglobin with a reversible and selective DNMT1 inhibitor.
Haematologica [Epub 25 June 2020]

Newton R, Waszkowycz B, Seewooruthun C, Burschowsky D, Richards M, Hitchin S, Begum H, Watson A, French E, Hamilton N, Jones S, Lin LY, Waddell I, Echalier A, Bayliss R, Jordan AM, Ogilvie D. (2020)
Discovery and optimization of wt-RET/KDR-selective inhibitors of RET^{V804M}Kinase.
ACS Medicinal Chemistry Letters 11(4):497-505.

Zhang M, Lai Y, Vasquez JL, James DI, Smith KM, Waddell ID, Ogilvie DJ, Liu Y, Agoulnik IU. (2020)
Androgen receptor and poly(ADP-ribose) glycohydrolase inhibition increases efficiency of androgen ablation in prostate cancer cells.
Scientific Reports 10(1):3836.

Joshi SR, Kitagawa A, Jacob C, Hashimoto R, Dhagia V, Ramesh A, Zheng C, Zhang H, Jordan A, Waddell I, Leopold J, Hu CJ, McMurtry IF, D'Alessandro A, Stenmark KR, Gupte SA. (2020)
Hypoxic activation of glucose-6-phosphate dehydrogenase controls the expression of genes involved in the pathogenesis of pulmonary hypertension through the regulation of DNA methylation.
American Journal of Physiology. Lung Cellular & Molecular Physiology 318(4):L773-L786.

Hashimoto R, Lanier GM, Dhagia V, Joshi SR, Jordan A, Waddell I, Tuder R, Stenmark KR, Wolin MS, McMurtry IF, Gupte SA. (2020)
Pluripotent hematopoietic stem cells augment α-adrenergic receptor-mediated contraction of pulmonary artery and contribute to the pathogenesis of pulmonary hypertension.
American Journal of Physiology. Lung Cellular & Molecular Physiology 318(2):L386-L401.

Hoang SM, Kaminski N, Bhargava R, Barroso-González J, Lynskey ML, García-Expósito L, Roncaioli JL, Wondisford AR, Wallace CT, Watkins SC, James DI, Waddell ID, Ogilvie D, Smith KM, da Veiga Leprevost F, Mellacharevu D, Nesvizhskii AI, Li J, Ray-Gallet D, Sobol RW, Almouzni G, O'Sullivan RJ. (2020)
Regulation of ALT-associated homology-directed repair by polyADP-ribosylation.
Nature Structural & Molecular Biology 27(12):1152-1164.

Cristea S, Coles GL, Hornburg D, Gershkovitz M, Arand J, Cao S, Sen T, Williamson SC, Kim JW, Drainas AP, He A, Cam LL, Byers LA, Snyder MP, Contrepois K, Sage J. (2020)
The MEK5-ERK5 kinase axis controls lipid metabolism in small-cell lung cancer.
Cancer Research 80(6):1293-1303.

EXTERNAL SEMINAR SPEAKERS 2020

The seminar series that we run is vital for the Institute, connecting world-class researchers across the broad spectrum of cancer research. Despite the challenges of maintaining connectivity with the research community remotely during the Covid-19 pandemic, we have still managed to enjoy meaningful scientific interaction with an excellent set of internationally renowned speakers via a digital platfrom. The postdoctoral researchers and technical staff at the Institute also continued to give weekly seminars, which were especially important in bringing our scientists together and to help integrate the entire cancer research efforts of the Institute.

Alberto Bardelli
The FIRC Institute of Molecular Oncology

Joan Seoane
Vall d´Hebron Institute of Oncology

Greg Hannon
CRUK Cambridge Institute

Ultan McDermott
Wellcome Trust Sanger Institute

Margaret Frame
CRUK Edinburgh Centre

Martin Eilers
University of Würzburg

Marco Gerlinger
The Institute of Cancer Research

David Adams
Wellcome Trust Sanger Institute

Peter Sarkies
London Institute of Medical Sciences

Gillian Griffiths
Cambridge Institute of Medical Research

Jonathan Houseley
The Babraham Institute

Kostas Kostarelos
The University of Manchester

Adele Fielding
University College London

Clare Isacke
Breast Cancer Now Research Centre

Bradley Bernstein
The Broad Institute

Elizabeth Patton
The University of Edinburgh

Sui Huang
Institute for Systems Biology

Uri Alon
Weizmann Institute of Science

Simon McDade
Queen's University Belfast

Sean Bendall
Stanford University

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 rose extremely well to the challenges imposed by 2020 and adapted quickly to remote ways of working to continue to provide an operational platform that facilitates the smooth running of the Institute. Early in the first lockdown, many of the team helped colleagues at the Medicines Discovery Catapult to set up the Lighthouse COVID-19 testing laboratory at Alderley Park, with over 30 members of CRUK MI volunteering in the first cohort of staff. While most of the Institute worked at home from the end of March to the beginning of June, our Logistics team continued to work on site to ensure our scientific infrastructure was properly maintained and to support the long-term studies in our Biological Resources Unit. Special mention should also be made of our Health and Safety team who worked tirelessly to set up COVID-secure ways of working for all. Throughout this time, we have benefited from discussions and pooling knowledge with our colleagues across the wider University of Manchester network as well as at our sister CRUK Institutes as we navigate adapting to these new ways of working.

Several members of the operations team formed the core of the Institute's COVID-19 management team, which met daily throughout most of the year to oversee our response and in parallel, carried out contingency planning with respect to a potential no-deal scenario following Britain's withdrawal from the EU. The resilience and disaster management experience gained from the Paterson Building fire in 2017 greatly informed our response to the events of 2020 and is also an experience that we continue to share with other institutes to help shape their emergency response planning.

During the year, the team welcomed Soraya Francis, Krar Haider and Christopher McCauley and they are yet to meet most of their colleagues in person. We look forward to welcoming them to the team properly during 2021 and to reuniting with the wider team and our colleagues across the Institute.

General Administration Team

Ruth Cox, Samantha Brandolani[2], Maria Belen Conti[3], Jayne Fowler, Soraya Francis[1], Delydd Jones[2]

[1]Joined in 2020
[2]Left in 2020
[3]Joint with Scientific Administration Team

This year the Administration Team have worked incredibly hard to ensure that the Institute has remained connected, engaged and productive while working from home. They quickly became proficient in organising Zoom meetings, seminars and workshops, and modified our regular events into a virtual format. As a team they support the Director and the Institute's Group Leaders day-to-day and have helped organised the Institute Colloquium, Christmas party, and supported a range of Education and Engagement events for staff and students throughout the year. They have also supported a safe return to work for lab-based staff by facilitating our new

booking systems for reduced occupancy transport and workspaces.

Belen Conti is Executive Assistant to the Senior Management Team, Jayne Fowler is Executive Assistant to the Director of the Drug Discovery Unit, and Delydd Jones was our Administration Services Coordinator until April. At the start of the year, we recruited Soraya Francis to replace Delydd, who took up the role of Personal Assistant to Professor Caroline Dive. Unfortunately, Soraya has not yet had the chance to work from our office in Alderley Park, or meet anyone face-to-face, but she has been a fantastic addition to the team, becoming an invaluable Zoom expert, organising our regular staff updates, and ensuring the external seminar series continued in a virtual format. We have hosted a varied programme of national and international speakers and are grateful to all of our invited speakers for committing their time to give talks. Details can be found at www.cruk.manchester.ac.uk/seminars.

We said goodbye to Samantha Brandolani, who temporarily covered Ruth Cox's role as Executive Assistant to the Institute Director and provided invaluable support during the challenges of the year. Towards the end of the year, we have been preparing to support Caroline Dive as the Interim Director of the Institute as Richard Marais steps down. Richard has been an inspiring, kind and supportive boss and we wish him all the best.

Finance and Purchasing

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

As the Institute began to settle into maximum functionality following the transition to Alderley Park after the fire, we were suddenly impacted by the breakout of the COVID-19 pandemic. The effect of the pandemic was largely two-fold on the Institute's finances. Firstly, this took shape in managing the transition to working from home for a large portion of the Institute, including additional IT spend, managing furlough funding and dealing with changes in procurement procedures, as well as adapting to the changes in supply and demand as many industries across the globe followed the same strategy.

The second and more difficult impact of the pandemic was on our core funding. Following the initial UK lockdown between March and June 2020, fundraising by charitable organisations saw a dramatic fall, leading to overall reduced charitable funding for research. Consequently, the finance team had to take immediate action to help plan and prepare budgets and financial statements to allow the Institute to re-assess its available funds and redistribute them to minimise as much as possible the disruption to our scientific research and staff.

The Institute continues to support the Director and the management of the £28m budget while providing costs and advice for new research proposals and contracts for all of our groups. A review of core facilities is still ongoing to assess and improve the financial management and facilitate maximum scientific output for the budget. Despite global financial pressures, we have been successful in receiving a number of new awards, with several million pounds flowing to the Institute in relation to outstanding research applications and agreements.

In addition to the pandemic, the exit from the EU continued to cause repercussions around our finances. We regularly review the changes to the financial regulations and procedures borne out of these circumstances and, given our continued collaboration with a large number of European entities, we manage the ongoing workload required in assessing and adjusting to the consequences of leaving the EU.

Human Resources

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

[1]Left in 2020
[2]Returned from maternity leave in 2020
[3]Joint with Scientific Administration

Over the past year, the HR Department has continued to deliver a high quality and 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. Throughout this year, the department has created, developed and adapted to new ways of working to ensure our proactive service continued to support staff and the Institute whilst working remotely.

During 2020, we successfully recruited 47 individuals into the Institute and facilitated the successful promotion of five individuals. The Institute has continued its commitment to develop our staff and ensure that Personal Development Reviews (Contribution Reviews) are undertaken, and in 2020, we had a 93.5% completion rate.

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 with the aim of ensuring consistency and benchmarking across the research sector.

An additional responsibility for the department this year has been supporting staff and the Institute throughout the COVID-19 pandemic. The department played an active part in the roll out of the new COVID-19 Health and Safety induction. We continued to provide advice and support to staff, with a specific focus on ensuring that wellbeing and mental health is supported, especially during the pandemic. Further, we have provided additional flexible working support to staff whilst working from home and those with childcare/caring responsibilities. We have also continued to provide support to our EU staff during the uncertain time as the UK prepared to leave the European Union.

Next year, the focus will be on a review of the Personal Development Reviews' process and the recruitment of a new research group in line with the Institute's research strategy.

Information Technology

Steve Royle, Matthew Young, Brian Poole, Krar Haider[1]

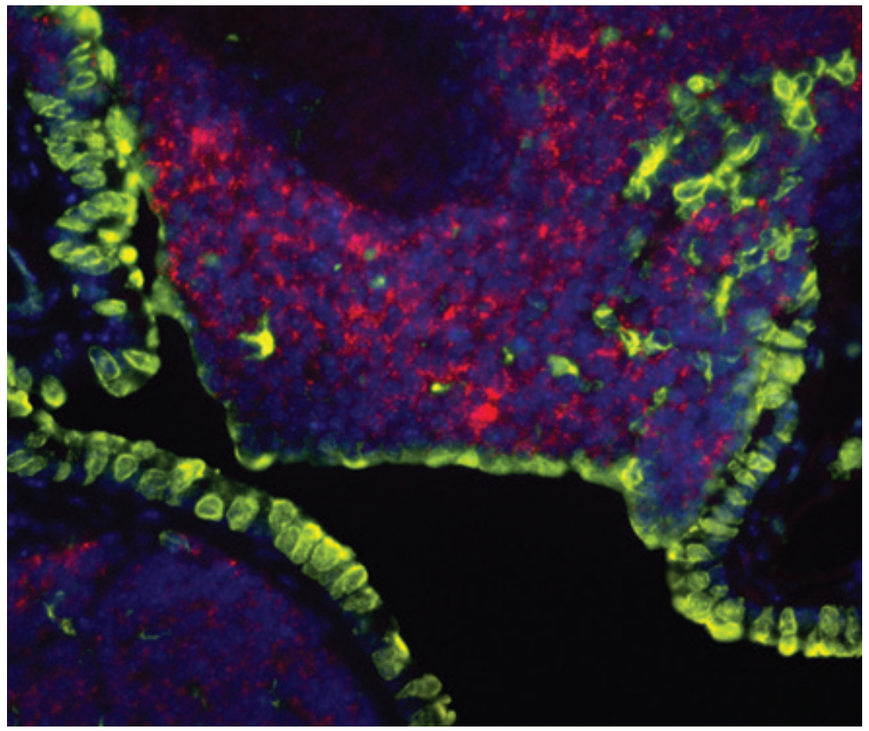
[1]Joined in 2020

The CRUK Manchester Institute Core IT is small team of four experienced IT professionals, who strive to ensure that we provide excellent IT support and customer service to the whole organisation. The team provides a wide range of IT support services to over 400 research and support staff, currently spread across several sites. This includes manned 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.

2020 was another year of change for CRUK MI Core IT team, not least due to the COVID-19

SCLC GEMM lung stained for the neuroendocrine (NE) marker ChromagraninA (CHGA) by in situ hybridization (red) which marks up the tumour, and CC10 (yellow) which marks up the lung airway. DAPI in blue.

Image supplied by Sarah Pearsall (Cancer Biomarker Centre) and Caron Behan (Histology)



pandemic and the changes we implemented to allow all our staff to work from home. During the pandemic, Zoom became our new virtual meeting room tool of choice, and our staff rapidly became competent with this video conferencing platform.

With the majority of our staff mostly working from home over the last 12 months, we have continued to provide the same high level of support using a selection of new and existing remote support tools. During the year we rolled out Office 365 across the Institute. This provided all our staff with the latest versions of the MS Office Apps and 'cloud' based email, plus another video conferencing app, MS Teams. During this period, we also continued with our Windows 10 upgrade programme. Whilst these rollouts did present some operational challenges, they were instrumental in supporting remote working and working from home.

We currently manage over 600 desktop computers, comprising a mixture of Windows PCs and laptops, Apple iMacs and Mac Books, plus a growing number of tablet devices, mainly Apple iPads and iPhones. All these devices are centrally authenticated, with access to a central file-store, a server farm and network printing. All desktop and portable devices are built on Windows 10, MacOS Big Sur or Catalina.

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

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. Further, our adoption of 'self-service' application installation now enables research staff to resolve a significant number of IT Service Requests themselves. Going forward, we plan to develop these and other services further to improve our IT support service.

Safety and Facilities Management

Colin Gleeson

Health and Safety

Colin Gleeson, Chris Bamber

Health and Safety initiatives over the previous twelve months have been mainly concerned with our response to the Coronavirus pandemic. This included managing the safe shutdown of the Institute in the first lockdown. This was then followed by significant engagement with Institute senior managers to establish a COVID strategy group, which focused on the development of our re-opening strategies. Health and safety formed a cornerstone of these developments so we could open as a COVID-safe workplace. Accordingly, a COVID risk assessment was developed, along with other plans and accompanying documentation around COVID-safe workplace arrangements, concerned with social distancing and hygiene measures, reduced occupancy laboratory work, supervision arrangements, and close proximity work where social distancing was not possible. This was conveyed to staff via regular Zoom-based staff updates and the re-Induction of all staff to our COVID-safe workplace. We re-opened cautiously and monitored the workplace for compliance with these new measures and also for any signs of clusters of infection. To this end we also put in place a bespoke track and trace system within our workspace, which could identify workers who may have been exposed to Coronavirus via a colleague who had subsequently tested positive. Utilising our track and trace system throughout the pandemic shows that we have no evidence of any onward transmission at work, demonstrating that our COVID-safe workplace arrangements were effective.
As the situation improved, we allowed an increase in laboratory occupancy whilst maintaining our COVID-safe workplace measures, including two-metre social distancing. Throughout the pandemic any work which did not require site access was undertaken at home. Home workplace and home office arrangements were assessed, and pragmatic advice given on how to best set up the home-working space; this included home and desk exercises to alleviate what could become a more sedentary work day for many people.

We have been minded to maintain contact with staff working from home throughout the pandemic via all-staff zoom staff updates. Individual research groups have also had regular zoom meetings. This has been, in part, to help alleviate the isolation of staff and help with overall wellbeing.

Electronics

Yunis Al-hassan

As part of the Institute's electrical and fire safety strategies, the electronics engineer continued working almost as normal throughout the pandemic. PAT testing and equipment repairs have continued albeit at a lower frequency due to low occupancy of the workplace during the pandemic. Thus, the repair facility continues to provide a significant economic benefit to the Institute in that unnecessary expenditure on replacement equipment is avoided. The Institute's 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.

Laboratory Services

Mark Craven, Busola Atuegbe, Corinne Hand, Petra Kubinova and Christine Whitehurst

During 2020, the Institute had to adjust to the disruptions caused by the impact of the COVID-19 pandemic.

When permitted, the department based at OCRB remained open under safe working practices and supplied the various sites with their required items. We supply sterile glassware, plastics and bespoke microbiological media to the scientists at OCRB and the expanded lab sites at the Tumour Implantation Facility, Kay Kendall Laboratory, WMIC Building, the Incubator Building and the Proton Beam Centre.

Whilst we are located at Alderley Park, we support our scientists, alongside the on-site Avantor team, with sterile plastics and bespoke microbiological media.

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

- maintain and service two photographic dark rooms, one in OCRB and one at AP
- provide a drop-in monthly Pipette Clinic at both OCRB and AP
- organise the delivery of clean, general and tissue culture lab coats across the 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[1], Wayne Howarth, Jonathan Lloyd, Robin Sherratt, Tony Woollam[1]

[1]Retired in 2020

The Logistics team has continued to deliver an efficient and reactive service, providing support for the research activity carried out at Alderley Park and OCRB. The team have also provided some level of support to staff based at the Incubator Building, WMIC Building, Proton Beam Centre and the MCRC Tissue Biobank team located in the Kay Kendall Laboratories.

The support provided includes the receipting, checking, booking in and distribution of goods ordered by staff. The team facilitate the delivery of dry ice and gas cylinders are monitored and replaced, as necessary. The team monitors the liquid nitrogen levels in the cell storage tanks and replenishes when required. In response to the impact of COVID-19, we have increased our gas holding stocks and increased the levels of nitrogen in the cell storage tanks to provide extra resilience.

One of the key tasks carried out by the team over the past year is the transport of samples and goods between various research locations and core facility groups. The team make daily collections of time-sensitive samples from the Christie NHS Foundation Trust CTU department and support the transportation of animals from the Incubator Building to Alderley Park at least once a week. The team have worked alongside Lab Services in delivering the sterile media and glassware and returning the empties and recyclable plastics.

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). To support safe working, new items of PPE such as hand sanitiser and disinfectant wipes, have been added to the stores catalogue.

The Logistics team also undertook preparatory work ahead of Brexit. We contacted our regular suppliers, requesting a 'Brexit statement' and contingency plans. We used this information and stockpiled where possible on the high demand products. This preparation has meant we have been able to maintain a good supply of stocked items in stores.

Scientific Administration

Caroline Wilkinson, Christopher McCauley[1], Maria Belen Conti Vyas[2], Gillian Campbell, Julie Edwards, Steve Morgan, David Stanier[3]

[1]Joined in 2020
[2]Joint with the General Administration Team
[3]Joint with HR

The team has all worked from home since end of March 2020 and has adapted well to converting many in person activities to online formats. A particular triumph was organising the annual Institute colloquium as a virtual event. We used the opportunity to introduce some new features which proved popular, including inviting some of our alumni for a careers' discussion. It was a pleasure to welcome back some familiar faces, now located across the world and working in both academic and industry settings.

In the summer we welcomed Web Developer Chris McCauley to the team. He has been busy updating some of our bespoke web applications such as our PhD recruitment portal and our staff recruitment portal, JobMarker, as well as supporting our intranet, *The Hub*, which provides a wide variety of functions including our HR reporting systems.

David Stanier was promoted to Information Governance Coordinator and Administrative Officer supporting the Institute's Information Governance Guardian, Caroline Wilkinson, with the management of information security, data protection and record management to ensure information governance disciplines are embedded within working practice across the Institute. To facilitate this, David regularly liaises with the University's Data Protection Officer and Information Governance Office over best practice. David also played a crucial role in co-ordinating arrangements for our new ways of working and logging records pertaining to our COVID-secure ways of working and co-ordinating our shuttle bus service to Alderley Park.

The team also manage communications for the Institute and over the year produced newsletters, this report, managed our social media accounts, our external website, oversaw press releases and liaison with CRUK and University Press Officers and approved any other external communications involving the Institute's staff and students. Belen Conti Vyas continued with her series of videos of staff and students describing life at the Institute which have been well received on twitter.

Gill Campbell is our Grants Adviser who provides support for the Institute's scientists to supplement their core CRUK funding through external awards. A total of 30 grant applications were submitted in 2020, with eight of those being successfully funded, plus a further three grants were also awarded that had been submitted in the previous year. Of particular note, Amaya Virós was awarded funding from the Royal Society to look at how sex shapes the molecular landscape of subcutaneous skin cancer; Caroline Dive will explore how liquid biopsies can be used to support the management of Ewing's Sarcomas with a grant from the charity Friends of Rosie; and finally, Dónal Landers and the digital ECMT were awarded Horizon 2020 funding as part of EU consortium 'Building Data Rich Clinical Trials' led by Vall d'Hebron Institute of Oncology to help deliver novel methods for the design and implementation of newer, more efficient and effective clinical trials in oncology. The grant application process is overseen by our Grants Committee, chaired by Iain Hagan, who provide critical input for all of our applications and provide feedback for practice interviews related to funding awards. Gill Campbell has also been part of an Institute team preparing an exhibit for the Royal Society Summer Showcase on the theme of the tumour microenvironment. The team, comprising post-doctoral research fellows, PhD students and representation from our core facilities had been accepted to present at this prestigious event in 2020, which was understandably cancelled but are now preparing digital content for an online public engagement experience in summer 2021.

Our Postgraduate Education Manager, Julie Edwards had a busy year helping co-ordinate extensions for PhD students due to lost time during the lockdown lab closure and managing all aspects of our PhD programme detailed elsewhere in this report. PhD vivas were conducted online while we managed to undertake the recruitment round in February 2020 at Alderley Park for our next cohort of students.

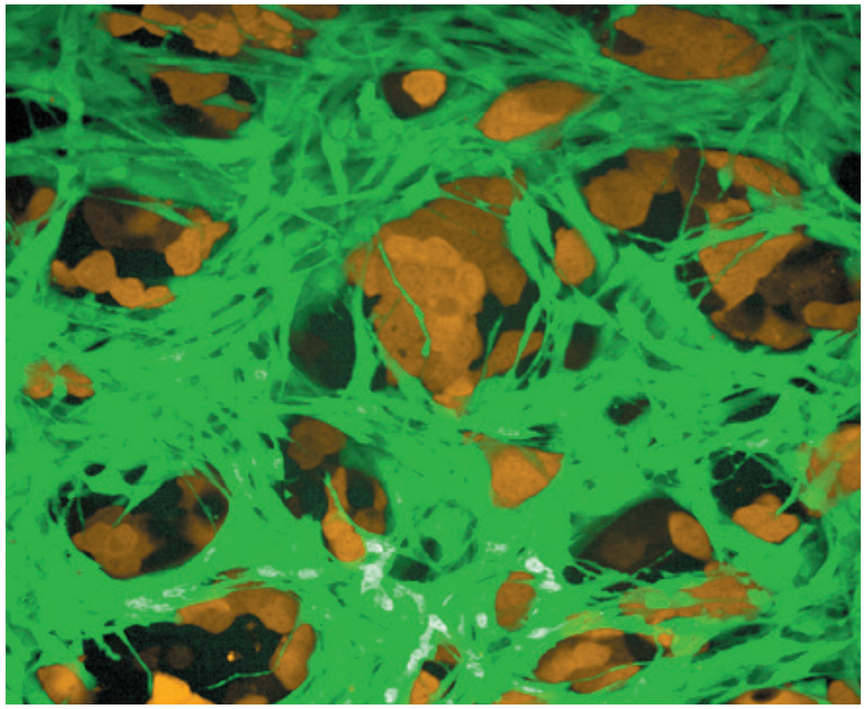
Steve Morgan returned to his reception duties at the Oglesby Cancer Research Building once the University's buildings started to open again post-lockdown and continued in his role there alongside staff from the University's Faculty of Biology, Medicine and Health running the reception service and the Institute's switchboard.

Towards the end of the year the team recruited a new member, Andrew Porter who begins in the new role of Research Integrity and Training Adviser in 2021 to support CRUK MI scientists in maintaining the highest research integrity and oversee additional training opportunities, particularly for the Early Career Researcher community.

Animal Welfare

Caroline Wilkinson, Establishment Licence Holder; **Simon Poucher**, Regulatory Liaison and Training Officer; **Janet Watson**, Animal Welfare and 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). The AWERB supports all staff involved with animal research, ensuring the provision of appropriate management structures and processes, staff training, and facilities for the care and use of mice, and encourages implementation of the 3Rs (replacement, reduction and refinement of the use of animals). It also reviews the ethics of proposed collaborations and grant



Fibroblasts (green) were co-cultured with mutant p53 cancer cells (red) in 2D. A431 mutp53 cells were co-seeded with NFG.

Image supplied by Lobsang Dolma and Patricia Muller (Tumour Suppressors)

applications involving animal research.
The arrival of the pandemic and lockdown in 2020 provided challenges to ensuring the safety of our staff and care for our animals, whilst preserving our research activity as far as possible. Animal studies were limited to the most critical or ongoing long-term, and breeding stocks of transgenic mice were kept to minimal numbers to preserve stock. To ensure the continuity of animal care in the case of enforced staff isolation, several teams of technologists were created to work alternately, including appointing additional Named Animal Care and Welfare Officers. Veterinary inspections of the animal unit moved online, allowing our Named Veterinary Surgeon successfully to see animals and continue advising scientists – a useful practice we shall continue in combination with on-site visits. Similarly, the activities of the AWERB and the twice-yearly meetings for all licensees continued virtually, this format allowing for possibly wider involvement of staff and easier attendance by our Home Office inspector. Applications and amendments to licences continued uninterrupted. Overall, there was a reduction of 25% in the numbers of mice used in regulated procedures under the Act in 2020 (a total of 22,733) compared to 2019. The lull in research activity did, however, provide the opportunity to develop some refined methods, including improved anaesthesia and surgical techniques, ultrasound-guided injection into the liver, non-surgical injection of tumour cells into the mammary fat pads of male mice and the adoption of less aversive handling techniques for mice. The Institute continues to uphold high standards of regulatory compliance, promptly reporting any unexpected findings or incidents to the Home Office Animals in Science Regulation Unit, which have been quickly resolved with their inspector.

Towards the end of the year, our licensing arrangements with The University of Manchester, where our transgenic mouse breeding colony resides, changed to bring this area of our operations under the same Establishment Licence as the Institute, thus affording better oversight of all animal research activities. We continue to work closely with the University to ensure full regulatory compliance.

Despite the inability to interact in person, our scientists have taken part, by invitation, in online forums and conferences and contributed to expert groups arranged by national bodies, such as the NC3Rs, RSPCA and LASA, to further the sharing of knowledge and advice on laboratory animal use.

Cancer Research UK Commercial Partnerships

Martyn Bottomley

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

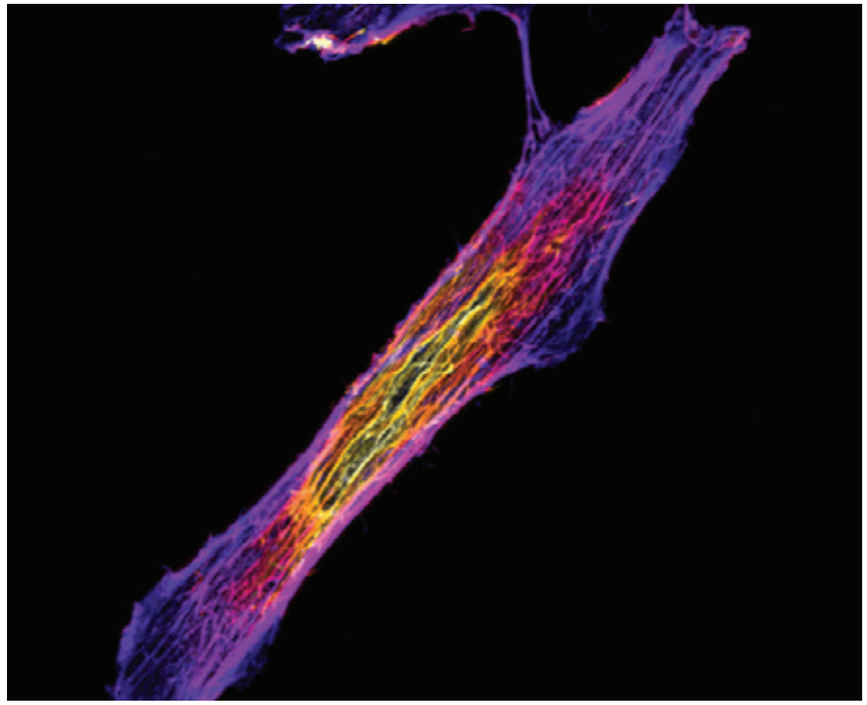
CRUK is aware that the ability to translate new discoveries into patient benefit has not progressed at the same pace as discovery research. This disconnect is linked to several factors related to academic culture, entrepreneurial mindset and the skills required to move discoveries forward. The CRUK-PACE 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 are a partner in the Alderley Park Oncology Development Programme that was launched in December 2020. The Programme is a national programme designed to develop and progress start-up oncology projects. Funded by Innovate UK and Cancer Research UK, the programme brings together a unique collaboration of global pharmaceutical and healthcare companies, research institutions and public bodies to identify and progress exciting oncology innovations that will improve the diagnosis and treatment of cancer. Its goal is to bring forward viable oncology projects much more quickly in order to significantly increase their likelihood of commercial success, and ultimately, patient benefit.

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 facilitate the identification and translation of oncology research, we are recruiting a joint role to focus on oncology research across Manchester. The recruit will work closely with Martyn Bottomley, a CRUK CP Translation Lead, who is also based in Manchester to provide oncology-focused expertise in technology evaluations, patent applications and management, funding for development, commercialisation, drug discovery, market intelligence, and project management. The person will also work closely with the Manchester Innovation Factory, Business Engagement Team, MCRC, CRUK Manchester Institute and the Christie NHS Foundation Trust to maximise the opportunities arising from the research.

Currently, CP is also actively managing a broad portfolio of development programmes and exciting licensing opportunities originating from the Cancer Research UK Manchester Institute that continue to attract commercial partners. The projects include a number of drug discovery assets from the Drug Discovery Unit, a novel pan-cancer treatment response biomarker from Santiago Zelenay's group and a next generation sequencing technology from Caroline Dive's Cancer Biomarker Centre. 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.

Immunofluorescence image of actin fibres in a KRAS-mutant non-small cell lung cancer A549 cell taken using the Airyscan confocal microscope. Basal actin stress fibres are shown in purple and the apical actin cap fibres are shown in yellow.

Image supplied by Hannah Reed (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 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 2020, we welcomed ten graduate students and two clinical research fellows to our PhD programme, working in a variety of fields including leukaemia biology, skin cancer and ageing, cancer biomarkers, cell plasticity and epigenetics, translational oncogenomics, cancer inflammation and immunity, tumour suppressors, stem cell biology and cell signalling.

It was also particularly gratifying to see that, over the past twelve months, some of our PhD students and clinical research fellows had published original research as first authors in *Peer J, Elife*, J*ournal of Thoracic Oncology, Molecular & Cellular Oncology, EMBO Molecular Medicine*, and *BMC Cance*r. First author reviews were also published in *Biochemical Society Transactions* and *British Journal of Radiology.*

The Cancer Research UK Manchester Graduate Programme

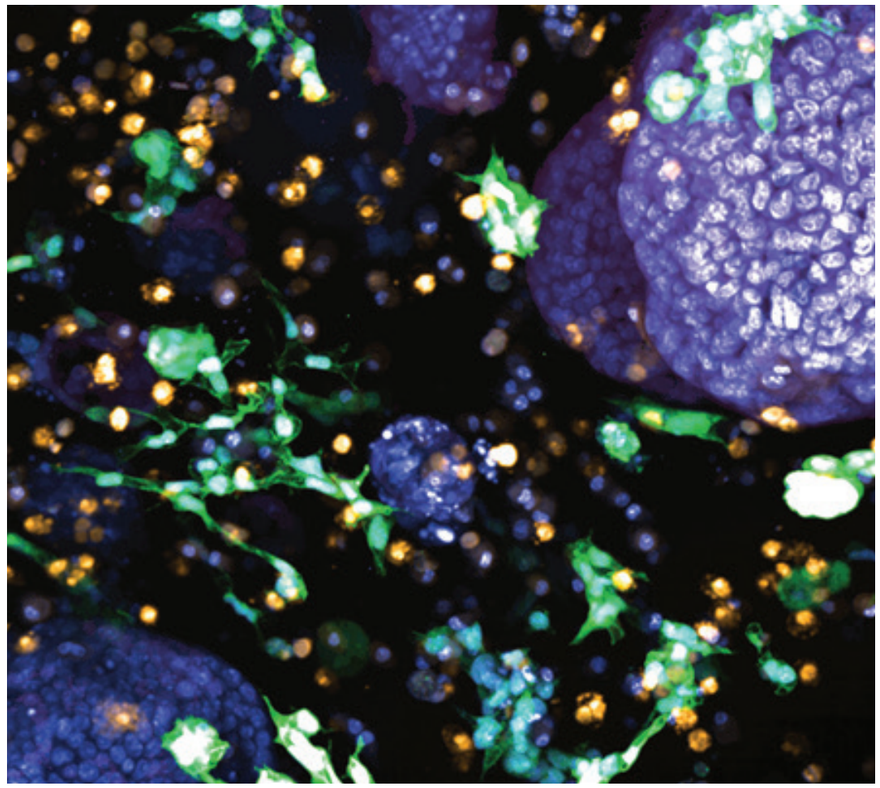
We aim for each student to receive high quality training in scientific research through an intellectually demanding but achievable research programme. Each project is peer-reviewed in advance and monitored throughout its course through a mixture of oral presentations, written reports and progress meetings. 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 and communication skills that are fundamental to a career 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, which is similar to a personal tutor on an undergraduate programme, and 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 Manchester Institute runs an external seminar series featuring talks from leading scientists in cancer research, and all our students benefit from these events. 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. The seminars and student talks continue to play an essential part in connecting colleagues across the Institute and despite the restrictions caused by the COVID-19 pandemic, the talks continued successfully using the virtual platform.

Staying connected with peers and colleagues was an especially important factor throughout the lockdown. During the initial months of the pandemic, a creative Education and Engagement Group was formed to discuss, implement and deliver a programme of events that would connect and engage everyone during this difficult time. The team comprised of senior scientists, operational staff, education and STAy committee members. Assessing the needs of everyone via surveys, the group worked hard to deliver a variety of seminars, courses and in-house training sessions, including an 'Introduction to Photoshop', 'Beginners introduction to R' and a 'Crash statistics course'. An 'Underpinning Elements of Cancer Research' seminar series was devised

Immunofluorescence image of a co-culture (Pancreatic ductal adenocarcinoma organoids, macrophages and fibroblasts) in PEG-hydrogel. Cell types stained for tumour (blue), fibroblasts (green) and macrohpages (orange).

Image supplied by Joanna Kelly (Systems Oncology)



that included lectures from local cancer researchers from The University of Manchester and clinicians from The Christie NHS Foundation Trust, as well as guest speakers from further afield from Queen's University Belfast and the University of Huddersfield. This was a prime opportunity for students to engage in a broad variety of research topics, and to gain insight into the concepts from other disciplines, such as pharmacology, drug discovery, radiation biology, hypoxia and carcinogenesis.

The group also acknowledged the impact of the lockdown on mental health and wellbeing of staff and students and distributed a survey to ascertain essential areas where additional support was needed. Advice and resources were regularly emailed to support the wellbeing of our staff and to equip managers in the implementation of that support.

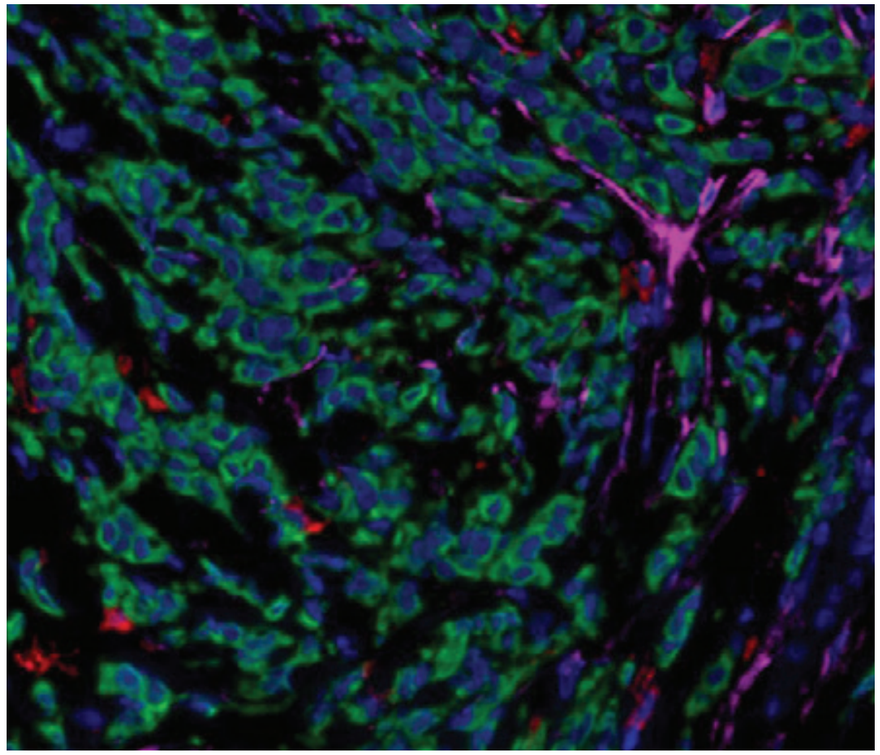
STAy (Science TakeAway) is a committee group run by junior scientists and students in the CRUK Manchester Institute with the aim of providing a forum for discussions and training related to research, communication of scientific engagement and development of social and networking opportunities. STAy are keen to encourage networking, career progression and personal growth of early career researchers and this has been key during the lockdown to keep the whole research community well connected. Activities included virtual quizzes, escape rooms, tabletopia and coffee mornings to keep students, scientific staff and postdocs well connected during this period. STAy also posted regular advice and resources to support staff and student's wellbeing during this time.

The CRUK Manchester Institute Colloquium usually takes place in September at Lancaster University, however this year we held the event virtually. Despite the challenge of translating a retreat-based event onto a virtual platform, the Colloquium was a great success and still provided an excellent opportunity for our new intake of students to interact with 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, and we were still able to host 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, which this year was awarded to Eimear Flanagan.

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 14th IPSCC due to be held at the Beatson Institute, Glasgow in June 2020 was postponed due to the pandemic. We are looking forward to joining the Beatson students virtually in June 2021 and travelling to the German Cancer Research Centre (DKFZ) for the IPSCC in June 2022.

Despite the difficulties in 2020, our activities have thrived virtually and remain a key part of the CRUK Manchester Institute's basis for expanding knowledge.



Multiplex immunofluorescence was carried out using triple negative breast cancer FFPE tumour samples. Nuclei are labelled in blue, cancer cells in green, and two different stromal cell markers in purple and yellow.

Image supplied by Christopher Bromley (Cancer Inflammation and Immunity)

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 Oglesby Cancer Research Building, Manchester.

Education Committee 2020

The Education Committee (EC) acts for postgraduate students and consists of group leaders, the Chief Operating Officer, the Postgraduate Tutor and the Postgraduate Education Manager from the CRUK Manchester Institute.

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

Education Committee Members

Tim Somervaille, Postgraduate Director & Chair, Education Committee
Angeliki Malliri, Postgraduate Tutor
Caroline Dive, Ex-Officio Member
Wolfgang Breitwieser
Julie Edwards, Postgraduate Manager
Claus Jørgensen
Elaine Kilgour
Georges Lacaud
Amaya Viros
Caroline Wilkinson

Student Representatives

Callum Hall[2]
Ryan Guilbert
Melissa Frizziero[1]

[1]Joined in 2020
[2]Left in 2020

THESES

Denys Holovanchuk
Molecular Oncology

Addressing the gaps in melanoma treatment: NRAS mutant and brain metastatic melanoma

Colin Hutton
Systems Oncology

Stromal Heterogeneity in pancreatic cancer

Mairah Khan
RNA Biology/Radiotherapy Related Research

Non-coding RNAs as functional regulators and biomarkers in cancer

Joe Maltas
Cell Signalling

The nuclear roles of the Rac activator Tiam1 in non-small cell lung cancer

Mark Williams
Leukaemia Biology

ABCB1 and chemotherapy resistance in acute myeloid leukaemia

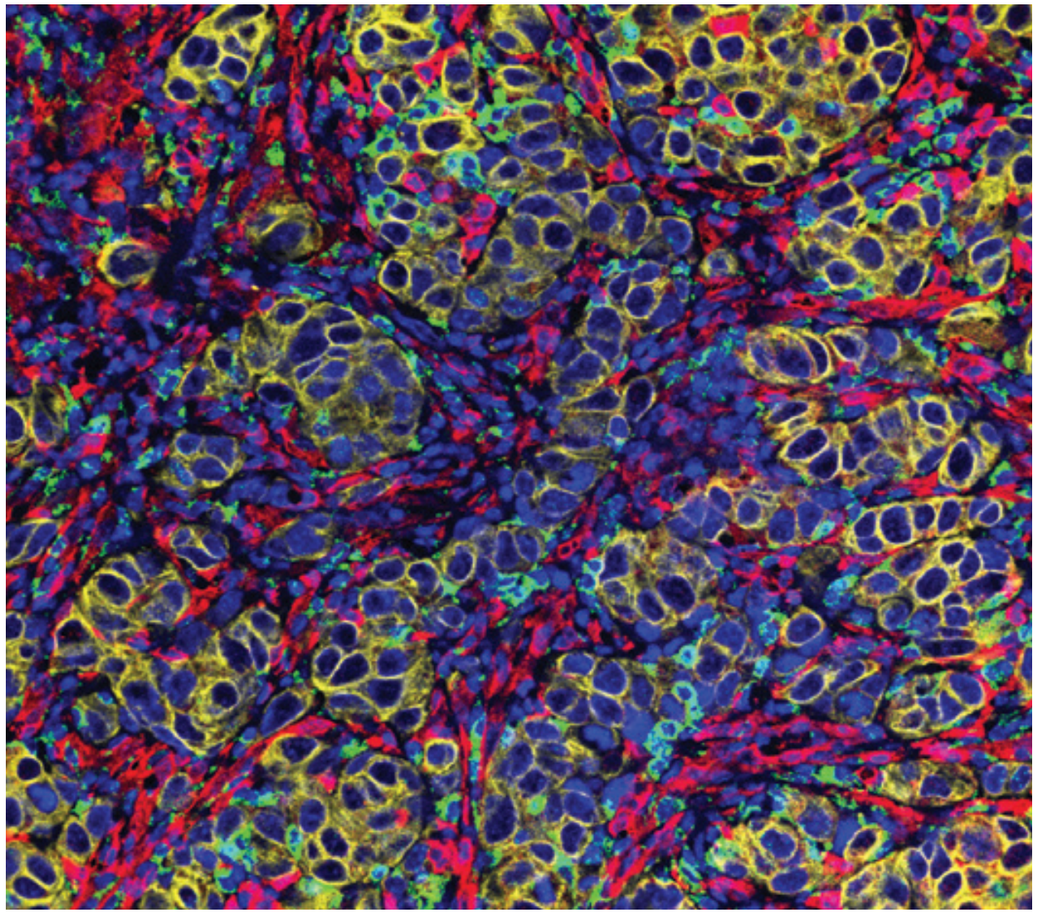


Image showing the contribution of normal cells from the host in red and green (mouse) in a (colourful) mammary tumour. Image is of an implanted (non-fluorescent) mouse mammary tumour with the tumour cells in yellow and cells from the host (mouse) in green (GFP) and red (Tomato).

Image supplied by Sjors Kas (Molecular Oncology)

Philanthropy webinar: Caroline Dive appears in a CRUK Philanthropy webinar, alongside colleagues from across the UK

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 and the public. The team works with researchers across the UK to engage and inspire, driving local and national interaction with life-saving research through compelling research content.

The vast majority of engagement activity planned for 2020 was cancelled due to the COVID-19 pandemic. Despite this disruption, scientists and staff at the CRUK Manchester Institute have continued to support the charity in its communications with supporters and the public.

In a year which saw the charity's income drop by 30%, it was never more important to remain connected to its valued supporters and volunteers.

The year also saw the world become accustomed to communicating via their computer screens, giving CRUK's Research Engagement Team new avenues for making meaningful connections.

Cancer research during COVID-19: Richard Marais and Steve Bagley appear in CRUK's online video

Images left to right:

Victoria Foy: Victoria Foy's story of returning to the NHS frontline appeared in local media

Dominic Rothwell: Dominic Rothwell supported CRUK's regional media work, sharing his views from the Lighthouse Lab

Richard Marais and Steve Bagley supported a video to demonstrate how research was initially affected by the onset of social restrictions. This was viewed over 2,200 times and serves as an historical snapshot of the cancer research community at the time.

A number of MI scientists also supported the work of the charity's Regional Media team, sharing stories of returning to the NHS frontline, supporting local hospitals with 3D-printed PPE and volunteering for the COVID-19 testing efforts at the Lighthouse Labs. The resulting news articles were viewed thousands of times across the Greater Manchester region and beyond, and were shared directly with supporters.

Working with the Philanthropy team at the charity, Institute scientists have been involved in direct engagement with donors, appearing in online webinars as part of a panel featuring key researchers from across the UK. One such webinar resulted in a direct 5-figure donation to the charity in response to Caroline Dive's presentation.

CRUK's own staff had the privilege of hearing directly from the Drug Discovery Unit's Ali Raoof during an all staff meeting, in which he spoke of his own experiences during lockdown and shared some of the latest developments in his work.

Everyone at CRUK remains hugely grateful to all the volunteer group leaders, researchers, scientists and staff who donate their time, energy and enthusiasm to support its engagement activities.

CRUK Staff Meeting: Ali Raoof talks to CRUK staff about his experiences during lockdown

ACKNOWLEDGEMENT FOR FUNDING FOR THE CANCER RESEARCH UK MANCHESTER INSTITUTE

The total funding of the CRUK Manchester Institute for 2020 was £28.1m. The major source of this funding was awarded by Cancer Research UK via a core grant of £12.4m plus additional strategic funding of £5.8m. 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.

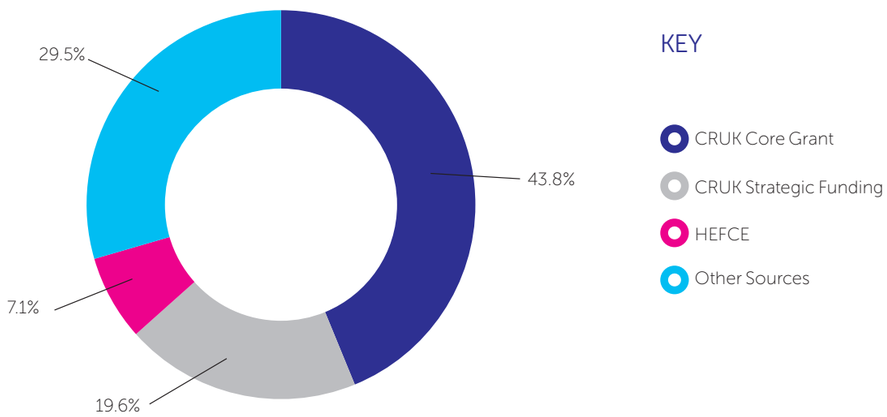
The balance of the Institute's funding is received from a number of additional sources. The research carried out through these additional projects enhances and supports the research undertaken by the core funding.

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 2020



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.

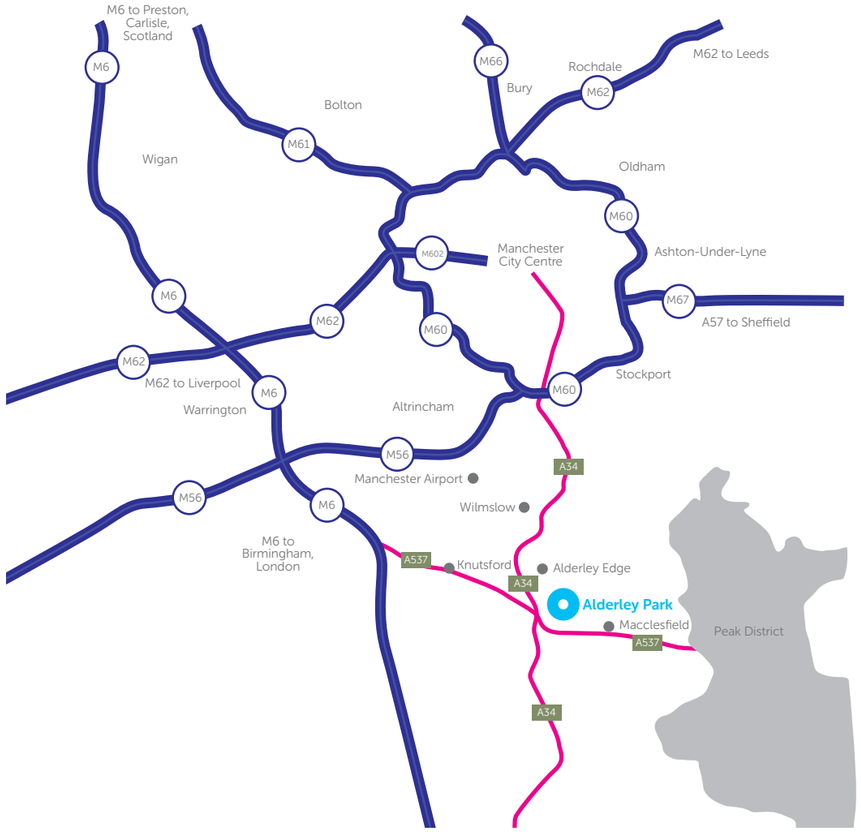
Opportunities exist at a number of levels in the Institute. We have a well-established programme of degrees by research which is described in the section on Postgraduate Education. We encourage applications from suitably qualified graduates to apply to join either the PhD or MD programmes. Graduates 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 (https://www.cruk.manchester.ac.uk/recruitment/candidate/searchvacancies), but suitably qualified and enthusiastic individuals should contact the Institute at any time to enquire about career possibilities.

CONTACT DETAILS



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

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