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)
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 16 total 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 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 work.

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 Oncology Process Research. They have developed two new inhibitors to pursue with CRUK M.I colleagues (Hakan (cancer cell cycle target) and Clas M. Jorgensen (tumour stroma target), and new interactions with Caroline Datuna 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-XRT inhibitor SB535 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 assay, now used worldwide, to advance SCLC research. Their T7 ctDNA pipeline now includes cDNA 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 ctDNA from human blood samples, providing a tractable and convenient approach to determine which patients are likely to respond to these treatments. In this vein, the CRUK, Cancer Inflammation and Immunity group published in the journal Immunology 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 repair. 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, quaternary nucleotide exchange factor (GEF) TIAM1 also regulates centriole duplication. Centrosomes are composed to two centriols, 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 increased. This was associated with 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 Comm. Also moving into the prostate, the Seri Cell Biology group found that RUNXI 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 LS31 inhibitor ORY-1003 (now called CEP-97241) reported in Cancer Inflammation and Immunity that the compound has a good safety profile. The Tumour Suppressor Group identified an intriguing role for copper in...
DIRECTOR’S INTRODUCTION (CONTINUED)

...by developing powerful tools for their research into 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, Derrys Holovanchuk (Molecular Oncology) won an AACR Annual Meeting Travel Award, and Hannah Reed (Cell Signaling) 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, unfailing 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.

REGULAR 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.


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 can be identified early during treatment, 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 TIE 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)
Oncology group reported that its BRAFV600E/treatment. In recent years, the Molecular genetic background, lifestyle and prior lines of plasticity of the immune system in a human markers of response in patient-derived responses for some. However, the biological patients, and eliciting long-term durable and PD-1/PD-L1 improving survival for many with checkpoint blocking antibodies to CTLA-4 revolutionised the treatment of melanoma, individual patients.

Tailored to achieve the best outcome for responding patients so that treatment could be immunotherapy that could be used to identify genetic, 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. These signatures were validated in two independent early on-treatment anti-PD-1 patient cohorts. Together, these data suggested 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.

Four consensus subtypes were proposed recently for molecular classification of SCLC, defined by the expression of NE transcription factors ASCL1 and NEUROD1, the POLR2A secretory tuft cell lineage marker and YAP1, a transcriptional activator with diverse roles, including in-proliferation, 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 thus restricted to localised clusters of minority non-NE cell subpopulations. Together, these data indicate that YAP1 and its target YAP1F1 in SCLC also contain YAP1-expressing subpopulations in SCLC. These models, YAP1 expression was co-localised with REST, and thus restricted to localised clusters of minority non-NE cell subpopulations.

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

Chemoresistance oncogenic functions. SCLC driving 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 µg/m²/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 diarrhea.

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.

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 ABCB1high 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 ABCB1low AML. HiC27Ac ChIP sequencing demonstrated significant activity of native promoters in all cases of ABCB1high 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.

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.


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.
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.

**Cancer Research UK Manchester Institute Cancer Biomarker Centre**

The CRUK Manchester Institute Cancer Biomarker Centre (CBC) is a centre for the discovery and validation of biomarkers and digital solutions that optimise personalised cancer medicine. The CBC is supported by the Cancer Research UK (CRUK) and is part of the Manchester Institute for Biotechnology.

The CBC is located in the Christie Hospital, Manchester, and works in close collaboration with the Christie Hospital Foundation Trust, the University of Manchester, and other academic and clinical partners.

The CBC has a multidisciplinary approach, bringing together experts in cancer biology, translational medicine, bioinformatics, and data science. The CBC aims to translate fundamental research into clinically relevant biomarkers and digital solutions that can improve patient outcomes.

**CBC Case Studies**

- **Nature Cancer** and the Journal of Thoracic Oncology: 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, and how such studies can guide SCLC research.
- **Advancing SCLC Research:** The CBC is working on developing new assays and methods to study SCLC, including the use of circulating tumour cells (CTCs) and transcriptomic profiling of tumour biopsies. The CBC is also collaborating with other academic and clinical partners to develop a standardised assay on the clinically available tissue and patient-relevant models.

**Intra-tumoural functional heterogeneity in SCLC**

Using our CDX biobank, the Preclinical Pharmacology team continues 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, vascular mimicry, and tumour microenvironment.

**Biomarkers to inform immunotherapy trials**

The Cells and Proteins team will continue to expand and develop the CBC immune biomarker ‘toolbox’. Through a new partnership with TheratioMaybe, our latest effort involves the development of a novel platform for the screening of potential novel markers for SCLC.

**In vitro co-culture models**

Intra-tumoural 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 is a VM surrogate pathway via active integrin signalling leading to collagen deposition and remodelling (Figure 1).

**In vitro co-culture models**

Figure 1. CDX non-NE cells form tubules with hollow lumens when cultured on Matrigel. A, Tubule formation of CDX1716 non-NE cells visualised via brightfield (b) and fluorescence microscopy (c). B, C, Confocal imaging of CDX17 non-NE cells forming hollow tubules visualised using anti-mouse antibody staining, showing a defined outer tubule wall (b), a hollow lumen (b, c) and cross sections of CDX17 non-NE cells showing a defined cell containing an outer tubule wall (c), and continuous layer of cells forming the hollow centre (C and G). All images are representative.

**A potential mechanism of immune evasion**

Generation of new CDX models with the aim of extending the nutrient supply to 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 Prof Santiago Zelenay (supported by a MRC Confidence in Concept Award) to develop a standardised assay on the clinically available 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 Zelenay group and CBC, to explore the potential for the CDX-inflammation 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 ECMAT team and have demonstrated the feasibility of detecting cytokines by ELISA. In a pilot study, we have shown the potential 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 underlying this approach. This will be used in the NOIATION study to evaluate biomarker and response to therapy in patients receiving immunotherapy and advanced T-cell therapies. We are also working with Dr Phil Monaghan, to deliver transfer of our technology and S1ELISAs to the Christie Hospital Diagnostic Biochemistry Laboratory for routine NHS use and deployment in the upcoming.
CANCER BIOMARKER CENTRE (CONTINUED)

VATI1a 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 ofctDNA 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.

cDNA analysis within the CACTUS and DETECTION trials

Over the last year the NAB team has performed GCP-compliant droplet digital PCR (ddPCR) analysis of cDNA within the CACTUS Trial (Circulating Tumour DNA Guided Therapy Switch, CI Paul Robertson UoM/CFT) for advanced cutaneous melanoma phase II patients. The ddPCR cDNA assay measures mutated BRAF levels to instruct treatment switch from targeted to conventional chemotherapy and measures 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 III/IV BRAF or NRAS mutant, with wildtype Melanoma after surgery).CI, Paul Lorigian. This trial involves ddPCR cDNA analysis to detect early relapse/micro-metastatic disease and select patients for targeted therapy and will open to recruitment in Q1 2022. NAB has now validated the lower levels of detection for 4 BRAF mutations and 3 NRAS mutations, with validation of 4 additional AML/MT2 mutations ongoing.

Methylation profiling of ctDNA for the early detection of cancers

We have further refined our genome-wide cDNA 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, those incorporating UMs, our method results in superior performance in the cDNA 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 ongoing 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 cDNA from the same patient using a panel of 8 CDX tumours and the cDNA 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 cDNA samples to determine the sensitivity and specificity of the SCLC classifier which will 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 cDNA analysis of 37 HNVS and 51 cancer patient samples using a pan-cancer signature consisting of 1221 differentially methylated regions if CUP and 1192 if C3C. 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 tumour specific methylation profiles are currently ongoing.

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 VATI1a trial that seeks to qualify our liquid biopsy measuring S100.2 and 1022 BRAF pan-cancer signature cDNA methylation detected across T7 cancer patients (95% specificity) across 4 tumour types. The PROACT Study was delivered, which investigated the feasibility of implementing an app for capturing patient reported outcomes and experiences electronically and remotely. The study home, 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 ECMAT 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 is the development of the CORONET tool (COVID-19 risk in Oncology Evaluation Tool) by digital ECMAT (with assistance from the BBC 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 established. As part of this, the REACT tool was repurposed to enable hospital data be visualised.

The digital ECMAT, along with our EU colleagues, including those from Fondazione IRCCS Istituto Nazionale di Tumori Milano and Instituto di Investigacion Oncologica de Vall de Hebron, Barcelona was awarded a CRUK Accelerator Award to develop the 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 proven to be ethically and medically valuable. The digital clinical trial data acquisition and data interpretation from the network of (currently 24) participating Experimental cancer medicine centres is 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.
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 prominent 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 evaluation combination regimens of PD(L)-1 blockade 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, the current understanding of the basis underlying therapy response, resistance or relapse is limited. Likewise, little is known about the mechanisms that drive tumour evasion of the adaptive immune response. It is now clear that regulatory T cells (Treg), myeloid suppressor cells (MSC) and other suppressor cells can prevent antigen-specific T cell responses and contribute to tumour escape. Immunotherapy has the potential to target these pathways;

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 PGE2 is an intratumoural inflammatory reprogramming that attracts and stimulates the differentiation of anti-cancer effectors T cells. Activated NK cells undergoing a global single cell RNA sequencing analysis of tumour infiltrating immune cells revealed that the transcriptional profile 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 an acute depletion of NK cells, the transcriptional profile of tumour infiltrating myeloid cells showed a marked enrichment in signalling pathways that are strongly associated with malignant tumour growth. In agreement, tumours that spontaneously regressed in wild-type immune competent mice, grew progressively in mice lacking NK cells. Lastly, the use of genetically-modified mice in which NK cells are selectively

invasive to prostanoglandin E2 (PGE2) effects, the main factor that drives immune escape in our tumour models (Zelenay et al. Cell 2019), established NK cells as the main target of cancer cell-derived PGE2.

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/).

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 human cancers, and in particular in cancer types in which tumour mutational burden or PD-1 expression do not, such as in clear cell renal cell carcinoma, the most common type of kidney cancer.

Building on these from 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 the first step 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.
CELL DIVISION

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

The DNA-damage sensing approaches of chemotherapy and radiation 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 cellular 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.

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 phosphatase then permits mitotic entry. A trigger level of Cdk1-Cyclin B-Bkin promotes a positive feedback loop that enables 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 block Wee1 and inhibit Cdc25 activities. Once the damage is repaired, or the replication is completed, the block to mitotic commitment is relieved and cells divide.

Cerebrosides nucleate all the microtubules in the cell to generate the interphase cytoskeleton and the bipolar mitotic spindle that physically segregates the chromosomes. However, cerebrosides may organise more than just the microtubule spindle. The initial appearance of active Cdk1-Cyclin B on human cerebrosides, 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 cerebroside equivalent, the spindle pole body (SPB), provide 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 cut25 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's 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 Cdc25 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 are also reflected in the 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 Cdk-Cyclin B, Cdc25-Cyclin B, PP1, and Cdc25 (Figure 1). Remarkably, although these kinases play a key role in regulating, committing, and maintaining 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 clinical trials. 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 mitotic 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 first step we have constructed a panel of transformed lines exhibiting 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.

**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.

**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 through 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 completed, the block to mitotic commitment is removed to generate 4N DNA between 10 and 12 h after drug removal to generate 4N DNA profiles, after which cells divide to return to display 2N profiles 25 h after release from the arrest.

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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 HRASG12V 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. Instead, 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 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 GE/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 GE/GEF TIAM1 has been shown to be required for the formation and growth of KRAS-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. The reasons why RAC promotes tumour formation and growth and which mechanisms contribute to RAC-promoted tumorigenesis are still not fully understood. In particular, the role of RAC in tumour progression is not clear. Some studies have shown that RAC promotes tumour progression, while others have shown that RAC inhibits tumour progression. In this review, we will focus on the role of RAC in tumour progression, with a particular emphasis on the role of RAC in the formation of KRAS-induced tumours.

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

Even though, as mentioned above, TIAM1 inactivates RAC, its role in tumour progression is not clear. Some studies have shown that TIAM1 increases RAC activity, while others have shown that TIAM1 decreases RAC activity. In this review, we will focus on the role of TIAM1 in tumour progression, with a particular emphasis on the role of TIAM1 in the formation of KRAS-induced tumours.
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 Ian 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’s biomarker discovery scientists 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 all 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.

Kinetin 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 adenocarcinomas (PDAC), colorectal carcinomas (CRC) and non-small cell lung cancer (NSCLC), proliferation requires both RAF and SRC signalling. Using this knowledge, we discovered CCT3833, a pan-RAF 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 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 collagen and elastin, promoting primary tumour growth and metastatic spread in breast, PDAC and CRC. Uncontrolled ECM regulation plays a key role in establishing fibrotic lesions, so pathological wound scarring that can be caused by infections including COVID19. Notably, LOX and LOXL1 play a key role in establishing fibrotic lesions, so LOXL1 is both an anti-cancer and an anti-fibrotic therapeutic target. In collaboration with Richard Marais, we have discovered LOX and LOXL1 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 of lung fibrosis. We are currently selecting the best drug candidates to progress to toxicity 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 in 2021.
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.

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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-safe environment as autumn approached, 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 MCRU/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 preclinical evaluation and future patient benefit. This year marked something of a milestone for our work on a historic 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 published 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 this 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 translycopiprine-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 12 months. 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 important signs of efficacy in 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 promising efficacy with this combinational 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 therapeutic approach in myeloid leukaemia. We established a direct collaboration with Fabrizio Camera and Bradley Revell at the University of Manchester in collaboration with CellCentric, a novel therapeutic approach in myeloid leukaemia. We discovered that these translycopiprine-derivative inhibitors of LSD1...
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 scientists and clinicians. Those ties also provide access to patient issues, 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 to 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 recent findings in a patient to characterise the molecular changes in the microenvironment that 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. The results show that tumour cell interactions with the tumour microenvironment are complex and heterogeneous, 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.

Conversely, in 2018, we reported that about 15% of common cutaneous melanoma genomes present large-scale genome gains and losses that are not thought to be UVR-driven. These data suggest that melanomas can develop along distinct molecular pathways and critically, patients with rare melanomas still have limited treatment options. To investigate if UVR can drive melanoma, we performed whole genome sequencing on 10 conjunctival melanomas because this form of melanoma is UV-exposed mucosal membrane. We compared our conjunctival melanoma genomes to those of 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 conjunctival melanomas, we posit that conjunctival melanoma genomes present 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 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 conjunctival melanomas, we posit that conjunctival melanoma genomes present 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.

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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.

LY6D is a gene with as of yet no established role in prostate development or cancer. It is a member of the Ly6C/PAR 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 organo- 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.

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 samples 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.
This year we have progressed our understanding of 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 modify 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 responses 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.

Publications listed on page 68
Biology and Medicine, University of Manchester

the Division of Developmental Biology

Georges Lacaud

Funded on an MRC Doctoral Training Partnership, part of the Division of Developmental Biology and Medicine, University of Manchester

Group Leader

Postdoctoral Fellows

Divya Malik
Muhammad Maqbool
Nao Wen Hao

Scientific Officers

Ranaa Alasi
Michael Lie-a-Ling

Graduate Students

Renadu Mevel
Muhammad Faruqul Islam
Ewan Sinclair
Steven Mages

Funded by Blood Cancer UK

Sponsored by an ERC Doctoral Training Partnership

STEM CELL BIOLOGY

Genes encoding the AML1/RUNX1 transcription factor and its cofactor CBFB 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 (lincRNAs) have also emerged as important regulators of gene expression. In this context, we more recently started the investigation of lincRNAs 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 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 (lincRNAs) could also be implicated in regulating gene expression. LincRNAs represent a large fraction of the human genome (Figure 1). Through acting as tethers for epigenetic machinery, or participating in genome (Figure 1). Through acting as tethers for epigenetic machinery, or participating in chromatin looping, lincRNAs participate in gene regulation. In addition to the THP-1 cell line, we also generated other MLL-rearranged AML cell lines (MOLM-13 and MV-4;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 lincRNAs (10 guides for each lincRNA). Transduced cells were selected and then grown in culture for 20 cell doublings. Of the 3882 lincRNAs screened, a total of 19 were identified as significantly influencing cell proliferation. Of these 19 hits, five including 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 lincRNAs important in leukaemic maintenance. We selected the top 6 hits and confirmed their screen phenotype individually, experimentally using a internally controlled growth assay. Of these six hits, five including Lnc79 expression was higher in AML patient samples (AML) as compared to healthy samples (green). C. Lnc79 expression was higher in AML patient samples (LAML, red) than in healthy samples (green). Lnc79 expression is higher in AML samples (representation of cells expressing Lnc79 sgRNA is shown). A. Downregulation of Lnc79 in AML.

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 lincRNA 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 knowledge of these LncRNAs 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 molecular mechanisms regulated by them.

Besides this approach, we have identified several lincRNAs 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 lincRNAs that display conserved expression patterns in both species and may play a conserved role in human/mouse normal and malignant haematopoiesis. We are currently functionally evaluating the role of these lincRNAs in the maintenance of leukaemia. The successful identification, and characterisation of lincRNAs essential in AML could offer possible new avenues for therapeutics.

Use of antisense oligonucleotides (ASOs) complementing to our target lincRNA to specifically downregulate our lincRNA transcript without modifying adjacent chromatin as with CRISPRi. To start identifying their molecular functions, we are currently determining which proteins are associated with our lincRNA, 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 lincRNA protein partners will help direct further experiments, such as changing analyses in epigenetic modifications upon lincRNA knockdown, using chromatin immunoprecipitation (ChIP).

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

Figure 1

LincRNAs represent an important fraction of the human genome. A. Representation of lincRNA compared to other RNAs. B. General features of lincRNAs.

Figure 2

Lnc79 is upregulated in AMLs. A. Downregulation of Lnc79 by CRISPRi approach impairs the proliferation of THP-1 human AML cell lines. B. Lnc79 expression is higher in AML patient samples (AML), real than in healthy samples (LAML, red), Lnc79 is associated with poorer overall survival.

Figure 3

LincRNAs are over 200 nucleotides in length different from mRNA and miRNA and are non-coding. They are processed similarly to mRNA, but are enriched in large exons and display greater translation complexity.

Figure 4

A. Downregulation of Lnc79 by CRISPRi approach impairs the proliferation of THP-1 human AML cell lines. B. Lnc79 expression is higher in AML patient samples (AML), real than in healthy samples (LAML, red), Lnc79 is associated with poorer overall survival.
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 stroma-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 signaling 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 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 substitution of a therapy that is matched to specific characteristics of individual tumours, has benefited 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. These methodologies may be used to define optimal combination of therapies for tumour and stromal cells.

Publications listed on page 69
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 tumourigenesis, impacting not only cell proliferation and survival but also cell motility, invasion and metastasis. We identified and characterised a KRAS-responsive lncRNA, KIMAT1 (ENSG00000287039) and showed that it correlates with KRAS levels both in cell lines and in lung cancer specimens (Figure 2).

Mechanistically, KIMAT1 is a MYC target and drives lung tumourigenesis by promoting the processing of oncogenic miRNAs (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 during lung cancer progression and provides a proof of principle that interfering with KIMAT1 could be a strategy to hamper KRAS-induced tumourigenesis (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 MicrotubuleLike 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, alecinib, brigatinib and lorlatinib. Even though the objective response rate for the ALK inhibitors crizotinib and alecinib in the clinic surpasses 60%, patients typically develop resistance to these inhibitors and relapse soon thereafter.

In order to mimic the context of acquired resistance to ALK inhibitors in vitro, we utilised cell lines with acquired resistance to crizotinib (CixR), ceritinib (CerR) and alecinib (AleR) by long-term exposure to these drugs. RNA-seq analysis identified a cell cycle dysregulation in crizotinib-resistant cells, evidenced by 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-33c, 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).

Publications listed on page 69
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 tumours have an increased probability of producing incurable metastases which may go undetected at the point of diagnosis, and hence 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 radical prostatectomy or surgery due to local resistance and/or systemic spread. Clearly, there is a need to develop new biomarkers that give an insight into heterogeneous biology of outcomes in prostate cancer patients. In this regard, there is growing interest in the potential of genes involved in maintenance of genome stability. However, to date the potential use of these genes to predict risk and also guide their treatment. This approach holds much promise. Indeed, it is already known that tumours harboring 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 having very high sensitivity to androgen blockade, may also be targetable by checkpoint inhibitors. Furthermore, enhanced screening of patients harbouring germline mutations could lead to the earlier detection of cancers suitable for surgery. Understandably, there is great excitement in the potential clinical utility of genetic testing in prostate cancer and the cancer cascade is to consolidate prior studies with further evidence, both to support biomarker development, and to provide a mechanistic framework for gene associations. This will allow assessment of hypoxia in biopsy or pretreatment specimens 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 allowed to a detailed pathological examination to define the relationship between 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. This syndrome is characterised by dynamic gradients of oxygen diffusion and consumption, leading to sub-regions of hypoxia in a tumour with an MSI phenotype. 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 uniquely 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 (Fmoczaolig) 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.

Germline mutations in MMR genes are associated with increased risk of developing prostate cancers that progress to incurable, castrate-resistant disease (mCRPC) 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, EPAC1, 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 PARP 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 cancer. In this instance, it is characterised by dynamic gradients of oxygen diffusion and consumption, leading to sub-regions of hypoxia in a tumour with an MSI phenotype. A recent report has shown that 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 uniquely 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 (Fmoczaolig) 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.
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 cisplatin and etoposide. 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 cisplatin and etoposide. Loss of mutant p53 or RCP expression coincided with an increase in cleaved caspase 3 when mice were challenged with cisplatin.

Interestingly, restoration of RCP expression in RCP knockout cells restored sensitivity to cisplatin and etoposide, but expression of RCP in p53KO 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 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. 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’s 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.
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)
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 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.

Biological Resources Unit
Transgenic Breeding
Team Leader: Jennifer Hughes
Dan Bennett, Tim Blake, Carl Conway, Ali Jammoul\(^1\), Howard Kendrick\(^2\), Edyta Kijak\(^1\), Wesley Moore, Kerry O’Shea, Vicky Prestori, Rose Storey, Lauren Street\(^2\), Natalie Varley\(^1\)

1Joined in 2020
2Left in 2020
3Maternity 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 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...
RESEARCH SERVICES (CONTINUED)

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

We have now had our first full calendar year of using the ticklab system for securely recording all of our breeding and stock details, as well as providing an option for delivery of simple instructions to users. This continues to work well and as information accumulates over time it becomes an ever more useful tool for tracking historic data 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 ticklab 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 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 has 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 carried 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 become 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 imaging-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, Antoni Banyard, Yasra Elaggi, Michael Rennie

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 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 Cglsby 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, Antoni 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 populations. 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 unlocking 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 then cell fate determined; such research should 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 analysts 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 Navios flow analyser and CyTOF has helped Cancer Immunology and Immunity’s study of the action of inflammatory immune sub-types and their influence on tumour progression.

The ability of our analysts to rapidly perform cell cycle analysis has aided Cell Division’s pursuit of elucidating cyclin kinase 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, quantity and sort T cell populations used in this study.

Histology

Gary Anton, Caron Abbey, Marta Madureira da Graça, Uman Mahmood, Emma Watson, Katherine Lally, Deepthi Wikle, David Millard

Left in 2020

Haematological Malignancy Biobank

Whist 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 are conducted in combination 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 CLUK/M, 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 tissue 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 CDR 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 TMAs 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 UV radiation 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, RNAcope has 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.

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, RNAcope 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 HiSeq2500 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 sequence 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 chromatin applications, including high coverage whole genome sequencing as well as single cell transcriptome sequencing.
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 transcription 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 transcription 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 phosphate position and subsequent quantification of phosphorylation isoforms is a challenging problem in proteomics-based mass spectrometry, notably because of the need for ‘site-determining ions’ (unambiguous fragmentation ions corresponding to specific isoforms). 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 isoform 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, Kevin Doyle, Nadeem Baig, Stephen Kitcatt, ZhiCheng Wang**

1 Joined in 2020
2 Left 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 environment and to prevent cross dependencies.

A CytoKit2 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 tissue 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 Chiura pipeline used by both institutes for the analysis of High Definition Single Cell analysis. The software was adapted to work with Phoenix’s HOADB/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 M11’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...
Multiplex immunofluorescence was carried out using triple negative breast cancer FFPE tumour samples. Nuclei are labelled in blue, cancer cells in purple and yellow, green, and two different stromal cell markers in purple and yellow.

(RESEARCH SERVICES (CONTINUED))

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 discrete groups of cell types. SciCom improved the framework’s code base and developed a Shiny app that allows users to visualise, irradiation and analysis core facility, the Flow Cytometry core facility and the Visualisation, Irradiation & Analysis core facility. 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.

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 their activity on our most recent service: archiving and running. 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.

Transgenic Production Facility
Natalia Moncaut, Athina Papaemmanouil, Lauren Street1 and Satish Arcot-Jayaram2

1 Joined in 2020
2 Left in 2020

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) 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 48 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.

A collaboration between facilities (VIA, Histology, Molecular Biology and Computational Biology) 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.
Cancer Biomarker Centre
(page 36)
Caroline Dive

Refereed research publications


Other publications


Cancer Inflammation and Immunity
(page 20)
Santiago Zelenay

Refereed research publications

Image supplied by Santiago Zelenay (Cancer Inflammation and Immunity)
RESEARCH PUBLICATIONS (CONTINUED)

Cell Division
Iain Hagan

Referred research publications

Drug Discovery
Caroline Springer

Referred research publications

Cell Signalling
Angeliki Malliri

Other publications

Leukaemia Biology
Tim Somervaille

Referred research publications

Molecular Oncology
Richard Marais

Referred research publications


Other publications

Prostate Oncobiology (page 36)
Esther Baena

Refereed research publications

Other publications

Stem Cell Biology (page 41)
Georges Lacaud

Refereed research publications

RESEARCH PUBLICATIONS (CONTINUED)
RESEARCH PUBLICATIONS (CONTINUED)

Other publications

Translational Oncogenomics
(ref page 46)
Rob Bristow
Other publications

Tumour Suppressors
(page 48)
Patricia Muller
Refereed research publications

Select additional publications

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 platform. 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

Uiltan 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

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 Sonaya 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.
OPERATIONS (CONTINUED)

bookings 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 year, we have been preparing to support the Institute throughout the COVID-19 pandemic. The effect 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 has had to take immediate action to help plan and prepare budgets and financial statements to allow the Institute to reassess its available funds and redistribute them to maximise 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*, Rachel Craven*, Andrew Haines, Julie Jarrett, Laura Jones, Emma Lloyd*, David Stanier*

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, Krat Haider*

* Joined in 2020

The CRUK Manchester Institute Core IT is a 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 Olgilsty 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 MiCore IT team, not least due to the COVID-19 pandemic. Further, we have provided

SCCL-GEMM lung stained for the neuropeptides (NE) marker ChromagraninA (CHGA) by in situ hybridisation (red), which marks up the tumour, and CC10 (yellow) which marks up the lung airway. DAPI in blue.

Image supplied by Sarah Pearall (Cancer Biomarker Centre) and Carson Betan (Histology).
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, 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, Mac OS 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 which is housed 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 workplace, 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**, Budiola Ategbue, Corrinne Hand, Petra Ribnikova 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 Proton Beam Centre and the MRCC Tissue Biobank located in the Kay Kendall Laboratories.

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 Proton Beam Centre, MRCC Building, Proton Beam Centre and the MRCC Tissue Biobank team located in the Kay Kendall Laboratories.

The support provided includes the receipting, checking, bookin in and distribution of goods ordered by staff. The team facilitate the delivery of dry ice and gas cylinders which are monitored and replaced, as necessary. The team monitors the liquid nitrogen levels in the cell storage tanks and replenishes when required. Response to the impact of COVID-19, we have increased our gas handling stocks and increased the levels of nitrogen in the cell storage tanks to provide extra resilience.
mutp53 cells were co-seeded with cancer cells (red) in 2D. A431 fibroblasts (green) were co-cultured with mutant p53.

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, Maria Belen Conti Vyas, Gillian Campbell, Julie Edwards, Steve Morgan, David Storier

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, JobMatcher, 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 practices. 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 Vints 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, Donal Landers and the digital ECMT were awarded Horizon 2020 funding as part of EU consortium ‘Building Data Rich Clinical Trials’ led by Val of 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 Ian 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 M 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 grants...
OPERATIONS (CONTINUED)

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, affording better oversight of all animal research activities. We continue to work closely with the Home Office Animals in Science Regulation Unit, which have been quickly resolved with 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 continued 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 UK’s Research and Innovation Directorate. The CP Team aims to maximise patient benefit from CRUK-funded research worldwide by advancing research discoveries into development with pharmaceutical and biotechnology companies. 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 RIBI 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 existing 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.
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, Journal of Thoracic Oncology, 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.

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 R’ and a ‘Crash statistics course’. An ‘Underpinning Elements of Cancer Research’ seminar series was devised.
POSTGRADUATE EDUCATION (CONTINUED)

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 Hichman 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 (SEM), 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.

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.
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)
CANCER RESEARCH UK’S RESEARCH ENGAGEMENT

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.

A number of MRC 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.

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.

Research Engagement Manager
Tim Hudson

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


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

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
- Angi Inc
- Astex Pharmaceuticals
- Astra Zeneca
- Bloodwise
- Carnick 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
- GiacomoSmithKline
- Harry J Lloyd Charitable Trust
- John Swallow Fellowship
- Kay Kendal Leukaemia Fund
- Lep 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
- Tahto Oncology Inc
- The US Department of Health and Human Services
- Welcombe Trust
- Worldwide Cancer Research

We are immensely grateful to all our sponsors.