SCIENTIFIC REPORT 2021

cruk.org

CANCER RESEARCH UK | MANCHESTER INSTITUTE

COVER IMAGE

Histological image showing the cutaneous papilloma from a female mouse following exposure to the carcinogen DMBA/TPA. This image featured on the front cover of journal *Clinical Cancer Research* (June 2021, Volume 27, Issue 11) Copyright © 2021, American Association for Cancer Research.

Image supplied by Amaya Viros and Tim Budden (Skin Cancer and Ageing group)

SCIENTIFIC REPORT 2021

MANCHESTER INSTITUTE

MANCHESTER 1824
The University of Manchester

CANCER RESEARCH UK | MANCHESTER INSTITUTE

CONTENTS

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

The Oglesby Cancer Research Building.

DIRECTOR'S INTRODUCTION

Professor Caroline Dive

Director of the Cancer Research UK Manchester Institute

During 2021, the Institute continued to face challenges of the SARS-CoV-2 pandemic and throughout this time I have been impressed by both the resilience of our staff and by the enduring quality of our research. I am grateful for everyone's cooperation in keeping us safe while we remain as productive as possible during these unprecedented times.

Pivotal to the smooth running of the Institute during the disruption caused by restrictions and remote working has been our operations teams. I would like to recognise them for their hard work behind the scenes in supporting the Institute's agile response to the new working practices necessitated by the pandemic.

Whilst we have worked remotely for periods of time and been unable to meet face to face, we adapted well to communicating using online platforms. As we became more expert in the digital arena, virtual meeting rooms not only connected us but encouraged us to explore new ways of working together.

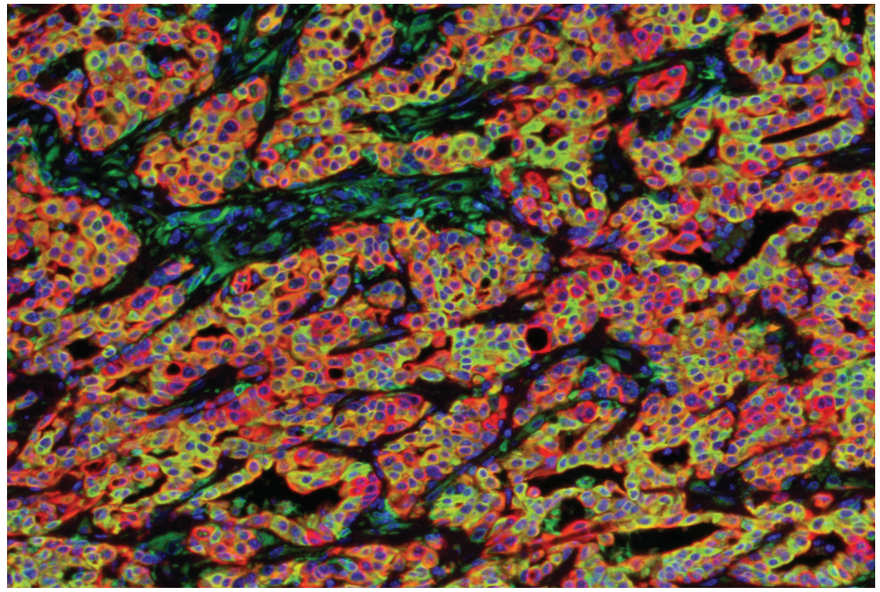
I am delighted that we have continued to recruit and appoint new staff and students and I would like to welcome you all. It has been an unusual time to join a new working environment and build relationships but this year we initiated virtual 'coffee club' sessions that were an alternative and enjoyable way to meet each other. I am especially grateful to the STAy Committee for organising a variety of entertaining online social events.

Recruitment of a new Junior Group Leader was a key objective for the year, and we look forward to welcoming Evangelos Giampazolias who will join us in January 2023 from the Francis Crick Institute to establish his group, Cancer Immunosurveillance. Evangelos will strengthen cancer immunology at the Institute while providing synergy especially with Santiago Zelenay, Claus Jorgensen and the Tumour Immunology and Inflammation Monitoring Laboratory within the Cancer Biomarker Centre.

Despite the challenges, we have accomplished an impressive publication output some of which is featured in our research highlights section. It was especially pleasing to see the first-author papers from PhD students Colin Hutton, Fabrizio Simeoni and Max Schenk completed under the challenging circumstances of the Paterson Building fire in 2017 and subsequent relocation to Alderley Park as well as the COVID-19 lockdown and ongoing restrictions.

Schenk et al. (*Nature Communications* 12(1):6652, 2021) defined a new mechanism of acquired chemoresistance in SCLC and identified a potential new therapeutic avenue for this recalcitrant tumour. Investigating the mechanisms by which Forkhead family transcription factor gene FOXC1 blocks normal myeloid lineage differentiation in AML, Simeoni et al. (*Cell Reports* 36(12):109725, 2021) discovered that RUNX1 - a critical regulator of myeloid differentiation - and FOXC1 interact through their respective DNA binding domains and that this protein:protein interaction could be a potential therapeutic target. Hutton et al. (*Cancer Cell* 39(9):1227-1244.e20, 2021) identified two novel pancreatic fibroblast lineages with distinct tumour permissive and suppressive function that have important implications for therapeutic efficacy.

Institute Fellow Amaya Virós saw the culmination of four years' work developing her new research programme result in several significant senior author papers. In Budden et al. (*Nature Communications* 12(1):2742, 2021) her Skin Cancer and Ageing group described the mechanism of UV damage to the dermis leading to decreased melanoma invasion, which translates to real-life patient outcome. In a second paper (Budden et al. *Clinical Cancer Research* 27(11):3125-3223) they described the sex bias in the immediate and late phases of the immune response to early and late skin carcinogenesis, using mouse models and real patient data to show that the immune response underpins the sex bias observed in incidence and outcome. Postdoc Tim Budden was recognised with the Institute's Award for the Best Young Scientist of 2021, The Edith Paterson Prize, which replaces the Dexter Award and is named after Edith Paterson, who together with her husband Ralston Paterson, made pioneering discoveries in cancer research and treatment in Manchester. Amaya also secured funding from Wellcome and the Melanoma Research Association.



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

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

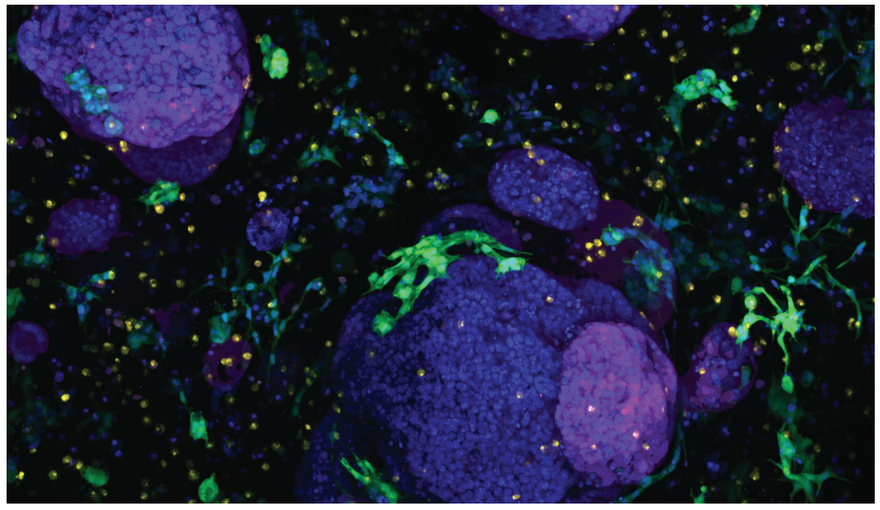
Carlos Lopez-Garcia was awarded a grant from the UK's National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3RS) and has set up his own research group as an Institute Fellow, focusing on lung squamous cell carcinoma, details of which are set out in this report. Carlos was a valued member of the Cancer Biomarker Centre for the past two years, and I am delighted that he is establishing his own independent group.

Other scientific highlights include work by the Cancer Biomarker Centre to develop a tissue of origin classifier using ctDNA methylation with potential for clinical impact in Cancers of Unknown Primary (CUP); a trio of liquid biopsy driven trials in melanoma; a significant expansion of our toolkit of immune based biomarkers, including the translation of Santiago Zelenay's COX-IS signature to clinical trials; and the open sourcing of six digital healthcare products to the UPSmart Accelerator Consortium of 24 early phase clinical trial sites in UK, Spain and Italy.

Our core facilities have been working together to bring new technological advances and innovation to our research platform capabilities. These include CO-Detection by indEXing (CODEX) – a method for single cell biology where simultaneous detection of up to 40 biomarkers in the same cell (Histology and Visualisation, Irradiation & Analysis), and the implementation of machine learning methods to analyse and understand high dimensional CyTOF output data (Scientific Computing, Flow Cytometry, VIA and the Systems Oncology research group).

Conferences are a highlight in the research calendar, and whilst we have missed attending the international meetings, we held regular virtual seminars and internal meetings including our annual colloquium. Hosting the event on the virtual conference platform, Gather Town encouraged informal scientific communication, where 'social spaces' allowed more meaningful encounters with our colleagues. As well as talks and posters from our scientists, Doug Lauffenburger joined us online from the Massachusetts Institute of Technology to describe how systems biology approaches can help explain the effect of immune cells in tissue microenvironments.

After a delay of more than a year, our research was showcased at the prestigious Royal Society Summer Science Exhibition. In July, a team of researchers presented a raft of digital activities to help explain the complexity of cancer. The team engaged the public online with an introductory video encapsulating the research undertaken at the Institute; an interactive 3D tumour model; and a YouTube 'lightning lecture'. Over 15,000



Pancreatic cancer organoids (purple) were co-cultured with pancreatic fibroblasts (green) and bone-marrow derived macrophages (orange) in a synthetic PEG hydrogel scaffold.

Image supplied by Joanna Kelly and Christopher Below (Systems Oncology)

users accessed the content during the main period of the event and the lecture has been viewed over 13,000 times on YouTube. I would like to thank our enterprising scientists for all their hard work, which was well received by the public.

Our annual 3Rs' poster prize event, which recognises developments in the principles of replacement, reduction and refinement of animal models, was once again held in conjunction with scientists from AstraZeneca and Agenda Life Sciences. Congratulations to Bianca Blochl from the Cell Plasticity & Epigenetics group who was awarded the prize for best poster describing a new screening technology that will help reduce the number of animals used in experiments.

It was with great pleasure that I opened the first face-to-face meeting of the year as we got together at the OCRB Lecture Theatre to celebrate the remarkable life and scientific contributions of our first Director, Professor Laszlo Lajtha (who led the Institute from 1962-83) – a pioneer in stem cell biology and haematology. Joint with the Consul General of Hungary in Manchester, we brought together Mancunian and Hungarian cancer research, learned about the latest scientific innovations, and connected with colleagues old and new.

In October, the cancer exhibition, 'Cancer Revolution: Science, Innovation and Hope' opened at the Science and Industry Museum in Manchester where it will remain until March 2022. This world-first exhibition illustrates the revolution in science that is transforming cancer care and I am delighted that it features research from the Institute.

The replacement of the Paterson Building, our former home damaged by fire in 2017, remains on track and is due to be completed in December 2022 with our relocation from Alderley Park anticipated in early 2023. We have worked with our colleagues in The University of Manchester's Division of Cancer Sciences to plan where our research teams will be based to best exploit scientific synergies and have begun programmes of work to plan the relocation in detail to minimise disruption to research.

My first year as Interim Director has certainly been a challenge but also hugely enjoyable. I am particularly grateful to Deputy Director Iain Hagan, the Senior Management team, and to my administrative assistants for all their support. The resilience of the Institute through first the fire, followed by relocation and now the pandemic has been considerable, and it is a pleasure to work with everyone at the Institute towards our goals of evolving and expanding our research to advance scientific progress and ultimately improve outcomes for people affected by cancer around the world.

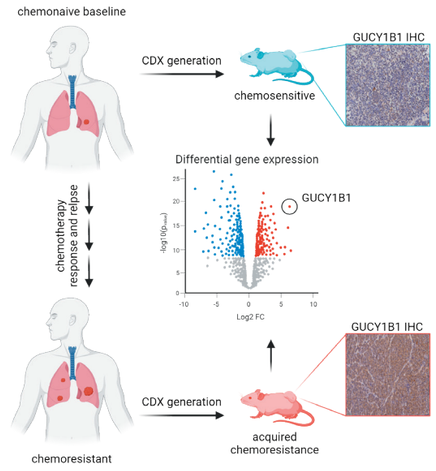
Professor Caroline Dive, CBE., FMedSci.
Interim Director, Cancer Research UK Manchester Institute

RESEARCH HIGHLIGHTS

In this section we highlight some research publications from 2021 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.

Schenk MW, Humphrey S, Hossain ASMM, Revill M, Pearsall S, Lallo A, Brown S, Bratt S, Galvin M, Descamps T, Zhou C, Pearce SP, Priest L, Greenhalgh M, Chaturvedi A, Kerr A, Blackhall F, Dive C, Frese KK. (2021)
Soluble guanylate cyclase signalling mediates etoposide resistance in progressing small cell lung cancer.
Nature Communications 12(1):6652.

Small cell lung cancer (SCLC) is an almost universally lethal disease for which the backbone of standard treatment remains the chemotherapy doublet cisplatin and etoposide. Although this treatment is frequently initially very effective, relapse is often rapid and mechanisms of acquired chemoresistance remain largely undefined. In this study, researchers from the Cancer Biomarker Centre utilised preclinical models derived from patient circulating tumour cells (CTCs), both before treatment and after the development of acquired chemoresistance, to uncover a novel mechanism of resistance to etoposide. Molecular profiling of such 'paired' models showed that although no DNA mutations could explain the acquisition of a chemoresistant phenotype, differential gene expression analysis revealed recurrent up-regulation of both subunits of the soluble guanylate cyclase (sGC) correlated with the development of acquired chemoresistance. Full activation of this pathway required both NOTCH-dependent transcriptional up-regulation and enzyme activation by nitric oxide (NO), resulting in downstream activation of protein kinase G (PKG), a known mediator of chemoresistance. Both CRISPR-mediated genetic ablation of sGC as well as pharmacological inhibition of either sGC or PKG re-sensitised cells to etoposide *in vitro*. These results were extended to demonstrate that both genetic targeting of sGC and pharmacological inhibition of NO production with the nitric oxide synthase inhibitor L-NMMA sensitised a chemoresistant patient-derived model of SCLC to cisplatin/etoposide. In summary, these data define a new mechanism of acquired chemoresistance in SCLC and provide a potential therapeutic avenue using clinically available nitric oxide synthase inhibitors.



Differential gene expression analysis of paired models generated both before treatment and after disease relapse revealed recurrent overexpression of the soluble guanylate cyclase subunit GUCY1B1. Protein overexpression was verified by immunohistochemistry.

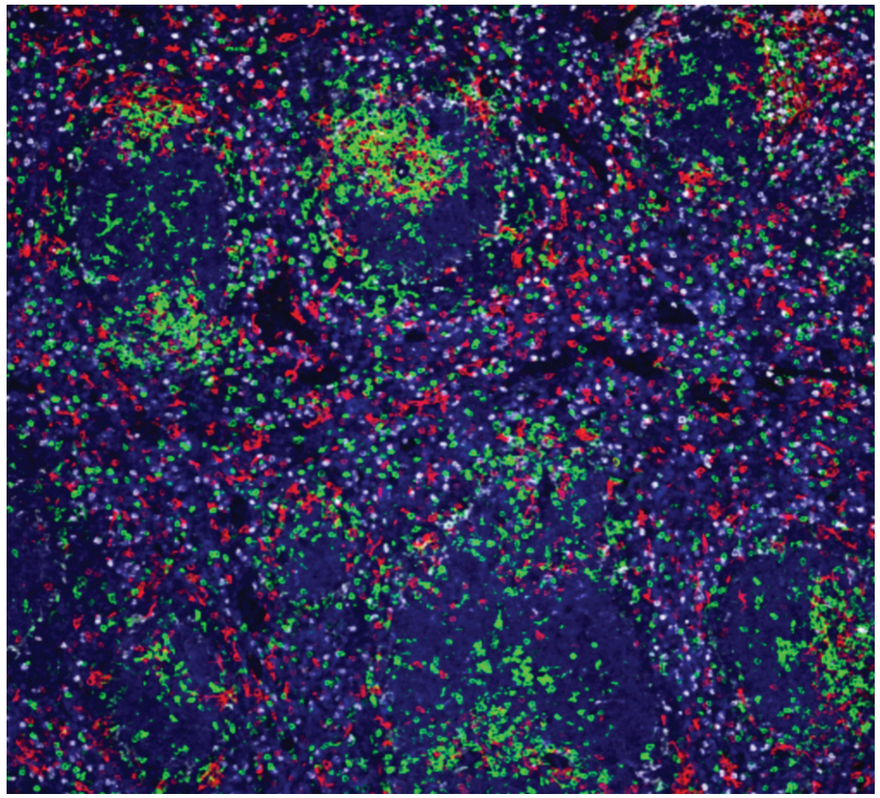
Image created by Kris Frese (Cancer Biomarker Centre)

Pelly VS, Moeini A, Roelofsen LM, Bonavita E, Bell CR, Hutton C, Blanco-Gomez A, Banyard A, Bromley CP, Flanagan E, Chiang SC, Jørgensen C, Schumacher TN, Thommen DS, Zelenay S. (2021)
Anti-Inflammatory Drugs Remodel the Tumor Immune Environment to Enhance Immune Checkpoint Blockade Efficacy.
Cancer Discovery 11(10):2602-2619.

The type of inflammation most commonly found within clinically apparent tumours promotes malignant cancer growth and is associated with worse outcome and resistance

to therapy. In previous work, the Cancer Inflammation and Immunity group showed that the inflammatory lipid prostaglandin E2 (PGE2), produced by cancer cells downstream of cyclooxygenase (COX)-2 activity, stimulates this type of protumourigenic inflammatory response and enables tumour cells to escape the anti-cancer function of the immune system. In this new study, the group has explored the use of widely used anti-inflammatory drugs that target the COX-2/PGE_2 pathway as a means to enhance the efficacy of immune checkpoint blockade (ICB) therapy – a type of immunotherapy approved for the treatment of various malignancies but where only a fraction of patients experience complete and durable responses. They now demonstrate that pharmacological inhibition of PGE2 synthesis, using a selective COX-2 inhibitor, or signalling, using antagonists of the PGE2 receptors EP2 and EP4, synergised with ICB therapy to promote tumour control in various preclinical models. Surprisingly, the synergy was also observed using 'immunosuppressive' corticosteroids, frequently given to cancer patients to limit the adverse effects that often arise following ICB. Interestingly, monotherapy with COX-2 inhibitors or PGE2 receptor antagonists induced a rapid IFN-γ-driven inflammatory response at the tumour bed similar to that seen in tumour biopsies from patients that respond to ICB in the clinic. A similar remodelling of intratumoral inflammation was also found adding COX-2 inhibitors to patient-derived tumour explants using a cutting-edge experimental system developed by collaborators from the Netherlands Cancer Institute. Altogether, the group's findings support the rationale to co-administered inhibitors of the COX-2/PGE2 axis to rapidly tilt the balance towards cancer-inhibitory inflammation and improve the efficacy of immunotherapies.

Payapilly A, Guilbert R, Descamps T, White G, Magee P, Zhou C, Kerr A, Simpson KL, Blackhall F, Dive C, Malliri A. (2021)
TIAM1-RAC1 promote small-cell lung cancer cell survival through antagonizing Nur77-induced BCL2 conformational change.
Cell Reports 37(6):109979.



Confocal multiplex IHC images of murine spleen stained for immune markers, CD8 (Red), CD4 (Green) and CD11b (White) and DAPI (Blue). Staining was performed using the Bond (Rx) staining platform (CRUK MI Core facility) and imaged using the Leica gSTED confocal microscope (Advanced Imaging).

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

Small cell lung cancer (SCLC), an aggressive neuroendocrine malignancy, has limited treatment options beyond platinum-based chemotherapy, whereafter acquired resistance is rapid and common. Two broad SCLC categories exist: in >80% cases (NE-type) cells display classical morphology and express neuroendocrine (NE) genes consistent with their cell of origin, whereas the remainder (non-NE-type) cells display variant morphology and lack NE gene expression. Further subtype classification was recently reported based on differential expression of NE or non-NE lineage transcription factors. This subtyping is a first step towards personalised treatment as subtype vulnerabilities have been identified in preclinical models. In this study the Cell Signalling group analysed expression data from SCLC tumors, patient-derived models and established cell lines, and showed that the expression of TIAM1, an activator of the small GTPase RAC1, is associated with a NE gene programme. Moreover, they showed that TIAM1 depletion or RAC1 inhibition reduces viability and tumorigenicity mainly of NE-type SCLC cells by increasing apoptosis. Interestingly, increased apoptosis is associated with conversion of BCL2 from its pro-survival to pro-apoptotic function via BH3 domain exposure. This conversion is dependent upon cytoplasmic translocation of Nur77, an orphan nuclear receptor. TIAM1 interacts with and sequesters Nur77 in SCLC cell nuclei and TIAM1 depletion or RAC1 inhibition promoted Nur77 translocation to the cytoplasm. Furthermore, mutant TIAM1 with reduced Nur77 binding failed to suppress apoptosis triggered by TIAM1 depletion. In conclusion, their data showed that TIAM1-RAC1 signalling promotes SCLC cell survival via Nur77 nuclear sequestration.

Valpione S, Mundra PA, Galvani E, Campana LG, Lorigan P, De Rosa F, Gupta A, Weightman J, Mills S, Dhomen N, Marais R. (2021)
The T cell receptor repertoire of tumor infiltrating T cells is predictive and prognostic for cancer survival.
Nature Communications 12(1):4098.

T cells are immune cells with the potential to recognise and kill cancer cells. Tumour infiltration by T cells is necessary for effective anti-cancer immune responses and data support their critical role as the agents of cancer cell killing induced by anti-PD1 check point blockade immunotherapy.

Each T cell has a receptor with a unique sequence that is the "fingerprint" for that particular T cell. These receptors can be used to identify individual T cells and any progeny derived from them that have expanded (clonal expansion) after recognising cancer cells. T cell receptor repertoire diversity is a measure of the variety of different receptors, or T cell clones, and thus of the potential to recognise many different targets, whereas T cell receptor repertoire clonality is a measure of whether there is a dominance of one or a small number of T cell clones, indicating an expansion of a narrower range of T cell clonotypes.

Members of the Molecular Oncology group hypothesised that the T cell receptor repertoire of tumour infiltrating T cells could provide clues about disease course at different stages in cancer progression. They studied the receptors of the T cells that infiltrate various tumour types and found that different patterns of T cell receptor repertoire relate to cancer patients' prognosis and response to immunotherapies. Specifically, the study showed that the diversity of the T cell receptors of tumour infiltrating T cells is prognostic in various cancers, including melanoma, breast cancer, and certain lung cancers. Moreover, receptor clonality of T cells infiltrating metastatic melanoma, before therapy, is predictive for response and survival after anti-PD1 based immunotherapy.

These results have important implications to improve the personalisation of cancer patients' treatments.

Budden T, Gaudy-Marqueste C, Craig S, Hu Y, Earnshaw CH, Gurung S, Ra A, Akhras V, Shenjere P, Green R, Jamieson L, Lear J, Motta L, Caulín C, Oudit D, Furney SJ, Virós A. (2021)
Female Immunity Protects from Cutaneous Squamous Cell Carcinoma.
Clinical Cancer Research 27(11):3215-3223.

Squamous cell carcinoma of the skin (cSCC) is the 2nd most common skin cancer in the UK, affecting approximately 40,000 people per year in the UK. There is a markedly higher incidence (62%) and rate of metastasis (75%) in men compared to women. The cSCC male bias has been dismissed as merely reflecting the increased exposure of men to ultraviolet radiation (UVR) associated with manual labour and delayed clinical presentation.
The Skin Cancer and Ageing group's research using mouse models and patient data revealed innate sex-linked biological mechanisms in the immune response that underpins the sex bias observed in incidence and outcome. In response to equal carcinogen dose and mutation burden, female mice had lower incidence and grade of tumours, driven by a higher influx of T cells and upregulation of anti-tumour immune pathways in the skin. The

group confirmed overlapping immune pathways in single cell sequencing of human skin from female cSCC patients, validating the stronger female immune response in the skin to carcinogens in humans. The importance of the immune system protecting against skin cancer was reinforced in a clinical audit of immunosuppressed and immunocompetent cSCC patients by sex. While in the immunocompetent population females had less severe disease and incidence than their male counterparts, in the immunosuppressed population both men and women had equal rates of skin cancer. Overall, this work establishes that the sex bias in skin cancer is a result of the stronger response of female immunity protecting against tumourigenesis.

Further understanding of the role of the immune system in cancer sex bias will impact potential therapy and preventative treatment in at risk populations and allow for patient stratification by risk of disease and progression. Critically, their research shows the importance of including both sexes in models for translational research, particularly for research involving immuno-oncology.

Budden T, Gaudy-Marqueste C, Porter A, Kay E, Gurung S, Earnshaw CH, Roeck K, Craig S, Traves V, Krutmann J, Muller P, Motta L, Zanivan S, Malliri A, Furney SJ, Nagore E, Virós A. (2021) Ultraviolet light-induced collagen degradation inhibits melanoma invasion.
Nature Communications 12(1):2742.

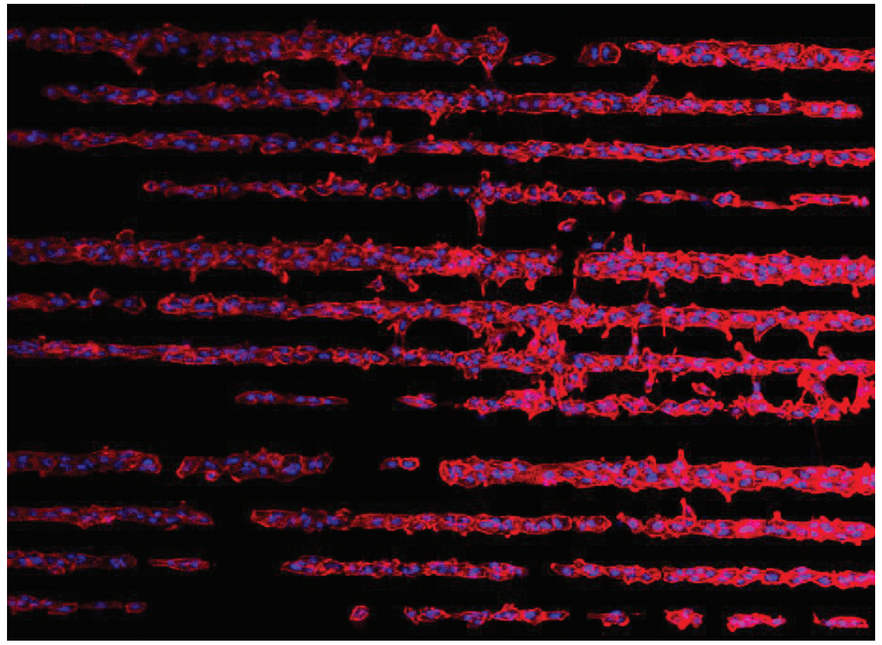
The ageing, sun-exposed dermis accumulates ultraviolet radiation (UVR) damage, and older patients develop more melanomas at UVR-exposed sites. As fibroblasts play key roles in both the stromal response to UV and in cancer progression, the Skin Cancer and Ageing group investigated whether long term UVR modifies dermal fibroblast function and how this affects melanoma invasion.

Chronic UVR exposure on dermal fibroblasts showed that extracellular matrix pathways, particularly those involved in collagen catabolism, were upregulated in the absence of acute UVR. Importantly, the expression of collagen-cleaving matrix metalloprotein-1 *(MMP1)* was persistently upregulated. This resulted in persistent degradation of collagen 1, and an overall degraded and disorganised matrix. Collagen degradation by MMP1 decreased melanoma invasion *in vitro*. Conversely, both inhibiting extracellular matrix degradation and MMP1, or higher collagen 1 expression, restored the invasion of melanoma through collagen.

This linked to the better outcomes of melanoma arising on chronically sun damaged skin. Significantly fewer cancer cells invade as single cells at the invasive front of melanomas arising in chronic sun damaged skin. The group show high collagen deposition and melanoma cell invasion in the dermis are robust predictors of poor melanoma-specific survival in three, international cohorts of primary melanoma. Thus, melanomas arising over UVR-damaged, collagen-poor skin are less invasive, and this reduced invasion improves survival. However, they discovered a subset of melanomas arising over collagen-poor, UVR-damaged dermis, have a poor outcome, and found that increased new collagen synthesis by melanoma-associated fibroblasts at the invasive front in these cases restores melanoma single cell invasion and drives poor outcome. Finally, they demonstrate high *COL1A1* gene expression is an early-stage biomarker of poor outcome across a broad range of primary cancers. This study highlights the prognostic power of the collagen architecture in aged primary melanoma tumours, making it a clinically accessible and useful biomarker for melanoma patient outcome.

Simeoni F, Romero-Camarero I, Camera F, Amaral FMR, Sinclair OJ, Papachristou EK, Spencer GJ, Lie-A-Ling M, Lacaud G, Wiseman DH, Carroll JS, Somervaille TCP. (2021) Enhancer recruitment of transcription repressors RUNX1 and TLE3 by mis-expressed FOXC1 blocks differentiation in acute myeloid leukemia.
Cell Reports 36(12):109725.

Acute myeloid leukaemia (AML) is a blood cancer characterised by a block to normal myeloid lineage differentiation. Although new treatments are now available, it still remains an often incurable disease and new therapeutic approaches – including those which promote differentiation of leukaemic blast cells - are required. The Leukaemia Biology group previously reported that the Forkhead family transcription factor gene FOXC1, a critical regulator of normal mesenchymal and mesodermal differentiation, is highly expressed in around 20% of cases of AML. In vitro and in vivo experiments demonstrated that FOXC1 contributes to a block in monocyte/



We can use micropatterns - defined patches of extracellular matrix where cells can adhere - to control the shape and position of cells. Here lung cancer cells are seeded on micropattern strips of different widths (thickest at the top) as part of an experiment studying cell migration. Cells stained to show the nucleus (blue) and actin cytoskeleton (red).

Image by Hannah Reed and Andrew Porter (Cell Signalling)

macrophage differentiation and enhances clonogenic potential. However, the mechanisms by which FOXC1 confers a differentiation block remain largely unexplored.

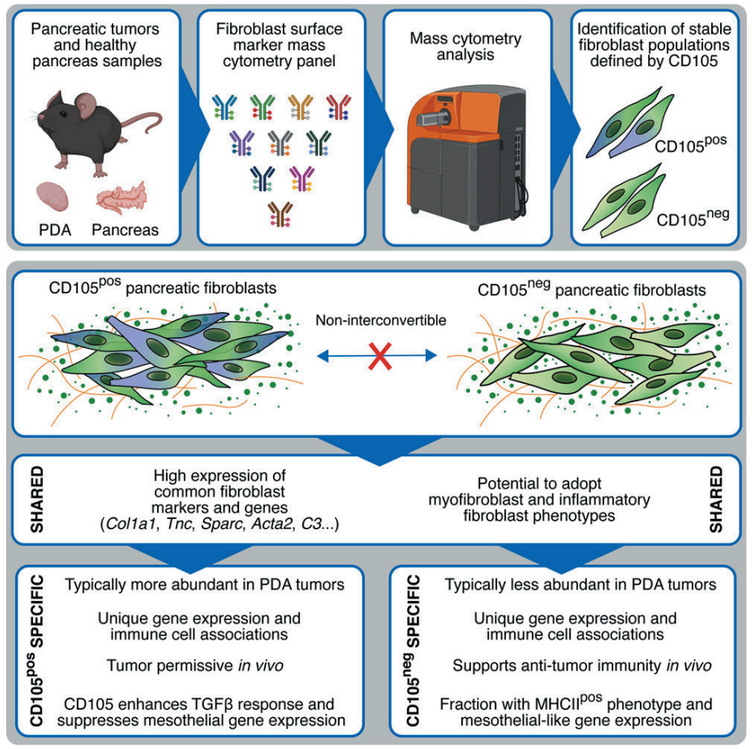
To address this in AML, the Leukaemia Biology group performed an integrated analysis of the protein-protein interactions and genome-wide binding sites of FOXC1 in human myeloid leukaemia cells. The group first profiled FOXC1's protein interactome on chromatin by Rapid Immunoprecipitation Mass spectrometry of Endogenous protein (RIME) in AML samples and discovered that RUNX1, a critical regulator of myeloid differentiation, and FOXC1 interact through their respective DNA binding domains. The two factors co-occupy primed and active enhancers distributed close to differentiation genes to limit enhancer activity. To do that, FOXC1 stabilises a repressor complex including RUNX1, HDAC1, and Groucho family member TLE3. Depletion of FOXC1 induces loss of the repressor complex from enhancers, permitting up regulation of a mature myeloid gene programme. Furthermore, depletion of FOXC1 triggers genome-wide redistribution RUNX1 and TLE3 from enhancers and toward promoters, leading to repression of self-renewal genes, including MYC and MYB.

This study suggests that therapeutic targeting of the protein:protein interactions of FOXC1 with RUNX1, or RUNX1 with TLE3, in appropriate patients may potentially prove advantageous.

Fadlullah MZ, Neo WH, Lie-A-Ling M, Thambyrajah R, Patel R, Mevel R, Aksoy I, Do Khoa N, Savatier P, Fontenille L, Baker SM, Rattray M, Kouskoff V, Lacaud G.
Murine AGM single-cell profiling identifies a continuum of hemogenic endothelium differentiation marked by ACE.
Blood [Epub 13 September 2021]

Haematopoietic stem cells (HSCs) sit at the apex of the blood system and, in the form of bone marrow transplants, are powerful treatment modalities for cancer and blood malignancies. Researchers in the Stem Cell Biology group established a unique and comprehensive atlas of the initial molecular events giving rise to HSCs.

The limited availability of bone marrow for transplants has made the efficient generation of HSCs in vitro an essential goal of regenerative medicine. Understanding the intrinsic and extrinsic cues that drive HSCs generation is critical for developing efficient in vitro HSC production protocols. HSCs arise in the midgestation embryo from rare specialised endothelial cells called haemogenic endothelium (HE). These cells become blood cells through an endothelial to haematopoietic transition (EHT). Although HE cells exist at multiple embryonic sites, they generate HSCs mainly in the dorsal aorta (DA). The rarity of the HE cells and the limited options for isolating these cells significantly impede the study of



Graphical Abstract from Hutton et al. Cancer Cell 2021 (doi: 10.1016/j.ccell.2021.06.017)

these processes. As such, the earliest molecular events driving the commitment from HE to HSCs remain poorly characterised.
Using transgenic HE murine reporter models, the researchers took advantage of new advances in single-cell technology to explore the canonical EHT differentiation continuum within the DA and the subaortic niche cells. The authors conducted these analyses in the presence and absence of the key EHT regulators RUNX1 and GFI1. They uncovered a pre-HE to HE continuum marked by Angiotensin-I converting enzyme (ACE). They unravelled that HE cells begin to enter the cell cycle near the time of EHT initiation. This study also revealed that a small subpopulation of RUNX1 expressing DA niche cells, consisting of vascular smooth muscle cells and PDGFRa+ mesenchymal cells, functionally support HSCs emergence. Overall, this novel dataset has already provided new insights into HE differentiation toward HSC and represents a unique and powerful resource to investigate these processes further.

Hutton C, Heider F, Blanco-Gomez A, Banyard A, Kononov A, Zhang X, Karim S, Paulus-Hock V, Watt D, Steele N, Kemp S, Hogg EKJ, Kelly J, Jackstadt RF, Lopes F, Menotti M, Chisholm L, Lamarca A, Valle J, Sansom OJ, Springer C, Malliri A, Marais R, Pasca di Magliano M, Zelenay S, Morton JP, Jørgensen C. (2021)
Single-cell analysis defines a pancreatic fibroblast lineage that supports anti-tumor immunity.
Cancer Cell 39(9):1227-1244.e20.

The tumour microenvironment of pancreatic cancers is highly complex and has been ascribed with both tumour promoting and tumour restrictive functions. Determining how interactions across the stromal subsets regulate tumour promotion versus regression is critical to identify which stromal targets to block or augment for improved therapeutic efficacy. To this end, the Systems Oncology group annotated the tumour microenvironment in pancreatic ductal adenocarcinoma (PDA) and identified specific populations of tumour permissive and tumour restrictive populations of pancreatic fibroblasts. Specifically, they used mass cytometry to annotate mesenchymal cell populations (endothelial cells, perivascular cells and cancer-associated fibroblasts) as well as immune cell populations in tumours from a mouse model of PDA. The team identified two fibroblast lineages, differentiated by the expression of the TGFβ co-receptor CD105, which were present in both normal and tumour bearing pancreas as well as in most normal and tumour-bearing tissues examined. Notably, whereas CD105^{pos} pancreatic fibroblasts were tumour permissive, CD105^{neg} pancreatic fibroblasts were tumour suppressive. The tumour suppressive effect of CD105^{neg} fibroblasts was dependent on a functional immune system. Critically the results identified, for the first time, pancreatic fibroblast lineages with distinct tumour permissive and suppressive function.

Phatak V, von Grabowiecki Y, Janus J, Officer L, Behan C, Aschauer L, Pinon L, Mackay H, Zanivan S, Norman JC, Kelly M, Le Quesne J, Muller PAJ. (2021)
Mutant p53 promotes RCP-dependent chemoresistance coinciding with increased delivery of P-glycoprotein to the plasma membrane.
Cell Death & Disease 12(2):207.

Previously, the Tumour Suppressors group had uncovered a role for mutant p53 in regulating RCP (Rab coupling protein/ Rab11-FIP1) to promote the recycling of integrins and growth factor receptors. An enhanced interaction between Rab11-FIP1 and these receptors allowed an increased signalling of the receptors to facilitate cell migration and cell invasion. In this paper, they discovered that another membrane protein, P-gp interacted with Rab11-FIP1 in mutant p53 cells. P-gp is known for its role in exporting chemotherapeutics and promoting chemoresistance. Many researchers have shown that expressing mutant p53 in cells makes cells more likely to become chemoresistant. Here they showed that the interaction between Rab11-FIP1 and P-gp is important in mutant p53 driven chemoresistance. Loss of mutant p53 or Rab11-FIP1 enhanced chemosensitivity. In response to chemotherapeutics, the team demonstrated an increased expression of P-gp on the plasma membrane and an increased efflux of a fluorescent P-gp substrate in mutant p53 cells.

von Grabowiecki Y, Phatak V, Aschauer L, Muller PAJ. (2021)
Rab11-FIP1/RCP functions as a major signalling hub in the oncogenic roles of mutant p53 in cancer.
Frontiers in Oncology 11:804107.

In a more recent, follow-up publication, the Tumour Suppressors group also identified ATP7B (a copper transporter that can also efflux cisplatin) as interactor of Rab11-FIP1, which re-located to the plasma membrane in a cisplatin/Rab11-FIP1 dependent manner in mutant p53 cells. Together these findings demonstrate that Rab11-FIP1 is a major hub in how mutant p53 regulates not only cell migration, but also chemoresistance. Future work will focus on how this regulation is orchestrated.

CANCER RESEARCH UK MANCHESTER INSTITUTE

RESEARCH GROUPS

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

Cancer Research UK Manchester Institute Cancer Biomarker Centre

Director
Caroline Dive

Staff Scientists
Alastair Kerr
Elaine Kilgour
Dominic Rothwell
Jonathan Tugwood

Associate Scientists
Kris Frese
Carlos Lopez-Garcia[2]
Kathryn Simpson

Service Manager
Tony Price

Postdoctoral Fellows
Katarzyna Bloch
Francesca Chemi
Ellyn Hughes
Sumitra Mohan
Maria Peiris-Pages

Bioinformaticians
Phil Carter
Saba Ferdous[2]
Steven Hill
Mukarram Hossain
Sam Humphrey[1,2]
Bedirhan Kilerci
Katarzyna Murat
Simon Pearce
Matthew Roberts
Cong Zhou

Clinical Informaticians
Fouziah Butt
Ciara Dwan[1]
Paul Fitzpatrick
Hannah Frost
Leanna Goodwin
Chris Grave[3]
Richard Hoskins[3]
Donal Landers
Anja Le Blanc[3]
Catherine McGuire[3]
Leanne Phillips
Paul O'Regan
Laura Stephenson
Julie Stevenson
Jason Swift
Jenny Ward
Magdalena Wysocka[1]
Oskar Wysocki[3]
Zili Zhou[3]

Clinical Fellows
Alicia Conway
Victoria Foy
Melissa Frizziero
Matthew Howell[2]

Graduate Students
Oliver Bartley

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 patients with small cell lung cancer to discover new targets and test new therapies. Here we highlight studies from our Preclinical Pharmacology (PP), Nucleic Acids Biomarkers (NAB), Cells and Proteins (CAP), Bioinformatics and Biostatistics (BBS), and digital Experimental Cancer Medicine Team (dECMT) teams. Our quality assurance, operations and administrative teams provide invaluable support to our research.

Small cell lung cancer

Small cell lung cancer (SCLC) is an aggressive, highly metastatic, and incurable neuroendocrine cancer. This disease forms an important focus for the Cancer Biomarker Centre and to contribute clinically relevant lung cancer research, CBC established a longstanding partnership with Prof Fiona Blackhall (UoM/CFT), a Professor in Thoracic Oncology and Lung Disease Group Chair at the Christie NHS Foundation Trust.

Patient derived preclinical models reveal novel biology of SCLC

The PP team continues to expand the diversity of our biobank of CDX (currently >60 models). Several CDX were derived from patients before treatment with immune checkpoint inhibitors and following disease progression, facilitating discovery of tumour intrinsic predictive biomarkers for response to this recently licensed therapeutic option. In 2020, they reported a new SCLC subtype driven by ATOH1, a neuroendocrine transcription factor not previously known to play a role in SCLC. They discovered that ATOH1 mediates a distinct transcriptional program, and both genetic and pharmacological targeting of this program increased cell death, implicating ATOH as a potential therapeutic target. They also exploited our pre and post chemotherapy CDX to discover a novel mechanism of acquired chemoresistance (an almost universal clinical occurrence), involving soluble guanylate cyclase that drives a nitric oxide-dependent signalling cascade culminating in activation of protein kinase G (see Research Highlights) another potentially tractable target.

SCLC Vasculogenic Mimicry (VM), perfusable tumour derived vessels

The PP team continued to study 'plasticity' of SCLC cells, in this case exemplified by their ability to mimic endothelial cell behaviours via VM. Having shown that VM vessels frequently contained red blood cells, they utilised a fluorescent lectin that interacts with glycoproteins in the basement membrane *in vivo* which labelled inner walls of both endothelial (CD31 positive) and VM (CD31 negative) vessels inferring perfusion with functional connectivity between VM vessels and the endothelium (Figure 1).

The first CDX Model of an Extra-pulmonary neuroendocrine carcinoma (EP-NEC)

EP-NECs have limited treatment options and biologically are poorly understood. In collaboration with Prof Juan Valle and Dr Mairead McNamara (UoM/CFT), the PP team generated and characterised the first CDX model of EP-NEC, and after histopathology (Figure 2) and RNAseq analysis, identified it as a Merkel Cell Carcinoma (MCC) EP-NEC, which prompted a review of the donor patient's original diagnosis. This CDX recapitulated the biology and chemotherapy response of the donor patient's tumour and holds potential as an avatar to guide future treatment.

SCLC Immunology

The CAP team developed methods for *ex vivo* co-culture of immune cells (peripheral blood lymphocytes or Natural Killer (NK) cells) and

Alessia Catozzi
Yitao Chen
Jakub Chudziak
Victoria Gernedl
Julia Ogden
Sarah Pearsall[2]
Sophie Richardson[1]
Max Schenk[2]

Scientific Officers
Sophie Atkinson
Diana Baxter
Dominic Birch
Kieran Bradbury
Samuel Bratt
Stewart Brown[2]
Debbie Burt
Holly Butterworth
Mathew Carter
Rebecca Carroll
Harry Clarke
Alex Clipson
James Copley
Joanne Felce
Lynsey Franklin
Melanie Galvin
Laura Glass
Molly Glenister-Doyle
Eleanor Gregory[2]
Eleni Grigoriou
Tatiana Guevara
Grace Hampson
Michael Hoffs
Elizabeth Hunter
Hayley Johnson
Alex Jordan
Noel Kelso
Andrew McLeod[2]
Simrah Mohammad[2]
Derrick Morgan
Karen Morris
Nicola Overton
Anthony Oojageer[2]
Safwan Patel
Rosalind Pearson[2]
Jackie Pierce[2]
Sabah Rashid
Alan Redfern
Mitchell Revill
Sophie Richardson[2]
Andrew Robb
Caroline Roberts
Jordan Roebuck
Karishma Satia
Daniel Slane-Tan
Karen Smith
Nigel Smith
Simon Topham
Daniel White

Junior Translational Scientist
Simon Hood

Laboratory Manager
Matthew Lancashire

Laboratory Support Technicians
Andrew Stevens
Mahrukh Khurshid

CBC Programme Manager
Ekram Aidaros-Talbot

COMPASS Study Project Manager
Jonathan Wake[2]

UpSMART Accelerator Project Manager
Akshita Patil

Translational Research Project Manager
Jackie Mitchell[1]

Administrative Coordinator
Suzanne Bickley

PA to the CBC Director
Delydd Jones[2]

[1]Joined in 2021
[2]Left in 2021
[3]Employed by The University of Manchester's IT Services or Department of Computer Science and fully or partly funded by CRUK Manchester Institute. The wider team includes Dr Andre Freitas (Department of Computer Science) and Dr Donna Graham (The Christie NHS Foundation Trust)

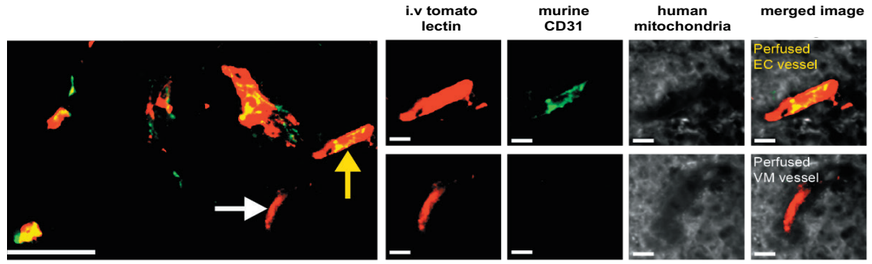


Figure 1. Intravenous tomato lectin injection to reveal perfusion of endothelial (EC) and vasculogenic mimicry vessels in CDX tumours.
Lectin labelled glycoproteins lining the inside of functional vessels and immunofluorescent staining of resected tumours are highlighted in both CD31+/(green)/lectin+ (red) EC vessels (yellow arrow, merge) and CD31-/lectin+ VM vessels (white arrow, merge). Human anti-mitochondria staining shows presence of CDX tumour cells. Representative image from CDX19, also observed in other CDX (data not shown). Scale bars, 50 µm.

disaggregated SCLC CDX cells to enable investigation of anti-tumour immune responses and resistance to immunotherapy (Figure 3). Early data suggest differential susceptibility between CDX models, as well as between the neuroendocrine and non-neuroendocrine CDX subpopulations within models, to NK cell-mediated killing. To complement these ex-vivo studies an IHC assay against the NK cell receptor NKp46 is being optimised for detection of tumour infiltrating NK cells. Generation of new CDX models (four so far), with banking of matched PBMCs from donors receiving immunotherapy, is also underway to progress co-culture research and study adaptive immune biology.

Subtyping SCLC via DNA methylation profiling of ctDNA

The recent description of molecular subtypes of SCLC with preclinical evidence of subtype vulnerabilities affords a 'horizon view' of personalised medicines for patients with this recalcitrant cancer. Given the paucity of adequate tumour biopsies, a blood test to subtype each patient will support this goal. The NAB and BBS teams combined their expertise to optimise our genome-wide circulating free (cf) DNA methylation assay (T7-MBD-seq), with a bespoke bioinformatics pipeline and R package for analysis. They applied this pipeline to profile DNA methylation in >1,300 samples, including 593 cfDNA samples, across several studies including SCLC CDX and cfDNA from patients with SCLC (in collaboration with Prof Charles Rudin at Memorial Sloane Kettering Cancer Center, New York). Together they developed a sensitive tumour/normal prediction classifier for disease monitoring and utilised differences in methylation patterns between the predominant molecular subtypes, based on NE transcription factors ASCL1, NEUROD1 and a double negative, to derive a molecular subtype classifier. These blood tests are now being validated in clinical cohorts.

Cancer of Unknown Primary – taking the U out of CUP

The NAB and BBS teams also studied DNA methylation to develop a tissue-of-origin classifier to support treatment decisions in cancer of unknown primary (CUP) study in collaboration with Dr Natalie Cook (UoM/CFT). CUP describes a metastatic cancer cohort, with unknown primary tumour, making selection of beneficial treatment challenging. Tissue-of-origin classifiers were trained on methylation array data from 8892 samples, across 33 cancer types. These classifiers are in the late stages of development (Figure 4).

Bioinformatics for cfDNA methylation assays

The BBS team used a Nextflow framework to create a robust and reproducible data processing workflow enabling capture of quality control metrics and metadata. Using a novel approach, BBS converted data from methylation arrays (an unsuitable approach for cfDNA) including published data on SCLC and data from a range of tumour generated within The Cancer Genome Atlas consortium (TCGA) to comply with NAB cfDNA sequencing data. Machine learning was

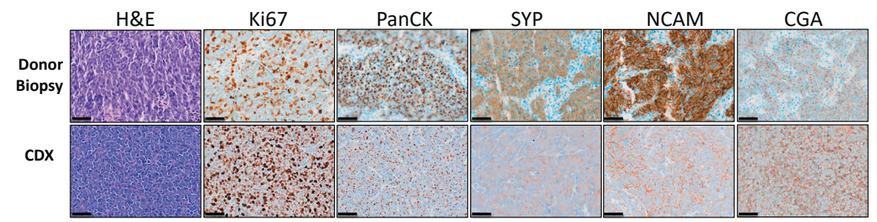


Figure 2.
Histopathology analysis of donor biopsy and derived CDX tumour showing similar neuroendocrine carcinoma morphology and expression of diagnostic markers (PanCKs, pan-cytokeratins, SYP, Synaptophysin, NCAM, Neural cell adhesion molecule, CGA, Chromogranin A). Scale bar (black line), 50 µm.

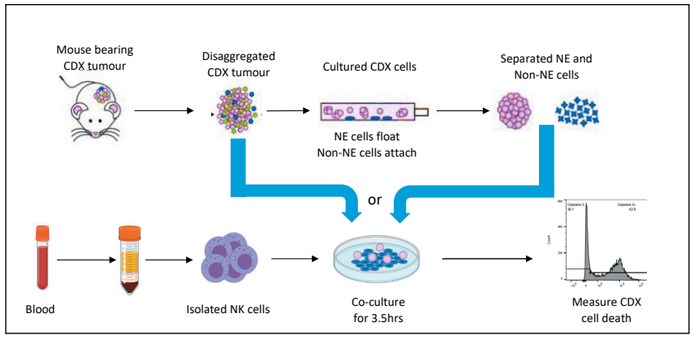


Figure 3.
Tumour cells are disaggregated for *ex-vivo* culture from CTC Derived Explant Models (CDX) grown in mice. Neuroendocrine (NE) tumour cells float in culture in contrast to Non-NE cells, which are adherent allowing physical separation of these differing phenotypes. NE and Non-NE tumour cells are co-cultured with Natural killer (NK) cells isolated from a healthy volunteer or patient's blood to determine the extent of NK-cell mediated tumour cell killing. This system is being developed to understand the mechanisms used by tumour cells to escape NK-cell killing and to test the effect of therapeutics.

applied to detect presence or absence of tumour-derived DNA in plasma (ctDNA), SCLC molecular subtypes and tissue-of-origin for CUP.

ctDNA liquid biopsy biomarkers to direct therapy decisions: CACTUS, DETECTION and DYNAMIC trials in Melanoma

Guided by basic and translational research from Prof Richard Marais' group (page 34), the NAB team, supported by our Quality Assurance team, developed our portfolio of GCP-compliant liquid biopsy trials including CACTUS, DETECTION and DYNAMIC trial in partnership with Prof Paul Lorigan and Dr Rebecca Lee (UoM/CFT). The *CACTUS* trial (CirculAting Tumour DNA gUided therapy Switch) uses a ddPCR ctDNA assay to measure mutated BRAF levels that instruct treatment switch from targeted to immunotherapy for advanced cutaneous melanoma: 37 patients have been screened and validated ctDNA data returned to clinic within 7 days. The *DETECTION* trial (Circulating tumour DNA guidEd Therapy for stage IIB/C BRAF or NRAS mutant- positive mElanoma after surgiCal resecTION) opened to recruitment in late 2021. DETECTION involves ddPCR ctDNA analysis to detect early relapse/micro-metastatic disease and select patients for targeted therapy using a panel covering 3 BRAF, 4 NRAS and 2 hTERT mutations. NAB delivers the first validated DETECTION assay in January 2022; sequential samples from up to 900 patients are expected over the next decade. NAB are also validating cfDNA ddPCR assays to monitor tumour activity and burden (TAB) levels for the upcoming *DYNAMIC* trial (Circulating tumour DNA guided Adaptive BRAF and MEK Inhibitor therapy), which uses BRAF V600 ddPCR assays to monitor TAB to inform adaptive BRAF-MEK inhibitor therapy in Stage III unresectable/IV cutaneous melanoma.

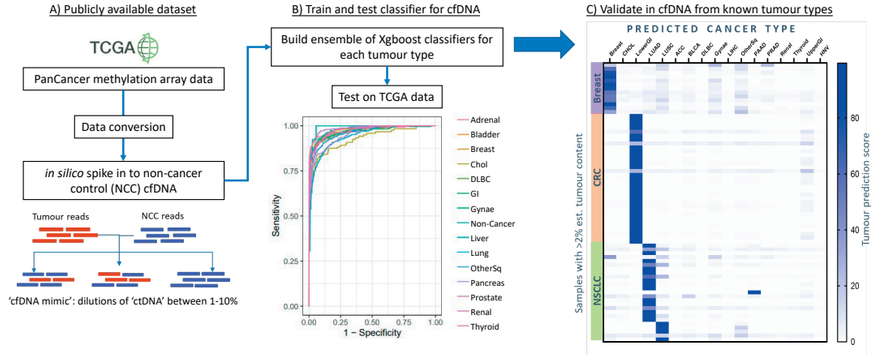
Biomarkers to inform immunotherapy trials

Reflecting unmet clinical need, the CAP team continue to expand the CBC immune biomarker 'toolkit'. Through a partnership with ThermoFisher, T-cell receptor sequencing assays have been optimised to reveal T-cell receptor clonality, diversity and convergence in blood samples. An early study using these assays involves a pilot set of longitudinal samples from NSCLC patients receiving immunotherapy/ chemotherapy combination with ongoing clonal analysis of patient profiles by the BBS team.

CRUK Manchester Institute basic science biomarker discovery to the clinic

Working with Dr Santiago Zelenay (see page 20), CAP has developed an assay to assess his COX_IS gene signature in FFPE patient samples using the clinically compatible Nanostring platform to validate its prognostic and/or predictive clinical value. Emerging data shows good intra and inter-assay reproducibility for analysis of clinical samples. Our ACED funded PhD student, also working jointly with Zelenay's group, has analysed COX-IS and other immune/ inflammatory gene signatures in early stage lung cancer resections to assess if there are associations with risk of disease relapse. A complementary COX2 IHC assay shows correlation between COX2 gene (Figure 5) and protein expression and is included in a multiplex IHC panel of 24 markers being established in collaboration with Prof Lisa Coussens (Oregon Health Science University) to enable assessment of expression levels and spatial distribution of immune cells within the tumour microenvironment and ultimately relationships with patient outcomes.

Figure 4.
Tissue of origin classifiers in cfDNA to support management of Cancers of Unknown Primary.
A) Pan cancer methylation array data undergo conversion and in silico spike into non-cancer control cfDNA reads acting as a 'cfDNA mimic'. B) An ensemble of Xgboost classifiers is trained on and then tested for each tumour type. C) Each tumour classifier is validated on cfDNA samples from patients with known tumour types and applied to cohort of cfDNA samples from patients with Cancer of Unknown Primary for tumour type prediction.



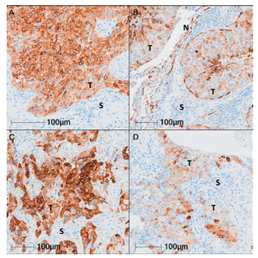


Figure 5.
COX-2 protein expression in non-small lung cancer measured by immunohistochemistry. A, B: Representative images of squamous cell carcinoma expressing high and low levels of COX-2 in tumour and normal tissue, respectively. C, D: Adenocarcinoma expressing high and low levels of COX-2 in tumour. T = tumour, S = stroma, N = normal lung tissue (pneumocytes).

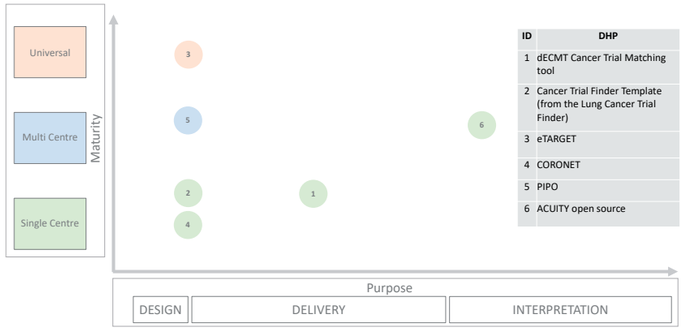
Community based blood sampling

The CAP and digital ECMT teams in partnership with Fiona Thistlethwaite (UoM/CFT) assessed the feasibility of detecting 15 cytokines by ELISA in as little as 30µl blood collected using a micro-sampler device and optimised workflows for sample processing and analysis. This approach is being applied in the *NOTION* trial to evaluate home-based blood sampling kits that allow assessment of cytokines as an 'early warning' system of adverse events and cytokine release syndrome in patients receiving immunotherapy and advanced T-cell therapies.

Bioinformatics and Biostatistics across the Cancer Biomarker Centre

The BBS team integrates bioinformatics and statistical methods for its many and varied projects including specialised methods for single cell analysis to classify CTCs. They advise on the statistical powering of experimental designs including for the *NOTION* trial (described above) and the *VALTIVE1* trial, the objective of which is to qualify plasma Tie2 for clinical decision making around anti-angiogenic agents in ovarian cancer in partnership with Prof Gordon Jayson (CFT/ UoM), and the Accelerometer trial as part of our CRUK UpSMART Accelerator Award, coordinated by digital ECMT (see below). The *TARGET* trial, led by CBC alumnus, Dr Matt Krebs (CFT/UoM), matching patients with a broad range of advanced cancers to early phase clinical trials is now complete. Within TARGET, the NAB and BBS teams analysed and reported somatic mutations and copy number alterations across a 641 cancer-associated gene panel in a single ctDNA assay. The latest BBS data processing workflow was used to rerun all the ctDNA analyses producing a rich, internally consistent retrospective dataset now available to researchers to test hypotheses.

Figure 6.
Summary of UpSMART Digital Healthcare Products (DHPs) currently available to the consortium at the end of 2021.



ID	DHP
1	dECMT Cancer Trial Matching tool
2	Cancer Trial Finder Template (from the Lung Cancer Trial Finder)
3	eTARGET
4	CORONET
5	PIPO
6	ACUITY open source

Digital solutions to support treatment for cancer patients

The digital ECMT seeks to digitally empower patients and healthcare professionals to innovate and design new cancer care pathways. They provide next generation patient cancer care through comprehensive data-driven evidence, enabling transformation of clinical decision-making, evolving the role of the patient and improving patient outcomes. This is achieved by listening to patients and healthcare professionals, understanding their needs and working proactively with them to develop ethical algorithms (AI) to support patient care, building digital solutions and evaluating technologies under clinical trial conditions (technology clinical trials).

Examples of digital ECMT's distinctive research include: (i) The *IN-HOME* study, assessing feasibility of Acute Kidney Injury (AKI) detection in the patient's home; Part A demonstrated feasibility and Part B is now opened to recruitment, evaluates potential for earlier diagnosis of AKI/change in renal function in cancer patients with intensive home monitoring. (ii) The *A-EYE* study, developing new AI methods to detect adverse retinal abnormalities associated with cancer treatment, now open to recruitment at the Manchester Royal Eye Hospital with >240 patients recruited. (iii) The *NOTION* study (with the CAP team), enabling dry blood spot technology to measure cytokine levels in the home for early detection of immune related toxicities. (iv) The *eTARGET* tool, a digital solution integrating clinical and genomic data, histopathology data and corresponding images in a single portal to support decision making by Molecular Tumour Boards and deployed in two clinical trials being led from the CFT *(TARGET National and CUP-COMP)*. eTARGET incorporated a link to the *digital ECMT Cancer Trial Matching tool* supporting clinical decision making by matching a cancer patient's tumour genetic profile to optimal clinical trials. (v) dECMT Artificial Intelligence (AI) researchers, business analysts and software engineers continued to improve the CORONET tool (COVID-19 risk in Oncology Evaluation Tool) using new datasets alongside input from the BBS team and our clinical colleagues. The *CORONET* team were recognised as "trailblazers in COVID-19 Research Response" at the NIHR Clinical Research Network Greater Manchester's Evening of Excellence in November 2021. (vi) With colleagues in Italy and Spain, digital ECMT continued to lead our CRUK Accelerator Award, *UpSMART* to enable SMART Experimental Cancer Medicine Trials. Six Digital Healthcare Products (DHPs) were made available to the network of 24 sites (Figure 6) and with six more DHPs prioritised for the coming year. (vii) digital ECMT joined a consortium led by Vall d'Hebron (Barcelona) to secure EU Horizon 2020 funding for *CCE_DART* (Building Data Rich clinical Trials) with partner sites across Europe.

Publications listed on page 64

CANCER INFLAMMATION AND IMMUNITY

Group Leader
Santiago Zelenay

Postdoctoral Fellows
Eduardo Bonavita
Agrin Moeini

Scientific Officer
Shih-Chieh Chiang

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

Clinical Fellow
Charles Earnshaw

[1]Left in 2021

The inflammatory response that takes place at the tumour bed has a dual role in cancer. The most common type of inflammatory profile measured in solid tumours is long known for fostering aggressive tumour growth and for its strong association with dismal prognosis. In contrast, the remarkable clinical responses that some patients experience following treatment with so-called immune checkpoint inhibitors (ICIs) have highlighted a type of inflammation with profound cancer inhibitory features. The latter inflammatory 'flavour' is less often present in clinically apparent tumours and is linked to increased infiltration by select innate and adaptive immune cells with key functions in anti-tumour immunity.

Our group investigates the principles and rules that control the establishment of tumour inflammatory environments that either promote or restrain cancer growth spontaneously or following treatment. We argue that improved understanding of the determinants of the intratumoral inflammatory profile will help design better cancer treatments, especially those that exploit the anti-cancer function of the immune system. In this context, we combine fundamental and translational research to inform the design of novel interventions that boost tumour immunity and improve the efficacy of cancer therapy.

Much of our group's current efforts are centred on investigating the role of the cyclooxygenase (COX)-2/prostaglandin E2 (PGE2) pathway in tumour inflammation and immunity. This pathway is very often upregulated across many tumour types, including in lung, colorectal, breast, and pancreatic cancers and has been implicated in promoting several aspects of malignant tumour growth. Importantly, our past work has identified PGE2 as a powerful instructive signal that stimulates a type of inflammatory flavour that fuels tumour growth and therapy resistance through enabling immune escape. Combining the use of genetically engineered *in vitro* and *in vivo* pre-clinical cancer models with the analysis of patient samples, our work specifically pointed to the inhibitory effects of PGE2 on the immune system as the basis for its protumourigenic role. Accordingly, genetic ablation of PGE2 synthesis on cancer cells, or of its receptors EP2 and EP4, on select immune cell subsets led to spontaneous immune-dependent control of tumours that otherwise grow aggressively in wild-type hosts (Zelenay et al. *Cell* 2015; Bonavita et al. *Immunity* 2020). Searching for the primary regulators of cancer inhibitory inflammation in these experimental systems, we showed that natural killer (NK) cells are direct targets of PGE2 activity *in vivo*. When NK cells were rendered insensitive to PGE2 through genetic ablation of EP2 and EP4, NK cells drove an intratumoral inflammatory remodelling characteristic of the so called 'hot' inflamed tumours that set the stage for T cell-mediated tumour control.

Based on this genetic evidence highlighting PGE2 as a primary immunosuppressive factor in the tumour environment, we have explored if and how pharmacological inhibition of COX-2 could equally modulate the inflammatory landscape at the tumour bed and enhance the efficacy of ICIs (Pelly and Moeini et al. *Cancer Discovery* 2021). To test the value of therapeutically inhibiting the pathway in clinically relevant settings, we first used celecoxib, a selective COX-2 inhibitor widely prescribed for the management of conditions like arthritis. Daily oral administration of celecoxib, at a dose considered safe for human use, alongside treatment with ICI led to greater tumour control compared with the single treatments. Across multiple experiments in independent cancer models, we found that ICIs and selective COX-2 inhibition synergised. About two-thirds of animals fully eradicated their tumours after receiving combination therapy, while around only 25% eradicated their tumours when monotherapy was followed with ICIs, and <5% achieved full tumour eradication following monotherapy with celecoxib. We obtained similar results using forefront antagonist of the PGE2 receptors, EP2 and EP4, establishing a prevalent role of PGE2, among other prostaglandins and COX-2 products, and a potentially safer more targeted approach to limit the immunosuppressive effects of PGE2.

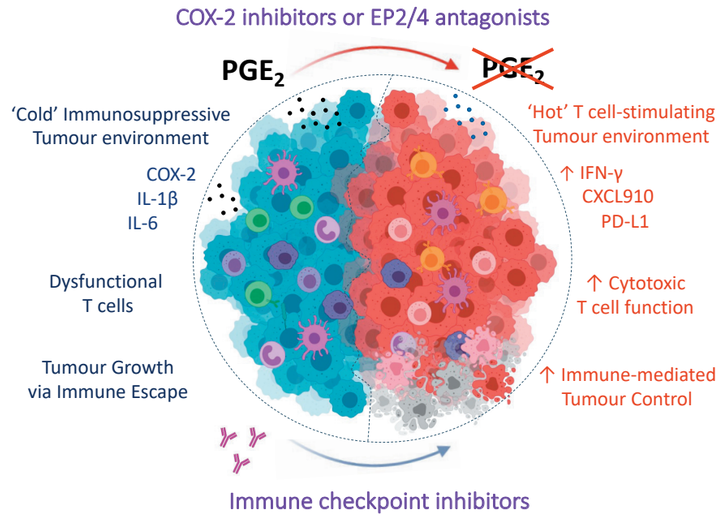


Figure 1.
Anti-inflammatory Drugs to Turn Up the Heat of Intratumoural Inflammation.
By performing in-depth inflammatory profiling of mice and human tumours, we have identified mechanisms by which anti-inflammatory drugs rapidly alter the tumour inflammatory profile, tilting the balance towards cancer inhibitory inflammation and enhancing the response to immunotherapy based on immune checkpoint inhibitors.

Most surprisingly, the synergy with ICIs was also observed when co-administering corticosteroids, widely considered to be potent immunosuppressants and frequently prescribed to cancer patients for the management of the adverse effects that often patients face following ICI therapy. These findings are of particular clinical relevance as they suggest that corticosteroids, while limiting the toxicities of ICIs, might concomitantly improve the anti-tumour response. Although it remains to be established in which exact settings corticosteroids might boost immune-dependent tumour control, our current work supports the notion that broad non-steroidal and steroidal anti-inflammatory drugs can somewhat paradoxically stimulate a cancer-restraining inflammatory response.

To investigate the underlying mechanisms by which pharmacological inhibition of PGE2 synthesis or signalling improved the efficacy of ICI therapy, we carried out deep cellular and molecular profiling of tumours early following treatment. In doing so, we found that both celecoxib and the EP2/EP4 antagonists can very rapidly stimulate a shift in the inflammatory profile, tilting the balance towards a type of inflammation that is also observed enriched in patients that respond to ICIs. Importantly, these rapid changes were also transient and waned over time unless we re-dosed the tumour-bearing animals with the COX-2/PGE2 inhibitors. This data exposed a notable plasticity in the intratumoural inflammatory landscape and implied that sustained inhibition of the COX-2/PGE2 axis might be required to maximise the efficacy of ICIs.

We demonstrated the relevance of our findings for human cancers by showing that addition of celecoxib also leads to a similar, rapid shift in the inflammatory profile of patient tumours using a recently established experimental platform developed by our collaborators Daniela Thommen and Ton Schumacher from the Netherlands Cancer Institute (NKI). Thanks to a CRUK travel award, Victoria Pelly, a former postdoc in the group spent four months at the NKI where she tested the effect of adding celecoxib to short-term cultures of freshly explanted surgical specimens from a variety of cancer types. She found, similarly as in the mouse cancer models, that celecoxib did not generally dampen inflammation but rather promoted an intratumoural inflammatory switch that was accompanied by enhanced T cell effector function.

Altogether, our findings establish PGE2 signalling as an independent immune checkpoint and support the rationale for using readily available anti-inflammatory drugs to tilt the balance toward cancer-inhibitory inflammation and improve the efficacy of current immune-targeting drugs (Figure 1). Certainly, these findings have significantly contributed to the design of two investigator-led clinical trials sponsored by The Christie NHS Foundation Trust. The first one, IMpALA (IMmunological effects of AveLumab and Aspirin) is funded by a Breast Cancer Now Catalyst Programme and will test in a neoadjuvant setting the combination of high-dose aspirin, a widely used anti-inflammatory drug with the ICI avelumab. The primary objective is to determine whether co-administering aspirin, a COX-inhibitor with ICI treatment switches the intratumoural inflammatory profile towards one that is more favourable to T cell-mediated tumor control as observed in our preclinical models. The second trial, LION (Lifting Immune CheckpOints with NSAIDs), is a pan-tumour basket trial funded by the J P Moulton Charitable Foundation and The Christie NHS Foundation Trust, in which we will test if addition of a selective COX-2 inhibitor enhances the efficacy of the standard of care immunotherapy in non-small cell lung cancer, triple-negative breast cancer and clear cell renal cell cancer.

Publications listed on page 65

CELL DIVISION

Group Leader
Iain Hagan

Postdoctoral Fellows
Asma Belbelazi[1]
Daniela Eckert
Zoe Edwards
Lenka Halova
Cynthia Li[2]
Pawan Singh
Sanjeeta Tamang[1]
Neslihan Zohrap[2]

Senior Scientific Officers
Eleanor Wendy Trotter
Keren Dawson
Pengbo Wang[3]

Graduate Students
Zoe Lee
Millie Jones
Alexandra Hendry[3]
Holly Greenaway[1]

[1]Joined in 2021
[2]Left in 2021
[3]Joined from Prostate Oncobiology in 2021

The inappropriate proliferation of cancer cells can arise from unchecked cell division, a failure to engage cell death pathways, or simultaneous changes in both. Understanding how the diverse cues are integrated to co-ordinate cell division and death is therefore key to understanding the biology of cancer. The DNA damaging approaches of chemotherapy and irradiation owe much of their success to the checkpoint pathways that ensure transition through the cell division cycle only occurs when genome integrity is guaranteed. We study these checkpoint pathways and the control of commitment to the physical process of genome segregation, mitosis. Because the regulatory networks that control cell division are highly conserved, we study these controls in human cells and unicellular fission yeast. The yeast work can identify key questions to ask in the context of complex 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 whether and when a cell leaves the non-cycling G0 state to enter the cell cycle by passing through a decision point of no return in G1 phase called the 'Restriction point' (Figure 1). Once cells are through the Restriction point, they are committed to the cycle and progress around the cycle, even if the pro-division cues that pushed them through this regulatory step are removed. Passage through the Restriction point and a second key regulatory step at the G2/M boundary is driven by the activation of distinct CDK-Cyclin protein kinase complexes. Successive waves of Cdk-cyclin activities drive different events as cells transit the cycle (Figure 2). The collective activity of all Cdk-Cyclin kinases must decline to zero in G1 phase because the recruitment of DNA replication proteins to origins of replication is inhibited by Cdk-Cyclin activity. If any Cdk-Cyclin activity persists in this crucial DNA replication licensing window, replication will be defective as some origins will fail to fire and others fire repeatedly.

In order to inappropriately drive cells through the restriction point and proliferate in the wrong circumstances, cancer cells boost Cdk-Cyclin. However, this de-regulated Cdk-Cyclin activity in the critical replication licensing window, when all Cdk-Cyclin activity should be repressed, causes DNA damage in a phenomenon that is known as 'oncogene induced replicative stress' (OIRS). OIRS can raise the degree of damage in cancer cells towards levels that are lethal. This is why the DNA damaging approaches of chemotherapy and radiotherapy are so effective in the clinic: the extra damage from therapy pushes the cancer cells over their lethality threshold.

A cancer's need to repair more damage than neighbouring tissue places greater reliance upon DNA integrity cell cycle checkpoints that repress Cdk-Cyclin activities to delay cell cycle progression while the damage is repaired. Once the damage is repaired, checkpoint signalling abates and Cdk-Cyclin activity is restored, enabling passage through the cell cycle to re-start. As these pathways are so efficient at blocking division, cancers invariably inactivate the primary DNA damage checkpoint in which p53 blocks passage through the Restriction point. Such p53 deficient cancer cells become entirely reliant upon a second checkpoint at the G2/M boundary. There is therefore considerable therapeutic interest in weakening this second G2/M checkpoint as it will force cancer cells into a lethal division, while their normal neighbours can use their functional p53 checkpoint to pause the cycle when they are damaged.

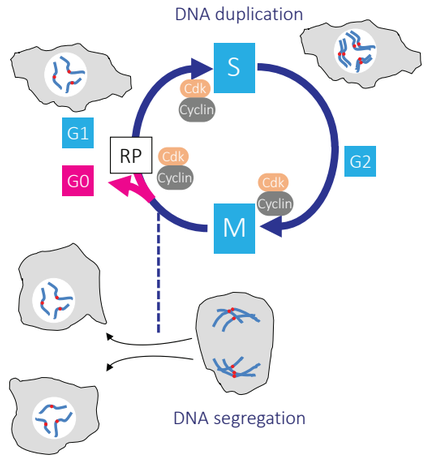


Figure 1.
The human cell cycle with Cdk1-Cyclin B control of the G2/M transition.
Passage through the Restriction point (RP) in G1 phase commits a cell to passage through the cell division cycle. DNA replication in S phase is separated from mitosis by a gap phase, G2. Transition through the major rate limiting commitment steps into the cycle, replication and division is driven by Cdk-Cyclin activities.

Figure 2

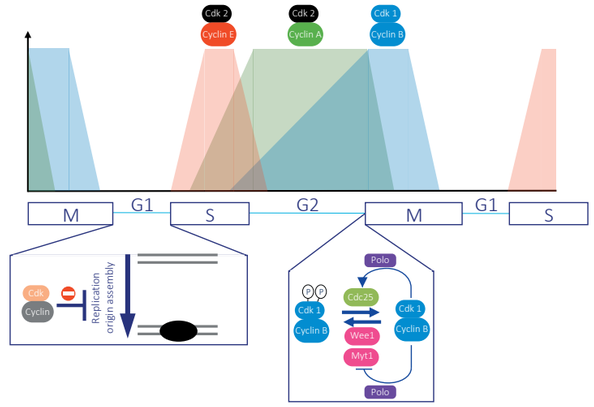


Figure 3

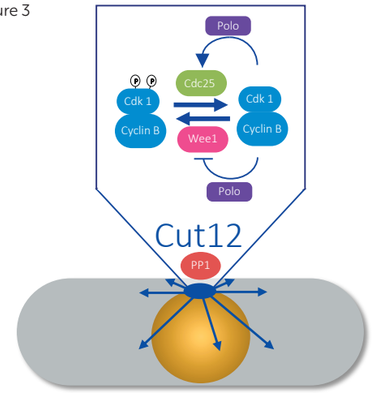


Figure 2.
Successive waves of Cdk-Cyclin activity drive cells through the cycle.
Once cells are driven through the restriction point by Cdk4-Cyclin D and Cdk6-Cyclin D activities, Cdk2-Cyclin E and Cdk2-Cyclin A promote DNA replication. Cdk2-Cyclin A activity persists until the beginning of mitosis, which it triggers alongside the principle mitotic regulator Cdk1-Cyclin B. The absence of any Cdk-Cyclin activity is critical to ensure that the DNA replication complexes can assemble ready for the subsequent division.

Figure 3.
The fission yeast spindle pole body triggers mitotic commitment.
Recruitment of PP1 to the SPB component Cut12 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 that drives cells through division.

The G2/M transition is driven by activation of the Cdk1-Cyclin B protein kinase. Wee1 kinases inhibit Cdk1-Cyclin B by phosphorylating the catalytic Cdk1 subunit. Removal of this phosphate by Cdc25 phosphatases then promotes mitotic entry. A trigger level of Cdk1-Cyclin B activation promotes a positive feedback loop that employs Polo kinase to boost Cdc25 and inhibit Wee1 activities to ensure that mitotic commitment is a rapid and irreversible switch from one state (interphase) into another (division) (Figure 2). The checkpoint pathways that block mitotic commitment when DNA is damaged, or replication is incomplete, do so by boosting the activity of Wee1 family kinases and repressing Cdc25. Thus, when thinking about how to target the G2/M checkpoint, there has been considerable interest in developing drugs that inhibit Wee1. Most of this interest has focused upon the founder member of this kinase family, Wee1. However, while we know a lot about Wee1, we know remarkably little about the biology of the closely related Myt1 kinase. We are therefore investigating how, when and why Myt1 is used to block the commitment to mitosis. Our findings will guide the application of small molecule inhibitors that are currently under development.

A second major interest of the team is understanding the role played by the centrosome in regulating the G2/M transition. Centrosomes nucleate all the microtubules in the cell to generate the interphase cytoskeleton and the bipolar mitotic spindle that physically segregates the chromosomes. However, we believe that centrosomes organise more than microtubules. The initial appearance of active Cdk1-Cyclin B on human centrosomes, before propagation throughout the cell, suggests that this organelle provides a specific microenvironment to trigger the G2/M transition. Our studies of the fission yeast centrosome equivalent, the spindle pole body (SPB), provide molecular insight into how and why this switch may operate (Figure 3). We have shown that release of Cdk1-Cyclin B, or Polo kinase activity at the SPB, will drive cells into division. In contrast, release of either kinase activity at any other location around the cell has no impact upon division timing. Our attempts to define the molecular basis for such a striking impact have been guided by lessons from the SPB scaffold Cut12. Simply blocking the recruitment of protein phosphatase 1 (PP1) to Cut12 enabled us to delete the *cdc25*$^{+}$ gene without compromising viability. This bypass of the requirement for an otherwise essential mitotic inducer arose from the impact of the Cut12/PP1 axis on Polo kinase activity. Polo activity was inappropriately elevated by the abolition of PP1 recruitment to Cut12. We are pursuing the hypothesis that Polo activity overcomes the need for Cdc25 because it boosts Polo's ability to inhibit Wee1 to such a degree that it completely silences Wee1. In this scenario, the absence of the kinase that places the phosphate onto Cdk1 removes the need for the phosphatase that normally reverses the missing phosphorylation event.

By understanding how the trigger to initiate mitosis is flipped and how cell status can restrain this step, we will gather new insights in how to develop new therapies and optimise the exploitation of existing approaches to treatment options.

Publications listed on page 66

CELL PLASTICITY & EPIGENETICS

Institute Fellow
Maximiliano M. Portal

Postdoctoral Fellow
Yelizaveta Shlyakhtina

Graduate Students
Bianca Blochl
Katherine L. Moran

As evidenced by numerous examples scattered across the various areas of biology, a cell phenotype is not solely determined by its genotype but is rather shaped by a multitude of dynamic non-genetic mechanisms. These include DNA and histones modifications, high-order chromatin architecture, gene expression dynamics and RNA-protein interactions, amongst others; all of them acting in concert to grant cells with the capability to dynamically adapt within an ever-changing environment. Despite the increasing recognition of the relevance of non-genetically encoded information for many biological processes, including cancer development, the underlying molecular details remain largely unknown.

Thus, our research focuses on the study of non-genetic information and its potential carriers, as we aim to unravel their genesis, dynamics, and inheritance as well as their role in responses to biological cues in models of epithelial-to-mesenchymal transition, oncogene-induced transformation and resistance to anticancer drugs. We believe that integrating these two crucial biological concepts – namely genetic and non-genetic information – and deciphering their interplay will drive our understanding of cancer evolution forward to ultimately translate our discoveries into more effective anticancer therapeutic paradigms.

Research Highlights

Accumulating evidence, including previous work from our group, has shown that even genetically identical cells (i.e. *in vitro* generated clonal cells), can be highly heterogeneous at the non-genetic level, displaying major differences in RNA and/or protein expression. Strikingly, this non-genetic heterogeneity could be linked to dramatically different cellular phenotypes, thus providing direct evidence for its biological relevance. Interestingly, it is widely accepted that extracellular cues from the environment can trigger dynamic intracellular changes at the non-genetic level which is often associated with phenotypic changes linked to adaptation to a given stimulus. Similarly, genomic mutations, which are often observed during cancer development, can be considered as intracellular effectors that result in changes in non-genetic compartments and thus modify phenotypic output (i.e. mutations might lead to the transformation of a normal cell into a malignant one). However, despite decades of research, the detailed molecular events that underlie malignant transformation are yet to be determined (Figure 1A).

Notably, and in spite of recent technical advances, the evolutionary path that tumours take is mainly inferred in a retrospective manner, mainly due to missing sampling from early stages of the disease and thus rendering the actual contribution of genetic and non-genetic components to cancer initiation unanswered. In order to fill this void in cancer evolution knowledge, our lab is currently developing a high-throughput platform with the aim to shed light on the molecular mechanisms of cancer onset promoted by the expression of hundreds of oncogenes and at single cell level (Figure 1B). Our experimental/computational pipeline enables us to combine information on the genetic level with non-genetic information encoded in the transcriptome, representing an important step to bridge the gap in knowledge on how these two frameworks interact and influence each other in the context of cancer evolution.

Importantly, our innovative approach can be applied to a variety of cell types allowing us to determine particular transforming oncogenes relevant for different cancer types in a quantitative manner. Indeed, by taking advantage of its quantitative nature we can rank transforming oncogenes – in a given cellular system of choice – based on their transforming potential, where the oncogenes displaying the most significant differences are further selected to be challenged in more complex models such as organoids or animal experimental settings.

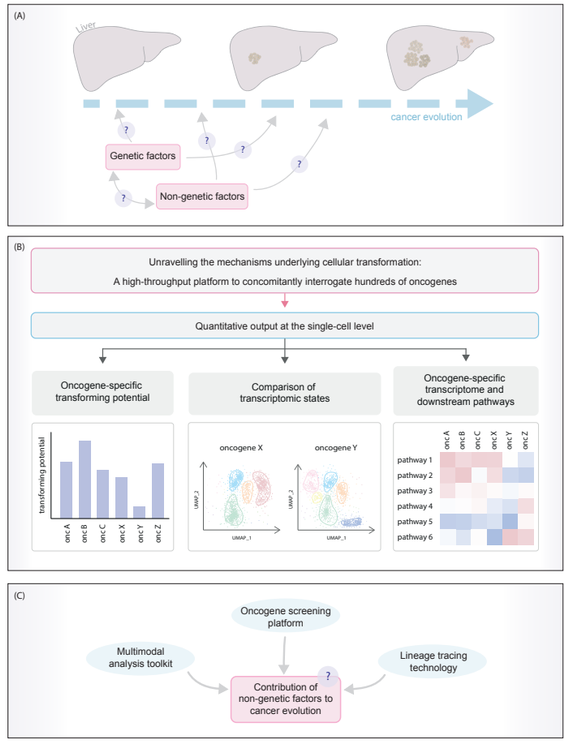


Figure 1.
A novel high-throughput platform to further our understanding of the role of non-genetic factors in cancer evolution.
(A) The precise molecular underpinnings of cancer evolution, shown here in the context of hepatocellular carcinoma, remain largely unknown. In particular, the contributions of non-genetic and genetic factors, and the interplay between these, are yet to be determined. (B) Our lab has developed a high-throughput platform to generate multiple outputs, as shown in the figure, which aims to further our understanding of the molecular mechanisms underlying cancer onset. (C) In addition to our oncogene screening platform, we will utilise a number of other novel approaches developed in our lab including a lineage tracing technique and multimodal analysis toolkit. Altogether, the knowledge generated by these approaches will help to uncover the role of non-genetic factors in the evolutionary trajectory of cancer.

Figure created by Katherine Moran.

Moreover, the non-genetic information gained from the transcriptome data for each cell can be linked to individual oncogenes, thus allowing us to establish a direct comparison between non-genetic characteristics (defined as transcriptomic states) and different oncogenes or oncogene families. Notably, changes at the level of transcriptomic states present before and after transformation can be identified and informatically mined to reveal intracellular pathways that are being activated or downregulated upon the cellular response to oncogene expression and/or the transforming event. Moreover, the resulting dataset can be further explored to gain a better understanding of the molecular mechanisms driving cellular transformation as well as potential starting points for the development of novel therapy strategies.

Having built and set up all the components of our platform, during the past year we have successfully completed our first round of pilot experiments comprising a reduced-complexity oncogene library of 26 RAS variants, a family of oncogenes commonly mutated in various cancer types. Notably, the transcriptome data of individual cells could be successfully assigned to each respective RAS variant and clustering of the data into the identified transcriptomic states was highly reproducible. Most strikingly, drastic differences in the transforming potential between the different RAS variants could be detected. These encouraging initial results demonstrate the capability of our platform to provide crucial insights into the dynamic mechanisms promoting cancer onset. Therefore, we are currently expanding our platform to investigate hundreds of oncogenes simultaneously and now include further experimental improvements which will allow us to explore the step-wise evolution following malignant transformation in a time-resolved manner. Importantly, our approach minimises the number of animal experiments dramatically by using an *in vitro* approach to select for successfully transformed cells and by pre-selecting the most relevant oncogenes before validating potential findings *in vivo*. Due to its relevance from the 3Rs perspective (reduce, replace, refine animal experiments) our efforts have been recognised at the joint CRUK MI-AstraZeneca 3Rs' poster event last year where we were awarded first prize.

Although many levels of non-genetic traits co-existing within a cell have been described, very little is known about the molecular rules underlying their establishment, dynamics and inheritance. One of the main aims of our lab is to shed light on these underlying mechanisms in order to better understand the role of non-genetic information in the establishment of biologically relevant phenotypes and their plasticity (the ability of the cellular phenotype to dynamically change/adapt). By using our in-lab developed lineage tracing approach, we are able to determine the transcriptomic states of thousands of individual cells and their direct progeny and thus can follow the dynamic evolution of metastable states along their lineages in a time resolved manner (Figure 1C). Strikingly, we uncovered for both transformed and pre-malignant (immortalised) clonal cell populations that cellular plasticity in terms of transcriptomic states is not random but instead is governed by molecular effectors that seem to restrict the dynamic transition between states. Our current efforts are directed towards deciphering the underlying molecular rules constraining non-genetic plasticity of cell populations and identifying key molecular players in this process. Along these lines, we are currently pursuing promising leads that suggest that non-coding RNAs and IDR-containing proteins (Intrinsically Disordered Proteins) segregate into phase-separated compartments and participate in the genesis and dynamic behaviour of transcriptomic states.

Finally, it is worth stressing that we have implemented a multimodal single cell analysis toolkit that enables us to explore non-genetic elements such as histone modifications or transcription factor binding concomitantly with the measurement of transcriptomic states (Figure 1C). Ultimately, we believe that by fusing together information about the various non-genetic determinants with classical genetics (i.e. oncogenes) we will create a comprehensive picture that will better reflect the complex intricacies of cancer evolution and its dynamics.

Publications listed on page 66

CELL SIGNALLING

Group Leader
Angeliki Malliri

Postdoctoral Fellows
Martin Baker[1]
William McDaid
Leah Wilson

Clinical Research Fellow
George Morrissey

Scientific Officer
Peter Magee

Graduate Students
Lucy Ginn
Ryan Guilbert
Hannah Reed
Joshua Searle
Kirsten Tinsley[1]

[1]Joined in 2021

The main focus of the Cell Signalling group is the identification of therapeutic targets in lung cancer, the most common cause of cancer related deaths worldwide. Lung cancer is divided into non-small cell lung cancer (NSCLC, approximately 85% of cases) and SCLC (~15% of cases). The most common histological subtype of NSCLC is adenocarcinoma, of which the most common driver mutation is KRAS. KRAS mutant lung adenocarcinoma (KRASm-LUAD) and SCLC treatments lag behind other lung cancer types, for which targeted therapies offer additional treatments prolonging patient survival.

No approved targeted therapies exist for SCLC, and until very recently, nor did they for KRASm-LUAD. However, recently direct inhibitors of KRASG12C (accounting for ~40% of KRASm-LUAD cases) have been approved, but resistance has been documented in relapsing patients. Other KRASm isoforms lack effective targeted therapies. Therefore, current drug development efforts focus not only on KRAS itself, but also on 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). Moreover, several studies using recombinant RAC and RAC GEF mice have shown that RAC is required for the formation and growth of tumours. In particular, RAC is required for the formation of KRAS-induced lung tumours in mice. Moreover, the RAC GEF TIAM1 is required for the formation and growth of HRAS-induced skin tumours (Malliri *et al.*, Nature 2002). Interestingly, TIAM1 and its homologue STEF/TIAM2 both contain a RAS-binding domain and are considered RAS effectors.

Role of TIAM-RAC1 signalling in lung cancer formation and progression

The role of TIAM1 and STEF/TIAM2 in NSCLC formation and progression is currently under investigation using *in vitro* and in vivo models and updates will be given in subsequent annual reports. SCLC is a highly aggressive malignancy broadly divided into neuroendocrine (NE, >80% of SCLC) and non-NE subtypes. Analysis of expression data from SCLC tumours, patient-derived models and established cell lines, showed that the expression of TIAM1 is associated with a neuroendocrine gene program (STEF/TIAM2 levels were very low in all SCLC tumours/cell lines). Moreover, we showed that TIAM1 depletion or RAC1 inhibition reduces viability and tumorigenicity of SCLC cells by increasing apoptosis associated with conversion of BCL2 from its pro-survival to pro-apoptotic function via BH3 domain exposure. We showed that this conversion is dependent upon cytoplasmic translocation of Nur77, an orphan nuclear receptor. Like in other cell types, TIAM1 is present in the nucleus of SCLC cells, where it interacts with Nur77 sequestering it in SCLC cell nuclei. TIAM1 depletion or RAC1 inhibition promoted Nur77 translocation to the cytoplasm. Importantly, mutant TIAM1 with reduced Nur77 binding failed to suppress apoptosis triggered by TIAM1 depletion. In conclusion, TIAM1-RAC1 signalling promotes SCLC cell survival via Nur77 nuclear sequestration (Payapilly *et al.*, Cell Reports 2021; see Research Highlights).

Role of RAC1 activators in migration and malignant progression

Although RAC seems always to promote tumour formation and growth, it may promote or antagonise malignant progression. There are cases where deletion of RAC GEFs leads to more invasive tumours and there are reports suggesting that reduced RAC activity levels correlate with more aggressive tumours (Porter et al., Small GTPases 2017). *In vitro* data have shown that activation of RAC may lead to opposing migratory phenotypes raising the possibility that targeting RAC in a clinical setting could exacerbate tumour progression. For these reasons it is important to identify the factors that influence whether RAC activation will promote or inhibit migration. One such factor that we have identified is the GEFs that activate RAC. RAC GEFs are multi-domain proteins with many binding partners. We showed that TIAM1 and another RAC GEF, P-REX1, have diametrically opposite effects on cell migration through RAC in certain epithelial cells and fibroblasts: TIAM1 promotes cell-cell adhesions to oppose cell migration while P-REX1 promotes migration. They perform these contrasting roles in cell migration by selecting RAC effectors (Marei *et al.*, Nat Commun 2016). Over-expression of specific GEFs, which occurs commonly in many cancers, can therefore drive different oncogenic signalling pathways. These data on TIAM1 are consistent with the fact that even though TIAM1 knockout mice were resistant to the formation of RAS-induced tumours, the few tumours which did form were more aggressive (Malliri *et al.*, Nature 2002). This highlights two distinct roles for TIAM1/RAC signalling: stimulating tumour formation but suppressing malignant progression. Early work on TIAM1's role in suppressing migration and invasion focused on its role in strengthening cell-cell junctions, associated with anti-migratory effects (Malliri *et al.*, J Biol Chem 2004). It was also shown that cell-cell adhesion disassembly and scattering of epithelial cells requires depletion of TIAM1 from cell-cell adhesions (Woodcock *et al.*, Mol Cell 2009; Vaughan *et al.* Cell Rep 2015).

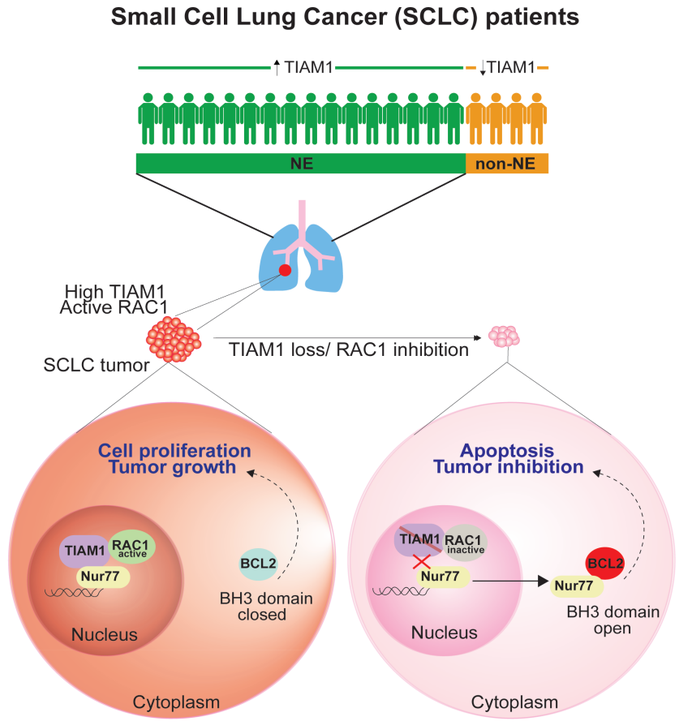


Figure 1.
Model depicting the role of TIAM1 in survival of SCLC cells: TIAM1 expression is upregulated in NE SCLC. TIAM1 sequesters Nur77 in the nucleus. Depletion of TIAM1 or inhibition of RAC1 activation by TIAM1 leads to cytoplasmic redistribution of Nur77. In the cytoplasm, Nur77 induces exposure of the BH3 domain of BCL2 promoting apoptosis.

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

More recently, we have uncovered a new role for TIAM1 in regulating the duplication of centrioles – structures at the core of centrosomes found at the poles of the mitotic spindle. Cells normally duplicate centrioles only once per cell cycle. Centriole overduplication is common in many cancers however, promoting aneuploidy but also increasing invasiveness of tumour cells. We found that TIAM1 localises to centrosomes and that its depletion leads to centriole overduplication, lagging chromosomes at anaphase and aneuploidy (Porter *et al.*, J Cell Sci. 2021), indicating another mechanism by which loss of TIAM1 could promote malignant progression.

Apart from these roles of RAC1 activators in antagonising cell migration, RAC1 activators are also associated with promotion of cell migration. Interestingly, activation of RAC by STEF/TIAM2 promotes migration (Rooney *et al.*, EMBO Rep. 2010), something we have seen in all cell types tested. STEF/TIAM2 localises at the nuclear envelope, co-localising with the key perinuclear proteins Nesprin-2G and Non-muscle myosin IIB, where it regulates perinuclear RAC1 activity. Interestingly, STEF depletion reduced apical perinuclear actin cables (thick actin bundles which run over the nucleus, constraining its height and guiding nuclear orientation), increased nuclear height and impaired nuclear re-orientation, which is required for optimal cell migration. Finally, STEF depletion reduced expression of TAZ-regulated genes, indicating an alteration in mechanosensing pathways as a consequence of disruption of the actin cap.

Publications listed on page 66

DRUG DISCOVERY

Director
Caroline Springer

Head of Chemistry
Dan Niculescu-Duvaz

Head of Biology
Graeme Thomson

Chemists
Mohammed Aljarah
Michael Brown
Efthymios Gavriil
Oleksandr Grytsai[2]
Chris Kershaw
Ralph Kirk[2]
Leo Leung
James Norris[1]
Ali Raoof
Deborah Smithen

Bioscientists
Diana Baxter[3]
Emma Crawley[1,2]
Joanna Grabarek
Anya Golder[1]
Nicola Hamilton[2]
Aisling Minard[1]
Julia Morris[1]
Alex Stowell[2]
Jessica Walker
David Waterhouse[1]

Computational Chemistry & Structural Biology
Chris Fitzpatrick
Jennifer Miles[4]

Graduate Students
Mihaela Ficu
Elizabeth Hogg[2,5]
Megan Mylrea
James Stratford

EA to Caroline Springer
Jayne Fowler

[1]Joined in 2021
[2]Left in 2021
[3]Joint with Cancer Biomarker Centre
[4]Joint with Richard Bayliss (University of Leeds)
[5]Joint with Systems Oncology

The Drug Discovery Unit (DDU) integrates medicinal, computational and synthetic chemistry with advanced biochemical and biophysical assays, in vitro cellular biology and in vivo capability to develop novel and first in class therapeutics for the treatment of unmet needs in cancer patients. The Unit has established facilities to enable state-of-the-art biological and clinical target assessment and validation, small molecule drug design and synthesis and the biological evaluation of the resultant compounds, and a track record of bringing projects from target validation to the clinic. The DDU builds upon fundamental biology discoveries made within the CRUK Manchester Institute to investigate novel drug discovery targets and to provide new chemical entities for the treatment of cancer.

Despite the impact of the coronavirus pandemic during the past year, research and laboratory work has continued apace during 2021, in a COVID-safe manner. We have strengthened our collaboration with colleagues in the CRUK Manchester Institute, and are working closely with Prof Iain Hagan, Prof Richard Marais and Dr Claus Jorgensen to advance drug discovery efforts against exciting targets involved in cancer cell cycle, in cancer cell stemness and resistance and in tumour stroma regulation, as well as progressing our suicide gene therapy for the treatment of solid tumours. We have actively sought to integrate our projects with Caroline Dive's biomarker discovery programme wherever possible, so that all nominated targets have selection and predictive biomarkers. We have fostered close collaborations with our clinical colleagues at the Christie NHS Foundation Trust on a number of late stage projects.

Research highlights of 2021 include new promising results with our lysyl oxidase (LOX) inhibitors in models of pulmonary fibrosis and metastasis, and the discovery and biological assessment of very potent, selective and bioavailable inhibitors of several collaborative cancer targets which are advancing towards or through lead optimisation.

In 2021 two of our late stage projects, the RAF+SRC inhibitor 3833 and the LOX inhibitors, were selected from nearly 90 submissions by the Alderley Park Oncology Development Programme, a national programme designed to develop and progress start-up oncology projects. The programme, funded by Innovate UK and CRUK and backed by a consortium of global pharmaceutical and healthcare companies, aims to identify exciting oncology innovations that will improve the diagnosis and treatment of cancer and to help accelerate their progression towards commercialisation. Both our RAF+SRC and LOX projects reached the final stage of the programme as part of a select group of only six projects from the whole of the UK.

RAS-driven cancers such as pancreatic ductal adenocarcinoma (PDAC), colorectal carcinoma (CRC) and non-small cell lung cancer (NSCLC) account for about one third of human cancers. Despite over 40 years of research, most RAS-driven cancers remain areas of unmet clinical need and patients with these cancers still generally receive conventional chemotherapy – often with limited efficacy and potentially high toxicity. Multiple signalling pathways are activated in KRAS-mutant cancers, and blocking only one target or one pathway is often ineffective or has paradoxical consequences. For example, drugs targeting mutant BRAF cause KRAS-mutant tumours to grow, because KRAS uses another RAF pathway protein, CRAF, instead of BRAF, to signal. In addition, RAS-mutant cells also require SRC, a controller protein for multiple parallel signalling pathways, to signal cells to proliferate.

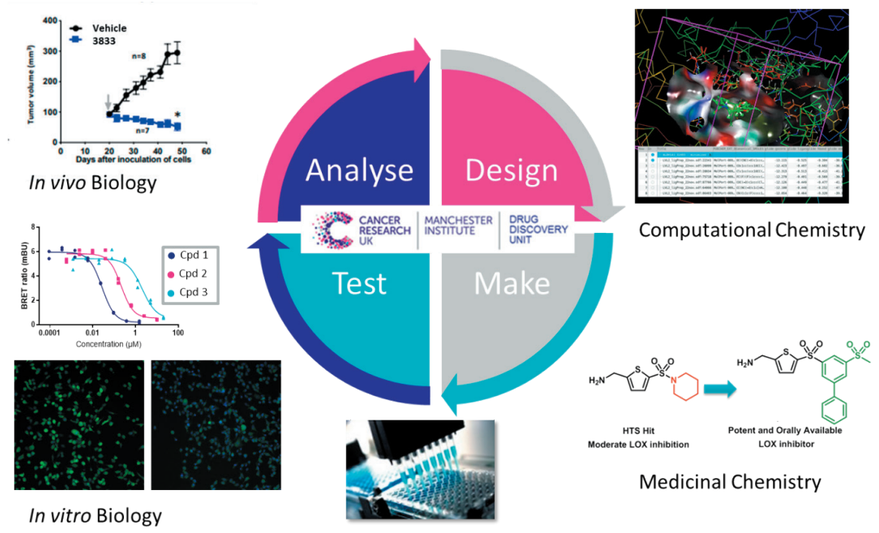


Figure 1.
The iterative cycle of drug discovery.
The Drug Discovery Unit has the capability, expertise and track record in taking a drug from target validation and hit identification through medicinal chemistry development and biological evaluation in vitro and in vivo to clinical candidate nomination.

The joint teams of Prof Springer and Prof Marais discovered 3833, an oral inhibitor for RAS-driven cancers with a unique mechanism of action as a bi-nodal RAF (BRAF and CRAF) + SRC inhibitor that targets these two key signalling pathways and so is effective in both BRAF-mutant and KRAS-mutant cancers (Figure 2A). A Phase I clinical trial (NCT02437227) at the Christie and Royal Marsden NHS Foundation Trusts, has shown that 3833 is well-tolerated by patients, with evidence of responses. 3833 significantly prolonged progression-free survival and partial response in a patient with a KRAS-driven spindle cell sarcoma who did not respond to the third-generation kinase inhibitor ponatinib and therefore had limited treatment options. This project was selected for the drug development programme at Alderley Park, with the aim of accelerating its progression to Phase II clinical trial and a subsequent route to approval.

Lysyl oxidases (LOX/LOXL1-4) crosslink collagens and elastin, stiffening the extracellular matrix (ECM). In collaboration with Prof Marais, we have discovered LOX and LOX family inhibitors with good pharmacological and pharmacokinetic properties. Our inhibitors have been designed to prevent the crosslinking of the ECM, which is a key step in the progression of pulmonary fibrosis, a disease that severely affects the quality of life of large numbers of patients. LOX also has important roles in the progression of tumour metastasis, which is the major cause of death in cancer patients. LOX inhibitors address large unmet needs as there is currently no effective therapeutic option for cancer metastasis and pulmonary fibrosis. We have already demonstrated therapeutic activity in primary tumour models of CRC, PDAC and breast cancer as well as anti-metastatic efficacy in preclinical models. New data in the bleomycin-induced model of pulmonary fibrosis have shown a marked reduction of positron-emmision

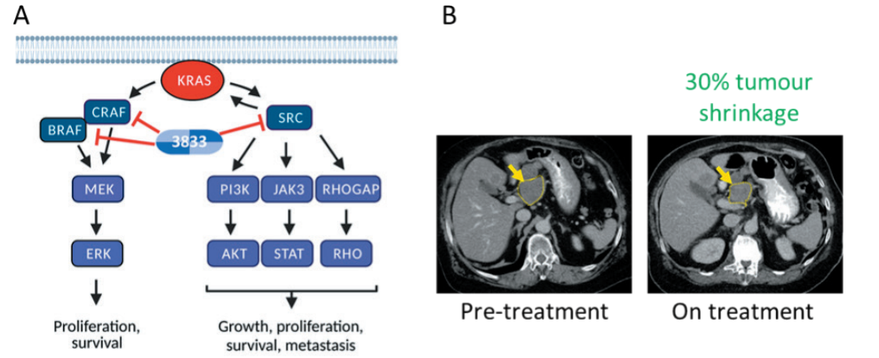


Figure 2.
(A) Clinical compound 3833, designed and synthesised in the Springer group, is a bi-nodal RAF+SRC inhibitor for RAS-driven cancers. (B) Tumour reduction after treatment with 3833 in RAS-mutant cancer patient resistant to standard of care drugs.

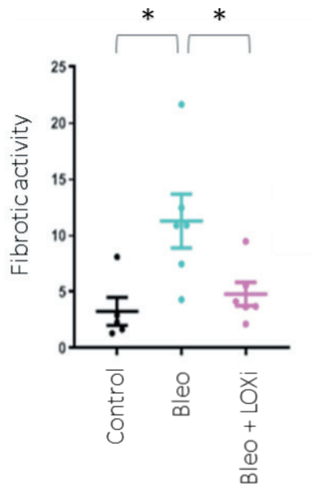


Figure 3.
In vivo FDG PET imaging of fibrotic activity in a bleomycin model of lung fibrosis shows reduction of fibrosis upon treatment with our LOX inhibitor.

tomography (PET) marker of fibrosis upon treatment with our LOX inhibitors (Figure 3). This is the second project selected by the Alderley Park Oncology Development Programme to be supported towards accelerating selection of a candidate drug to progress to toxicology and preclinical development studies before moving into early clinical trials in patients, as monotherapy and in combinations.

We have continued our collaboration with IDEAYA Bioscience and with Stephen Taylor at The University of Manchester on in vivo model assessment for our Poly(ADP-ribose) glycohydrolase (PARG) inhibitors, for the treatment of ovarian, gastric and breast cancer. IDEAYA Bioscience is planning to file an IND by Q4 2022.

The DDU has a healthy portfolio of advanced small molecule inhibitors and biologics projects that are in lead identification, lead optimisation or later stages (Figure 4). The exciting potential of many of our projects has raised great commercial interest and we are looking to accelerate the progress of these projects with commercial funding.

Publications listed on page 67

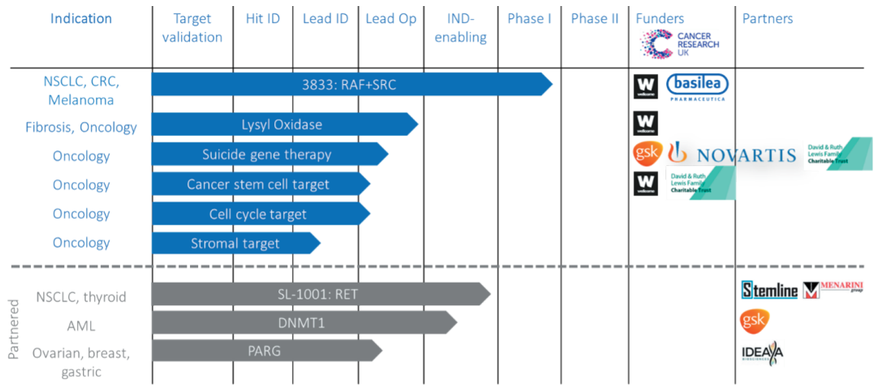
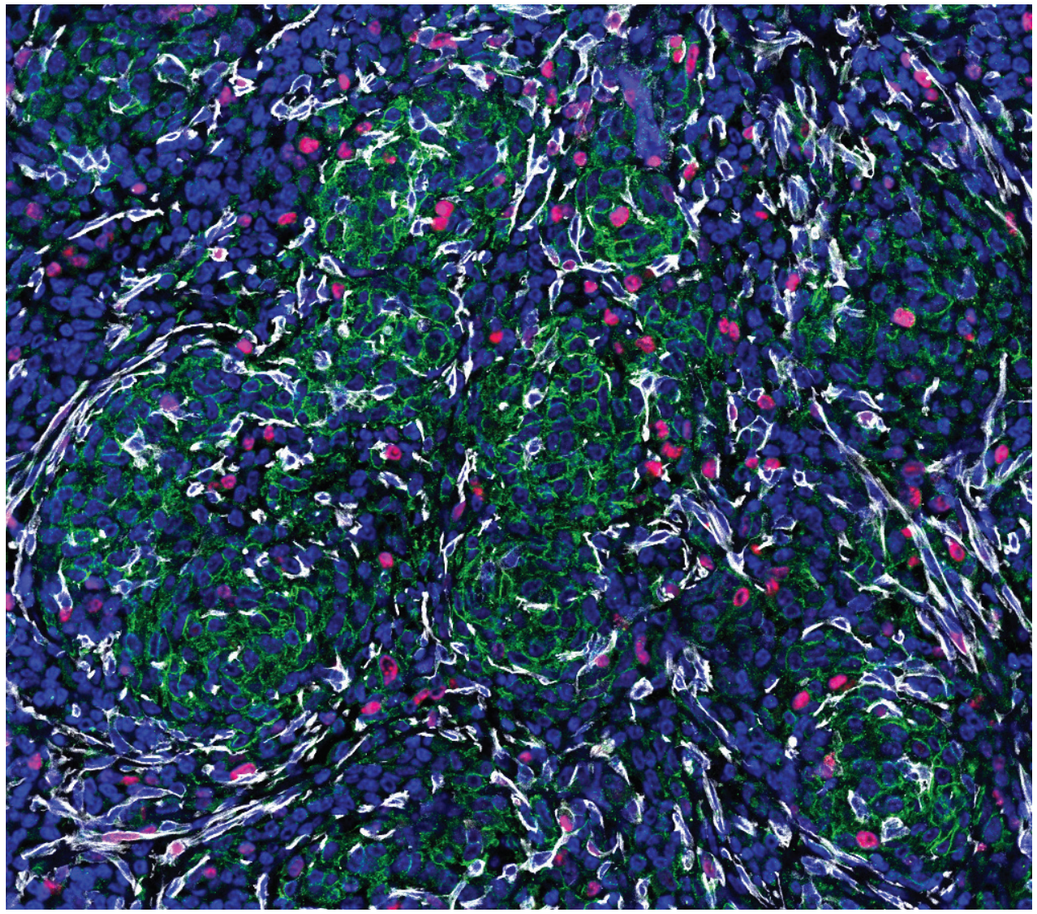


Figure 4.
Drug Discovery Unit portfolio of projects



Mouse pancreatic ductal adenocarcinoma stained with SMA (Red), LOX (Green), PDFR Beta (Grey) and DAPI (Blue).

Image supplied by Haoran Tang (former member of Molecular Oncology)

LEUKAEMIA BIOLOGY

Group Leader
Tim Somervaille

Postdoctoral Fellows
Luciano Nicosia[1]
Isabel Romero-Camarero[2]
Hannah Seberg
Bettina Wingelhofer

Bioinformatician
Fabio Amaral

Senior Scientific Officer
Gary Spencer

Graduate Students
Fabrizio Simeoni[3]
Francesco Camera
Bradley Revell
Naseer Basma
Oliver Sinclair[1]
Michael Jones[1]

MB Phd Student
Alexia Strickson[1]

[1]Joined in 2021
[2]Maternity leave June-December 2021
[3]Left in 2021

In keeping with the group's goal of understanding and identifying new disease mechanisms in myeloid lineage blood cancers, such as acute myeloid leukaemia (AML) and developing candidate therapeutic targets for development through to the clinic, we published two key studies in 2021.

In the first (Simeoni et al., 2021, *Cell Reports*) we report our new insights into how the Forkhead transcription factor FOXC1 confers a differentiation block in human AML: the work reveals a number of potential therapeutic targets for future pre-clinical analysis. In a second publication (Williams et al., 2021, *BMC Cancer*) we report a comparison of paired presentation and primary resistant AML samples, which reveals that AML stem cells are cycling cells with a transcriptional signature indicative of Forkhead factor FOXM1 activity.

Acute myeloid leukaemia (AML) is a blood cancer characterised by a block to normal myeloid lineage differentiation. This results in accumulation of myeloid blast cells in bone marrow (BM) and blood with consequent failure of normal haematopoiesis. While the range of balanced translocations, point mutations and indels associated with this malignancy is largely characterised, the mechanisms by which these genetic lesions confer a differentiation block is less well understood. This is emphasised by studies which show that many AML-associated mutations, including some chromosomal abnormalities, may be found in chemotherapy-treated patients in complete remission, in patients with myelodysplasia prior to evolution to AML or in ageing individuals with normal blood counts – the latter being the so-called clonal haematopoiesis of indeterminate potential. This is consistent with an emergent theme in AML that many disease-associated mutations promote expansion of haematopoietic stem and progenitor cells (HSPCs), which otherwise retain relatively normal differentiation potential, rather than immediately conferring a differentiation block. Few AML-associated genetic lesions are exclusively found in AML and even those such as FLT3 internal tandem duplications and NPM1 mutations yield prominent myeloproliferative phenotypes when modelled in mice.

The presence of certain combinations of genetic lesions within a long-lived progenitor cell is likely necessary for the generation of a differentiation block, but how mutations co-operate to arrest normal differentiation is often unclear. Improved understanding of the mechanisms involved is likely to facilitate development of therapeutic approaches to promote differentiation, an approach already exemplified by all-trans retinoic acid in the treatment of acute promyelocytic leukaemia. In addition to killing leukaemia cells with chemotherapy, induction of differentiation is a major goal of treatment.

We previously reported (Somerville et al., 2015, *Cancer Cell*) that the Forkhead family transcription factor gene *FOXC1*, which is a critical regulator of normal mesenchymal and mesodermal differentiation, is highly expressed in around 20% of cases of AML, but not expressed in normal haematopoietic lineages. High *FOXC1* expression in AML is almost invariably found in association with high *HOXA/B* gene expression, and ~30% of human *HOXA/B*-expressing AML cases (e.g. those with NPM1 mutations) exhibit high *FOXC1* expression. In vitro and in vivo experimental evidence confirms that FOXC1 confers a monocyte/macrophage lineage differentiation block and sustains clonogenic activity in both murine and primary human $FOXC1^{high}$ $HOXA^{high}$ AML cells. Co-expression of *FOXC1* with *Hoxa9* accelerates the onset of AML in murine modelling, with the resulting leukaemias exhibiting a higher level of differentiation block by comparison with those initiated by *Hoxa9* alone. Further, patients with high *FOXC1* expression exhibit inferior survival. More widely, high level *FOXC1* expression is also observed in a multitude of solid malignancies, including breast, colorectal, cervical, gastric and liver (Gilding & Somervaille, 2019, *Cancers (Basel)*) where functional experiments confirm that it promotes increased migration and metastasis and, as in AML, typically confers an inferior survival.

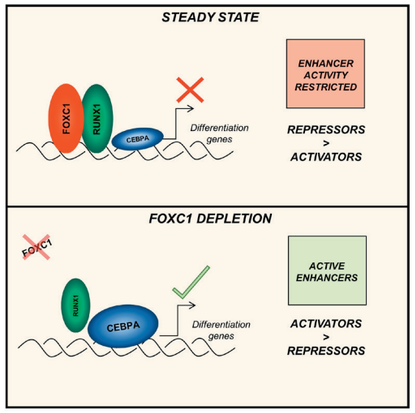


Figure 1.
Model illustrating activity of mis-expressed FOXC1 in sequestering repressive activity of RUNX1 and TLE3 at key enhancers controlling differentiation genes in leukaemia.

Despite the importance of FOXC1 in human AML, and more broadly in solid malignancies, the mechanisms by which FOXC1 confers adverse outcomes in human cancers remain largely unexplored. To address this in AML, we performed an integrated analysis of the protein:protein interactions and genome-wide binding sites of FOXC1 in human cell line and primary AML cells. In work led by Fabrizio Simeoni (Simeoni *et al.*, 2021, Cell Reports) we discovered that FOXC1 interacts with the key myeloid lineage transcription factor RUNX1 through its Forkhead DNA binding domain and that the two factors co-occupy a discrete set of primed and active enhancers distributed close to monocyte/macrophage differentiation genes. FOXC1 stabilises association of RUNX1, HDAC1 and the Groucho family repressor protein TLE3 at these sites to limit enhancer activity: *FOXC1* knockdown induced loss of repressor proteins, gain of CEBPA binding, enhancer acetylation and upregulation of nearby genes involved in monocyte differentiation, including *KLF2* (Figure 1). Furthermore, it triggered genome-wide redistribution of RUNX1, TLE3 and HDAC1 from enhancers to promoters, leading to repression of self-renewal genes including *MYC* and *MYB*. Our studies highlight RUNX1 and CEBPA transcription factor swapping at enhancers and promoters as a feature of leukaemia cell differentiation, and reveal that FOXC1 prevents this by stabilising enhancer binding of a RUNX1/HDAC1/TLE3 transcription repressor complex, to oncogenic effect. A number of the protein: protein interactions we identified in this study as required for retarding leukaemia cell differentiation are candidate therapeutic targets and will be evaluated in future pre-clinical studies. This work further emphasises the importance and multi-faceted roles of RUNX1 as a critical protein in human myeloid lineage blood cancers, over and above its well described point mutations and chromosomal translocations.

While development of drugs which relieve the differentiation block in AML is an important therapeutic approach, it remains the case that standard-of-care AML treatments are predominantly DNA damaging chemotherapies, which induce leukaemia cell death. Disease relapse remains sadly all too common following treatment of AML and is generally due to chemoresistance of leukaemia cells with disease repopulating potential. To date, attempts to define the characteristics of in vivo resistant blasts have focused on comparisons between leukaemic cells at presentation and relapse. However, further treatment responses are often seen following relapse, suggesting that most blasts remain chemosensitive. In work led by Mark Williams (Williams et al., 2021, *BMC Cancer*), we sought to characterise in vivo chemoresistant AML blast cells by studying their transcriptional and genetic features before and shortly after induction chemotherapy using paired samples from patients with primary refractory AML.

Briefly, we found that chemorefractory blasts from leukaemias with varied genetic backgrounds expressed a common transcriptional program. In contrast to the notion that LSC quiescence confers resistance to chemotherapy, we found that refractory blasts are both actively proliferating (Figure 2) and enriched with leukaemia stem cell maintenance genes. Using primary patient material from a relevant clinical context we also provided support for the role of the Forkhead transcription factor FOXM1 in chemotherapy resistance, proliferation and stem cell function in AML.

Publications listed on page 67

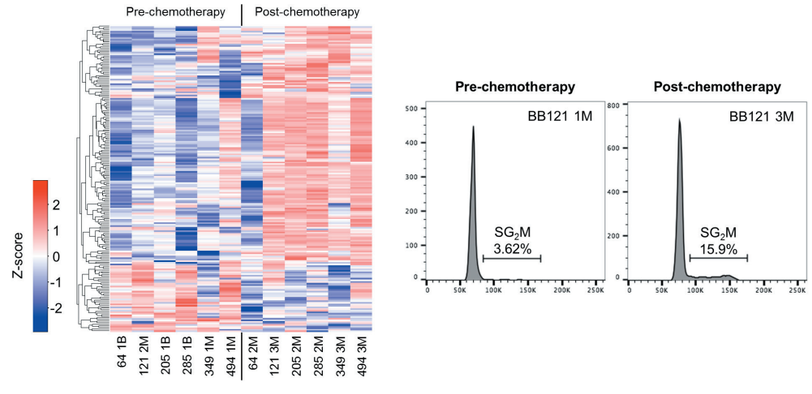


Figure 2.
Transcriptional and cell cycle features of paired patient samples of primary AML from presentation and at the end of the first cycle of chemotherapy. Left panel: heat map illustrates differentially expressed genes. Right panel: exemplar cell cycle profiles.

MOLECULAR ONCOLOGY

Group Leader
Richard Marais

Associate Scientists
Nathalie Dhomen
Valeria Pavet

Senior Clinical Scientist
Martin Cook

Postdoctoral Fellows
Daphné Brisard
Pauline Hascoët[1]
Sjors Kas
Patricia Pacios-Centeno

Clinical Fellows
Pablo Garcia Martinez
Zena Salih[1]
Sara Valpione

Scientific Officers
Christopher Chester
Darryl Coles
Megan Grant
Jessica Walker

Translational Research Project Manager
Jackie Mitchell[1]

Graduate Student
Luke Chisholm

[1]Left in 2021

Our multidisciplinary group studies cancer biology, from the basic causes of cancer to how patients respond to treatment. Over the past year, we described how patient blood can be used to monitor which patients will respond to immunotherapy, studies that have improved our understanding of the human immune system and provided new methods to identify which patients are likely to respond to therapy and which patients are unlikely to respond. We also identified new prognostic markers and new therapeutic targets in prostate cancer. Together, these studies provide new possible ways of monitoring cancer so that their treatment can be tailored for individual patients.

Immunotherapy has been transforming the melanoma treatment paradigm over the past decade, affording sustained survival benefit in some patients. However, we do not yet fully understand the mechanisms that determine response to immunotherapy and most patients with malignant melanoma still die of their disease. Our group has previously shown that in patients who respond to immunotherapy a small subset of circulating T cells that we have called immune effector T cells (T_{IE}) expand within 3 weeks of starting treatment. In the same study, we showed that changes in clonality (expansion of one or a small number of predominant T cell clones) or diversity (expansion of many T cell clones) of circulating T cells in response to immunotherapy also provided an indication of whether a patient would go on to respond to immunotherapy.

In the past year, we continued to study the immune system's response to cancer and immunotherapy. In one study, we sought to understand whether the extent of the T_{IE} expansion we previously described in patients that responded to immunotherapy correlated in some way with observed tumour shrinkage. Comparing tumour measurements at the start of therapy and 12 weeks after therapy, we confirmed an inverse relationship between tumour size and T_{IE} cell expansion, further validating T_{IE} cell expansion as a biomarker for immunotherapy response. We examined if the expansion of T_{IE} cells and the changes in T cell clonality and diversity were influenced by clinical variables such as sex, disease stage, BRAF mutation status, LDH levels or age. We found that whilst T cell expansion was not influenced by the first four clinical parameters, changes in the clonality and diversity of T cells in response to immunotherapy were influenced by age. Specifically, we observed that in patients younger than 70 years of age, T cells exhibited a trend towards increased diversity in response to immunotherapy, whereas in patients of 70 years and over, we observed a trend towards increased clonality. The increased diversity in younger patients but increased clonality in older patients is consistent with the reduction in thymus function as we age (thymus involution) and is important because it suggests that biological variation driven by age should be taken into account when developing immunotherapy approaches, new treatments or diagnostic tools.

In a separate study, we focused on the different types of T cells that reside within tumours, examining their clonality and diversity. We discovered that the diversity of T cell receptors in the tumour resident T cells is prognostic for survival not only in melanoma, but also in breast cancer, certain lung cancers, renal and testicular cancers, and thymoma, irrespective of whether patients received immunotherapy or not. This provides interesting new insight into the interactions between the immune system and tumour cells. Additionally, we discovered that the clonality of tumour resident T cells is predictive for response and survival after anti-PD1 based immunotherapy (Figure 1), providing a potential biomarker that can identify patients that are most likely to respond to these immunotherapies. There is a great need to identify markers of response to these therapies so they can be administered to the patients that will derive benefit from them, whilst sparing other patients the potential side effects of these therapies and affording them more time to explore other treatment options.

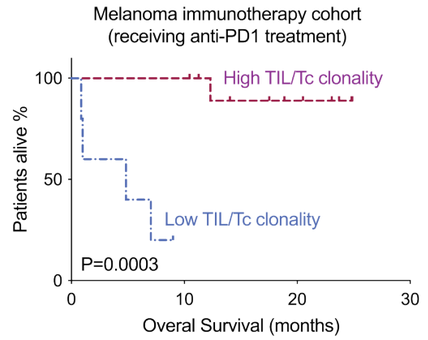


Figure 1.
Tumour infiltrating lymphocyte T cell (TIL/Tc) clonality is predictive of anti-PD1 response in melanoma.
Survival curves for a cohort of patients with metastatic melanoma treated with anti-PD1 immunotherapy drugs in Manchester or Italy, grouped according to the clonality of the tumour infiltrating T cells in their pre-treatment melanoma biopsy. Patients with high TIL/Tc clonality (purple) had a significantly longer survival (median not reached) compared to patients with low (blue, median survival=4.8 months) TIL/Tc clonality (n=16, cut-off calculated with *optimalCutoff* algorithm=0.06, log-rank P=0.0003). Image adapted from Valpione et al, *Nat Commun*, 2021;12(1):4098, https://creativecommons.org/licenses/by/4.0/.

Alongside these translational studies focused on identifying markers of response to therapy, we also continue our studies of the basic biology of cancer, seeking out vulnerabilities that may be exploited therapeutically. One such study of prostate cancer (PCa), the fifth leading cause of cancer deaths worldwide, revealed a 7-microRNA (miR) signature that identifies PCa primary tumours that are more prone to metastasise, providing a mechanism to help clinicians to stratify patients more accurately for follow up and therapy (Figure 2a, b). MiRs are small RNA molecules that regulate many biological processes, and analysis of our signature has provided clues to novel therapeutic options because we discovered that one of the miRs in our signature, miR-378a, impairs the metabolism of glucose by PCa cells. This is because miR-378a blocks the expression of a protein called GLUT1 (glucose transporter 1) in PCa cells, reducing their ability to use glucose to support their proliferation (division) (Figure 2c, d). These studies reveal that metabolism could be an exciting therapeutic target in aggressive prostate cancer.

In addition to understanding and developing therapeutic options for advanced cancer, we continue to seek ways in which cancer can be prevented or detected at earlier stages. Especially in the context of rare cancer types, such as melanoma of the eye, identifying their underlying drivers can be a key factor in preventing their development. Our studies of conjunctival melanoma, set in the context of a series of recent studies focused on the genetic changes that drive ocular melanomas, suggest that whilst different genetic events drive melanoma at specific sites (the skin, the eye, mucosal membranes), ultraviolet radiation (UVR) imposes additional events over these specific processes to accelerate melanoma development. It is generally accepted that this is particularly important for the skin, but our data show it is very important to remember that is can also happen in the eyes (particularly the conjunctiva and iris) and mucosal membranes that are sun-exposed, such as the lips. Our findings emphasise the importance of promoting UVR protection with sunblock for the skin, UVR protective lip balm for the lips and sunglasses for the eyes. The data also suggest that therapies currently approved for skin melanoma should also be considered for melanomas at other sites, guided by their underlying genetics.

Publications listed on page 68

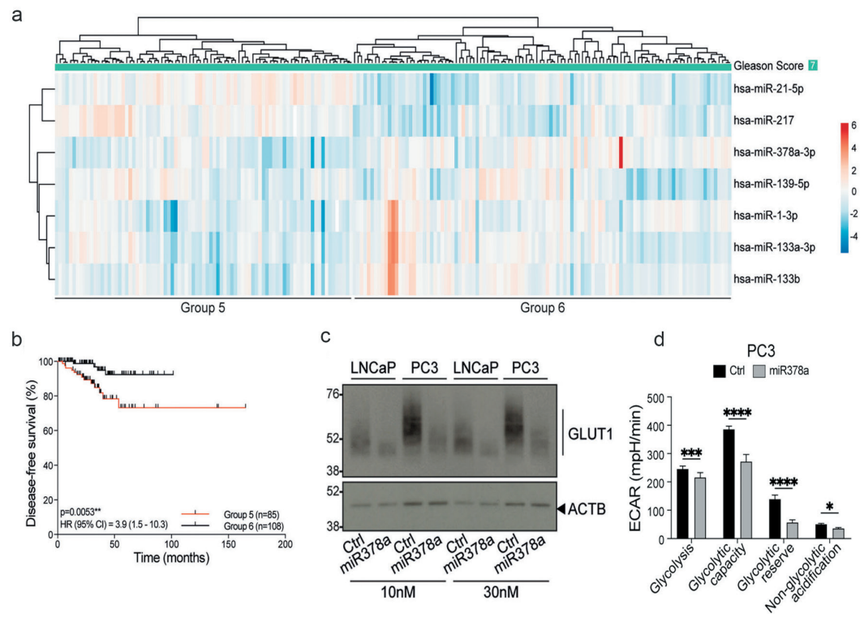


Figure 2.
Identification of a 7-miR prognostic signature and metabolism therapeutic target in prostate cancer
(a) Unsupervised hierarchical clustering TCGA N0 PCa patients presenting Gleason 7 score using expression (log2 RPM) of 7-miR identified from miR RNAseq data. Columns: individual patients, rows: individual miRs. Centering and unit variance scaling are applied to rows, and rows and columns are clustered using correlation distance and average linkage. The dendrogram shows Gleason score for each patient (G7: green). **(b)** Kaplan–Meier plot of disease-free survival in Group 5 and Group 6 patients (from a). The number of patients (n) in each group is indicated. **p = 0.0053; HR 95% CI = 3.9 (1.5–10.3); Mantel–Cox test. Median survival Group 5: not reached; median survival Group 6: not reached. **(c)** Western blot for GLUT1 and ACTB as loading control in PC3 and LNCaP PCa cells after transfection with two concentrations (10 nM and 30 nM) of the miR-378a-mimic or a non-targeting control (Ctrl). **(d)** Quantification of the glyco-stress test parameters in PC3 cells.

Images adapted from Cannistraci et al, Oncogene, online ahead of print (https://creativecommons.org/licenses/by/4.0/).

PROSTATE ONCOBIOLOGY

Group Leader
Esther Baena[1]

Postdoctoral Fellow
Teresita Flores Téllez[1,2]

Scientific Officer
Pengbo Wang[1,2]

Graduate Students
Ivana Steiner[1]
Alexander Du Feu[1]
Alexandra Hendry[1,2]

Clinical Fellow
Amin Ali[1]

[1]Left in 2021
[2]Joined another CRUK MI group

In localised prostate cancer, the spectrum of disease is broad from low to high-risk disease, clinically reflected by a wide range of treatments, including active surveillance and intensive curative treatments achievable through surgery or radiotherapy plus/minus androgen deprivation therapy. In contrast to localised prostate cancer, men diagnosed with metastasis will inevitably develop resistance to therapy (mCPRC) and succumb to their disease, despite multiple and improved palliative treatment options for these patients. Importantly, it is yet not possible to predict which patient will develop aggressive tumours versus more indolent cases. Therefore, the work of our group aims at understanding the onset of aggressive prostate tumours at their early, curable stages by identifying and characterising cells-of-PCa-origin to develop better therapies.

Research highlights

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

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

LY6D is a gene with yet no established role in prostate development or cancer. It is a member of the Ly6/uPAR family, characterised by their roles in cell proliferation, cell-cell interaction, immune cell maturation, and cytokine production, which are all essential components of tumour initiation and progression. We are currently defining the functional role of LY6D for tumorigenesis and tumour maintenance, which so far remains unknown. Our in vitro and in vivo data showed that LY6D+ cells in the luminal lineage represent luminal progenitors inherently resistant to androgen deprivation and enriched organoid-forming multipotent luminal progenitors. Taken together, these findings suggest that LY6D expression correlates with PCa initiation and progression to castration-resistant growth from the luminal lineage. Importantly, in support of this hypothesis, analysis of human PCa cohorts revealed that higher LY6D expression levels are associated with more aggressive disease and worse outcomes, suggesting that LY6D may serve as a prognostic biomarker for advanced PCa. Furthermore, our collaboration with Prof Georges Lacaud contributed to the identification of a distinct subset of castration-resistant luminal cells from early stages of prostate embryonic development. We showed that 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 complementary study, we are characterising localised high-risk prostate cancer patients based on their spatial distribution. We have built up a retrospective collection of specimens from patients with multisite lesions, for which matching clinical parameters are available. For the prospective collection of samples, we have completed the sample collection using our novel sampling method, established in Manchester in the previous year. 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 team is 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 novel curative approaches for prostate cancer patients.

Publications listed on page 69

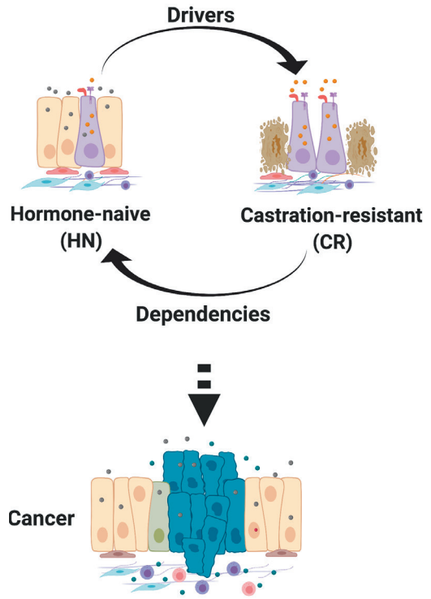


Figure 1.
The inherent resistance to androgen-deprivation therapy (aka. castration-resistance) in the prostate epithelium favours the onset of aggressive prostate cancer.

SKIN CANCER AND AGEING

Institute Fellow
Amaya Virós

Postdoctoral Fellows
Timothy Budden
Cian Vyas

Clinical Fellow
Sarah Craig

Graduate Students
Shilpa Gurung
Pedro Durao[1]

[1]Joined in 2021

The Skin Cancer and Ageing lab studies why skin cancer is more common and more deadly in elderly patients. The incidence and mortality rates continue to rise, as does the proportion of the population who is over 50 years old. Most skin cancer deaths and skin cancer complications affect the elderly, and mortality due to skin cancer is specifically increasing in the elderly. Older patients are more likely to suffer from multiple melanocytic and non-melanocytic skin cancers. We study the survival discrepancy between elderly and young patients, as age is a strong independent adverse prognostic factor.

We have first focused our research this year on understanding the changes in melanomas that drive more aggressive disease. We found that age and sex determine melanoma mutation rate, and we used mathematical approaches to model how age-mutations and sun-mutations accumulate by sex and age in melanoma. Similar to aged healthy, homeostatic tissues, we found that melanoma genomes decline in cell division with age. Critically, UV damage increases cell division, but the effect of UV on cell division was more pronounced in men. Thus, we discovered that males present more mutations independently of UV damage (Lotz et al, British Journal of Dermatology 2020). Additionally, we have focused on the genetic changes of the melanoma cell that make a tumour more aggressive. We found that a rapid rate of growth of primary melanoma, as observed by the patient, predicts poor survival; and described the molecular, epidemiological and clinical data of 200 cases of fast and slow growth melanoma cases with long term outcome. We found distinct mutations and environmental factors in the primary tumours and patients that identified the individuals at high risk of death (Gaudy et al, Journal of the American Academy of Dermatology 2021).

We have also focused on the features of the tumour microenvironment that affect melanoma outcome in the elderly population. We found collagen degradation in the aged dermis, following chronic UV exposure and damage to the connective tissue, inhibits melanoma invasion. We discovered collagen is necessary for primary melanoma cells to invade into the dermis. Thus, we found that melanomas that arise over sun damaged skin tend to have a better outcome. There is a clear exception to this rule in aged patients who upregulate new collagen synthesis and have a particularly bad outcome. We linked the ability to synthesise new collagen in some aged tumours to a greater presence of melanoma associated fibroblasts. Patients with new collagen thus die quickly of melanoma, and we showed this in multiple clinical cohorts measuring melanoma cell invasion, collagen synthesis and melanoma specific survival. Critically, we found other primary cancers expressing high collagen have a poor outcome as well in a pan cancer analysis using TCGA data (Budden et al, Nature Communications 2021).

We have also worked on the higher incidence of skin cancer in aged men compared to aged women, and undertaken first experiments to identify new strategies of adjuvant therapy. Specifically, we published that men and male mice are more susceptible to primary aggressive and metastatic cutaneous squamous cell carcinoma (SCC). We discovered that when female and male animals are challenged with the same dose of carcinogen, they have different transcriptomic responses. Critically, although both females and males repair the DNA damage from the carcinogen at equal rates, women and female mice activate distinct transcriptomic pathways linked to improved cancer immunity. We were able to validate these findings in mice and humans and showed that immunosuppressed women and mice have a similar course of disease to immunocompetent men. Our work had significant impact in the media and publication was selected for the cover of the *Clinical Cancer Research* June 2021 issue (Budden et al, Clinical Cancer Research 2021).

We are currently developing data and preparing to submit exciting studies on how the changes to the epidermis induced by sun damage affect melanoma onset and progression, how they change the tanning response in homeostasis, and how oral chemoprevention of non-melanoma skin cancers adversely impact the progression of melanoma. Lastly, we are preparing evidence on how aged and young subcutaneous fat contribute differently to melanoma metastatic potential.

Our postdoc Tim Budden was awarded the prestigious Edith Paterson 2021 Award from our Institute, which recognised his multiple contributions during this year. We have also been successful obtaining further research funding, with a new grant from the Melanoma Research Alliance and Rosetrees Trust, as well as further support from Wellcome to fund additional studies into the role of collagen, the dermis and patient outcome.

Publications listed on page 69

Figure 1.
Histological image showing the cutaneous papilloma from a female mouse following exposure to the carcinogen DMBA/TPA. The striking image was featured on the front cover of the June 2021 edition of Clinical Cancer Research.

Image supplied by Amaya Viros and Tim Budden, Skin Cancer and Ageing.

The image also appears on the front cover of this annual report.

STEM CELL BIOLOGY

Group Leader
Georges Lacaud

Postdoctoral Fellows
Neo Wen Hao
Divya Malik[1]
Muhammad Maqbool[2]

Scientific Officers
Rahima Patel
Michael Lie-a-Ling

Clinical Fellow
Mathew Sheridan[3]

Graduate Students
Liam Clayfield[3]
Renaud Mevel[1]
Muhammad Fadlullah Wilmot
Ewan Selkirk
Steven Mayers[4]

[1]Left in 2021
[2]Funded by Blood Cancer UK
[3]Joined in 2021
[4]Funded on an MRC Doctoral Training Partnership; joint with the Division of Developmental Biology and Medicine, University of Manchester

Genes encoding the AML1/RUNX1 transcription factor and its cofactor CBFβ are frequently rearranged or mutated in human leukaemia, such as acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL). Consistent with its implication in leukaemia, RUNX1 has also been shown to be critical for haematopoietic development. Similarly, the transcriptional co-activator MOZ is involved in independent myeloid chromosomal translocations fusing *MOZ* to the partner genes *CBP*, *P300* or *TIF2* in human leukaemia. Our group investigates RUNX1 and MOZ's function in haematopoietic development and maintenance to better understand how alterations of these functions might lead to leukaemogenesis. Besides these transcription factors and transcriptional activators, long noncoding RNAs (lncRNAs) have also emerged as important regulators of gene expression. In this context, we more recently started the investigation of lncRNAs essential for leukaemia.

Investigation of long noncoding RNAs in acute myeloid leukaemia

Transcription factors and epigenetic factors (writers, erasers or readers) regulate self-renewal and differentiation during normal haematopoiesis. Alterations of transcription factors and epigenetic factors are critical molecular events leading to leukaemia and other malignancies. Long noncoding RNAs (lncRNAs) are transcripts of over 200 nucleotides that display no coding potential. They represent a significant fraction of the human genome (Figure 1A). For many years, they were considered junk DNA and the result of spurious transcription. However, recent studies have revealed that lncRNAs can play essential roles in many cellular processes. Through mechanisms such as acting as tethers for the epigenetic machinery, lncRNAs have been shown to participate in gene regulation (Figure 1B). LncRNAs could therefore be essential factors in acute myeloid leukaemia (AML) development and represent a potential novel therapeutic avenue. However, our understanding of lncRNAs, and their functions in AML, is currently limited.

To identify lncRNAs important in the proliferation of leukaemic cells, we employed a CRISPR interference (CRISPRi) screening approach to induce sequence-specific repression of lncRNA expression. We selected the THP-1 cell line, a human AML cell line with an MLL-AF9 translocation, to express dCas9-KRAB (dead Cas9 fused to Krüppel associated box (KRAB) domain), which represses transcription at targeted loci. We screened 3882 lncRNAs, identifying 19 as significantly influencing cell proliferation. Within these 19 hits, we identified MIR17HG, a lncRNA that also encodes for the miR17-92a-1 cluster. The microRNAs within this cluster are essential factors in MLL-rearranged leukaemia, validating our screen's performance in identifying lncRNAs important in leukaemic maintenance.

We have worked on characterising the phenotype, and molecular mechanism of SGOL1-AS1, which we identified from this screen. As the subcellular location of a lncRNA can influence the mechanisms by which it can act, we looked to check the localisation of this lncRNA. We have shown this noncoding RNA to be enriched within the nucleus of AML cells, localising to discrete foci within the nucleus (Figure 2A).

As well as knocking down the transcript with CRISPRi, we have also used antisense oligonucleotides (ASOs) as an orthogonal

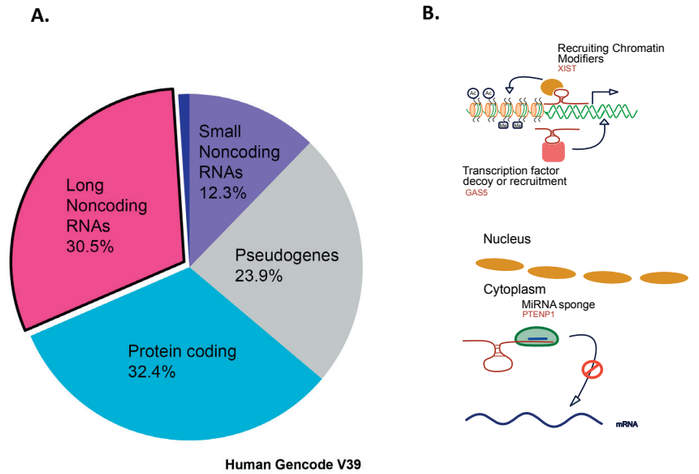


Figure 1.
LncRNAs represent an important fraction of the human genome.
A. Representation of LncRNA compared to other gene biotypes. B. General mechanisms of lncRNAs in control of Gene Expression.

Figure 2.
SGOL1-AS1 is upregulated in AMLs.
A. SGOL1-AS1 localises to discrete spots in the nuclei. B. Knockdown of SGOL1-AS1 reduces proliferation in liquid culture. C. Higher expression of SGOL1-AS1 is associated with poorer overall survival in AMLs. ASO-1 and ASO-2: anti-sense oligonucleotide against SGOL1-AS1. * p<0.05 and **** P<0.001.

knockdown technique. This approach has allowed us to target the transcript without affecting the local chromatin. Utilising these ASOs, we have shown that both the proliferation in liquid culture and the colony-forming potential of AML cells are dependent on the expression of SGOL1-AS1 (Figure 2B). Though knockdown of SGOL1-AS1 reduced the proliferation of cells, it did not affect the cells' differentiation or cell cycle state. Instead, reduced proliferation occurred via the induction of apoptosis. Utilising RNAseq, we identified genes that became differentially expressed upon knockdown of SGOL1-AS1. Notably, within these differentially expressed genes were those relating to innate immune programs. We showed downregulation of cytokine signalling pathways and several other programs pertaining to innate immune system cells.

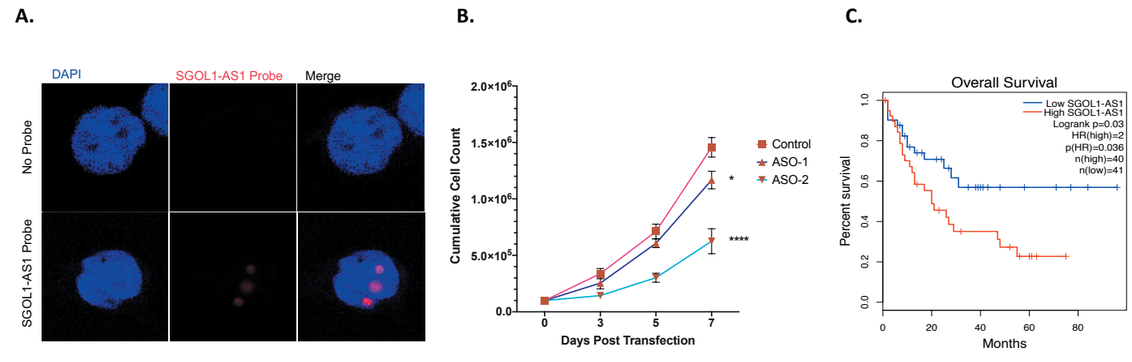
Having identified the possible downstream targets of SGOL1-AS1, we looked to identify any potential mechanism by which it may work. Using in vitro transcribed biotinylated RNA, we identified several proteins associated with SGOL1-AS1. A large number of these associated proteins are important regulators of heterochromatin, including components of both telomeric and centromeric heterochromatin and members of the PRC1 complex. Altogether, these data suggest that SGOL1-AS1 regulates the expression of innate immunity genes by regulating heterochromatin formation. We are currently further investigating this mechanism.

As well as our experimental characterisation of this lncRNA, we have also investigated the expression of this lncRNA in patients and its clinical importance through mining publicly available patient data (TCGA, Blueprint, GTEx). We observed that expression of SGOL1-AS1 is increased in AMLs compared to non-malignant haematopoietic progenitor populations, such as Multipotent progenitors, Common Lymphoid progenitors and Common Myeloid progenitors. Furthermore, expression of SGOL1-AS1 is increased in AML patients compared to healthy bone marrow. We also identified a correlation between higher expression in patients and poorer overall survival (Figure 2C). Together these data show that SGOL1-AS1 is upregulated in AML and suggests this may impact AML cell characteristics.

To gain further insights into these changes, we looked at genes that showed a strong correlation in expression with SGOL1-AS1 from the patient data. Similarly to our RNAseq data from cell lines, we observed an enrichment for genes relating to innate immunity. Therefore, we conclude that upregulation of SGOL1-AS1 in AML modulates the expression of gene programs relating to innate immunity.

Antisense oligonucleotide approaches for reducing gene expression have recently entered clinics, including clinical trials in haematological malignancies. This advance in antisense therapy could allow the targeting of a lncRNA essential for AML in patients. Thus, identification and functional characterisation of LncRNAs critical for AMLs could represent potential new therapeutic avenues.

Publications listed on page 70



SYSTEMS ONCOLOGY

Group Leader
Claus Jørgensen

Scientific Officers
Joanna Kelly
Xiaohong Zhang

Postdoctoral Fellows
Celia Cintas
Adrian Gomez
Nasir Haider
Stephen Kershaw[1]
Alexander Kononov[1]
Brian Lee[1]
Carol McMenemy[2]

Graduate Students
Christopher Below[1]
Catherine Felton
Felix Heider
Elizabeth Hogg[1]
Colin Hutton[1]
Louis Russell[2]

Clinical Fellows
Konstatinos Georgiadis[3]
Seung Hyun Lee[2,4]

[1]Left in 2021
[2]Joined in 2021
[3]Joint with Caroline Dive, CBC and Juan Valle, Christie NHS FT
[4]Joint with Juan Valle, Christie NHS FT and Lucy Foster MFT

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

Pancreatic Ductal Adenocarcinoma

Pancreatic Ductal Adenocarcinoma (PDA) is a dismal disease with an average five-year survival rate of 9%. PDA is the 11th most common cancer in the UK but the fourth 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 tissue stiffness and interstitial pressure, which results in decreased therapeutic efficiency. Moreover, the microenvironment contains an abundant fibroblast and myeloid cell infiltrate, which reduces immune surveillance and confers resistance to therapy.

Mapping the tumour microenvironment of PDA

The tumour microenvironment of PDA has been ascribed with both tumour promoting and tumour restrictive abilities. Stromal targeted therapies should therefore aim to inhibit the tumour promoting effects of the stroma while augmenting the tumour restrictive effects. We developed mass cytometry antibody panels recognising cell surface receptors and used this to assign subsets of cancer-associated fibroblasts (CAFs) and immune cells in tumours isolated from a genetic engineered mouse model of PDA. We observed that PDA tumours contain two separate populations of CAFs distinguished by the expression of CD105 (Endoglin). Isolation and characterisation of both CD105^{pos} and CD105^{neg} CAF revealed distinct expression of immune-regulatory signals. Moreover, the two stromal subsets expressed CD105 in a noninterchangeable manner and responded differentially to most exogeneous signals tested, suggesting the subsets may have distinct functional roles in the tumour microenvironment. Indeed, tumour cells co-implanted with CD105^{pos} fibroblasts grew similarly to tumour cells implanted in isolation, suggesting a tumour permissive role of CD105^{pos} fibroblast. In contrast, co-implanted CD105^{neg} fibroblasts restrict tumour growth, which was dependent on functional adaptive immunity. These data demonstrate that tumour promoting and tumour restrictive fibroblast subsets co-exist in the pancreas and provide molecular insights into the signals governing tumour development.

Development of a fully synthetic 3D model of human pancreatic cancer

Although tumour cells constitute less than 20% of the tumour volume in patients, most *in vitro* models do not support the study of tumour cells within an equally complex microenvironment. To improve how tumours can be modelled *in vitro*, we worked with Prof Linda Griffith (MIT - Massachusetts Institute of Technology) and Prof Martin Humphries (UoM) to adapt a fully synthetic scaffold to support growth of both tumour and stromal cells. Peptide ligands were used to mimic the adhesive signals found in the tumour microenvironment of pancreatic cancer, which enabled growth of both normal and tumour cells. Moreover, tumour cells grown in these scaffolds produce their own extracellular matrix, which we found engage integrin ligands in a similar manner to what is observed *in vivo*. Due to the synthetic nature of these scaffolds, they can be modified to recapitulate the entire stiffness range of patient tumours. We observe that tumour cells exhibit a different growth pattern and signalling depending on the scaffold stiffness, suggesting that incorporation of these models will be important to further address the impact of the environment on tumour cell function and to functionally interrogate stromal targeted therapies in patient derived models.

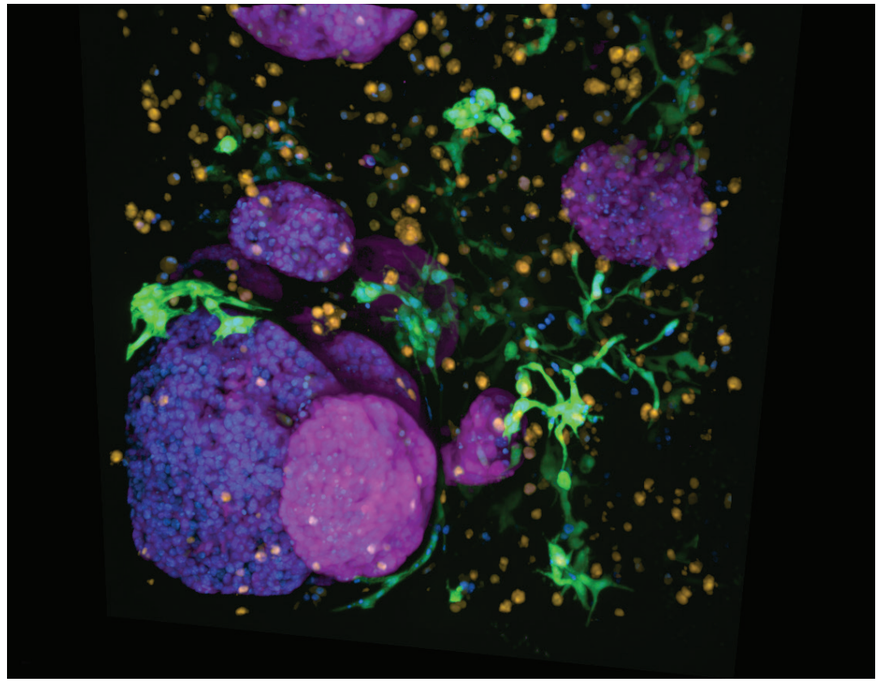


Figure 1.
Pancreatic cancer organoids (purple) were co-cultured with pancreatic fibroblasts (green) and bone-marrow derived macrophages (orange) in a synthetic PEG hydrogel scaffold.

Image supplied by Joanna Kelly and Chris Below.

Tumour stromal interactions control tumour growth and metastasis

Tumour cells co-opt stromal cells to secrete signals, which in turn expand the signalling network tumour cells can engage. Due to the complexity of the tumour microenvironment, we interrogated how stromal reciprocal signals depend on the stromal cellular composition. Interestingly, stromal fibroblasts behave in a highly adaptive manner to change the secreted signals depending on the cellular composition. Specifically, tumour cell secreted GM-CSF induces macrophages to secrete Oncostatin M (OSM), which in turn induce fibroblasts to produce multiple pro-tumorigenic inflammatory signals. This ensuing signalling environment induce a more mesenchymal tumour cell phenotype. Consequently, blocking OSM signalling *in vivo* reduce tumour growth and metastasis.

Publications listed on page 70

TRANSLATIONAL LUNG CANCER BIOLOGY

Institute Fellow
Carlos Lopez-Garcia[1]

Scientific Officer
Anthony Oojagger[1]

Graduate Student
Julia Ogden[1]

[1]Group formed in 2021

Lung squamous cell carcinoma (LUSC) is an aggressive type of lung cancer that originates in bronchial basal cells with limited therapeutic options. Apart from chemotherapy, only immunotherapies result in marginal improvement of survival in LUSC patients. Early detection is currently the most effective tool to prevent deaths by LUSC. Screening programmes by CT-scanning in high-risk populations have overwhelmingly confirmed this benefit. However, 40% of patients diagnosed with early-stage disease still die within five years, having failed to detect preinvasive lesions. These precancerous bronchial lesions show high risk of malignant progression but can be easily removed with minimally invasive procedures.

Hence, preventing deaths by LUSC requires the improvement of therapeutic modalities and early detection methods. These improvements depend heavily on more ambitious, innovative, and patient-relevant preclinical models that recapitulate the intra-tumour and inter-patient heterogeneities so frequent in this disease as well as developmental stages of LUSC progression (Figure 1C). However, existing LUSC models do not recapitulate those complexities, and this is a barrier to reverse the dismal landscape of LUSC.

Modelling the complexity of lung squamous cell carcinoma (LUSC)

Lung squamous cell carcinoma has been historically difficult to model using genetically engineered mouse models (GEMMs), and to date they are not sufficiently developed. The identification of *SOX2* (frequently amplified in LUSC and a component of the squamous differentiation pathways) as the most important LUSC driver and its incorporation in LUSC modelling strategies has made LUSC models more patient relevant. However, LUSC genomics is much more complex than *SOX2* amplification and additional modelling strategies are needed to develop models that represent this heterogeneity.

There is not a single targetable pathway that dominates the genomic landscape of LUSC. Instead, the most frequently altered pathways in LUSC are PI3K/Akt pathway (47%), squamous differentiation pathway (44%) and oxidative stress response (34%) (Figure 1A). Furthermore, analysis of LUSC genomes has not shown co-occurrence or mutual exclusivity in these dysregulated pathways. This suggests that none of the pathways are indispensable in driving LUSC, but also that they can cooperate. Deciphering the biology of this complex inter-patient diversity requires individual interrogation using appropriate models.

Understanding this extensive heterogeneity in LUSC involves addressing several key questions:

- Can these pathways drive LUSC tumourigenesis?
- Do they cooperate in driving LUSC tumourigenesis?
- Are LUSC cells addicted to these pathways?
- Are these pathways mutually dependent?
- What is the role of ITH drivers in driving adaptation to microenvironment challenges and therapy?

Answering these key questions requires intensive research programs that involve the manipulation of multiple loci. Approaches to implement the principles of the 3Rs (replacement, reduction and refinement) with respect to in vivo research are a responsibility of the scientific community, especially in the field of LUSC, where the new availability of more relevant mouse models will increase the number of projects involving animal research.

Primary human basal cells (HBCs) as an alternative to murine models to investigate LUSC

Human basal cells, the LUSC cells of origin, are a feasible and versatile alternative to mouse models

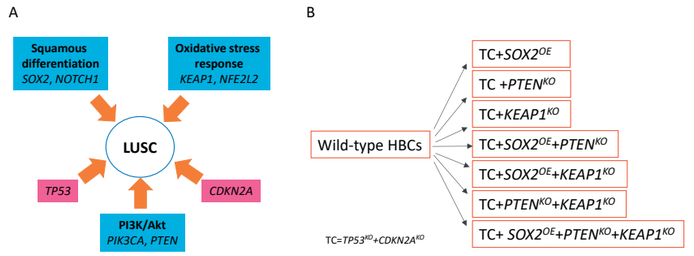


Figure 1.
A. Summary of the most relevant tumour suppressors (pink boxes) and pathways (blue boxes) involved in LUSC development, with examples of components of the pathways altered in LUSC.
B. Summary of the mutant HBCs that we are developing. All mutants contain TP53 and CDKN2A truncations with possible combinations of three pathways in order to assess individual and combined effects.

to interrogate LUSC evolutionary history and develop multiple allele combinations that reflect LUSC inter-patient heterogeneity. Current methodologies permit efficient expansion of HBCs, genome editing and development of organoids mimicking bronchial morphology. Using HBCs in combination with organotypic cultures (organoids) and other *in vitro* assays, we can interrogate how driver alterations induce epithelial perturbations indicative of LUSC initiation and progression.

Using HBCs to model LUSC presents multiple advantages beyond the replacement of mouse models. Importantly, modelling inter-patient heterogeneity is simpler in HBCs as they can be easily manipulated. They reflect human diversity better than mouse models and constitute a more adequate system to investigate the effect of exposures, mainly smoking.

In the Translational Lung Cancer Biology group, we have designed a genome engineering strategy whereby, using genetically modified HBCs, we intend to capture a large extent of the inter-patient heterogeneity that we observe in LUSC patients. To do this, we have generated double-mutant human bronchial epithelial cells (HBEC) bearing inactivating mutations in the tumour suppressors *TP53* (Figure 1B). Since inactivation of both genes is found in the vast majority of LUSC cases, we are using this double mutant background (referred to as TC from now on) to model the effect of the individual and combined effect of the activation of the squamous differentiation, PI3K/Akt and oxidative stress response pathways in LUSC development (Figure 1B). To activate those pathways, we have selected genes that are representative components of the pathways, namely *SOX2* overexpression, *PTEN* truncation and *KEAP1* truncation respectively.

Analysis of the mutant HBCs showed interesting phenotypes and interactions between pathways. We observed that, as described in the literature, *SOX2* overexpression is toxic for HBCs but that this toxicity is reverted by *CDKN2A* inactivation (Figure 2A). We intend to explore more carefully these interactions in the future as they can be the basis of clinically relevant synthetic lethalities. Analysis of air-liquid interface (ALI) organotypic cultures showed that SOX2 overexpression abrogates HBC differentiation and mediates the transition from low to high-grade premalignant stages (Figure 2B and C). *PTEN* and *KEAP1* mutation result in the expansion of p63-positive cells indicating a transition to more advanced stages (Figure 2C). We are currently focusing on extending our phenotypic characterisation to investigate other changes indicative of malignant transformation such as invasion, xenografts and anchorage independent growth. Additionally, we intend to test the reproducibility of our results with HBCs isolated from other donors.

In collaboration with the Bioinformatics and Biostatistics team from the Cancer Biomarker Centre (Matt Roberts), we have developed GEPreLUSC, an open source bioinformatic application that enables the user to investigate the biology of the LUSC premalignant stages. To this end, GEPreLUSC integrates multiple modalities of statistical analysis (gene-centred analyses, pathway and ontology analyses, custom made transcriptional signatures, etc.) and four different publicly available transcriptomic databases of LUSC premalignant lesions.

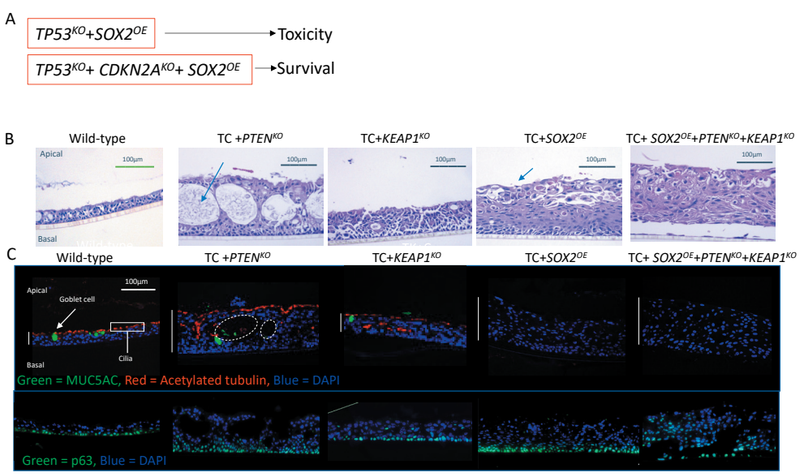


Figure 2.
A. *SOX2* overexpression induces cell toxicity in *TP53* mutant HBCs, but this toxic effect is rescued by the inactivation of CDKN2A.
B. Haematoxylin-eosin stained sections or air-liquid interface (ALI) HBC cultures from wild-type HBCs and 4 mutant HBCs. ALI cultures are bronchial organotypic cultures. C. Immunostaining of wild-type and mutant ALI cultures with parkers of bronchial differentiation. MUC5AC: goblet cells; acetylated tubulin (ciliated cells) and p63 (basal cells).

TRANSLATIONAL ONCOGENOMICS

Group Leader
Robert Bristow

Senior Scientific Officer
Steve Lyons

Postdoctoral Fellows
Maria Jakobsdottir
Maria Lizio[1,3]
Richard Rebello[2]

Graduate Students
Jack Ashton
Parsa Pirhady
Ronnie Rodrigues[2]
Alexandru Suvac

Clinical Fellows
Manas Dave[1,4]
Diego Sanchez Martinez[1,5]
Ashwin Sachdeva[1,6]
Martin Swinton[1,7]

[1]Joined in 2021
[2]Left in 2021
[3]Wellcome Institutional Strategic Support Fund Fellow
[4]Academic Clinical Fellow
[5]Clinical Research Training Fellow
[6]Collaborating, NIHR ACF
[7]MD Degree

Figure 1.
The HYPROGEN trial aims to characterise the role of hypoxia in driving the early metastatic spread of tumours. Patients with oligo-metastatic disease are recruited to the study prior to receiving any treatment. Hypoxia can be assessed by means of the tracer molecule, pimonidazole, which is administered before biopsies are taken from both the primary tumour and selected bone metastases. Genomics and transcriptomics will then be applied to provide insights into the relationship between hypoxia, genomic instability and metastatic spread.

Despite the use of stringent clinical criteria to place patients into prognostic groups, 30-50% of men can still fail precision radiotherapy or surgery due to local resistance and/or systemic spread. There is a need to develop new biomarkers that give an insight into heterogeneity of outcomes in prostate cancer patients. In recent years, there has been a growing appreciation of the role of DNA repair genes in the biology of prostate cancer (PCa).

In depth analyses of the prostate cancer genome have shown that somatic mutations in DNA repair genes are relatively frequent and are more common in incurable, castrate-resistant disease (mCRPC) than in primary cancers. Concordantly, it has been shown that men carrying germline mutations in such genes are at a higher risk of developing prostate cancers that progress to become metastatic. The presence of hypoxia in PCa is also 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. As hypoxia is tightly correlated with levels of genome instability across a range of cancer types, our lab studies genotype-phenotype interactions using primary or ex vivo human prostate cancer models for multi-omic TME and functional genomic studies.

Models for Chromosomal Instability: TME and Genetics

We are driving translational studies in prostate cancer - local and systemic aggression - within the PCUK-funded HYPROGEN trial in Manchester at the Christie NHS Foundation Trust (Figure 1). The biomarker assessments of this trial are designed to answer the question as to how hypoxia can drive LOH and chromosomal instability in the primary and metastases. We use a small molecule marker of hypoxia (Pimonidazole), which is administered to prostate cancer patients prior to their treatment with local and systemic treatment for metastatic hormone sensitive, M1 disease or high-risk localised prostate cancer. Detailed analyses with Prof David Wedge (Genomics, Division of Cancer Sciences) and Dr Pedro Oliveira (Pathology, Christie NHS FT) will clarify the relationships between levels of oxygenation and DNA repair, genome instability and metastatic spread. Parallel studies are investigating mutations and methylation in ctDNA and CTCs to determine whether aggressive clones can be detected at diagnosis using a liquid biopsy in collaboration with Prof Caroline Dive (CRUK MI Cancer Biomarker Centre).

In other work, we have shown that prolonged treatment of hypoxia in vitro can give rise to new genetic clones with varying growth in autonomy and invasion. This suggests that hypoxia can modify chromosomal instability and current efforts are underway to delineate the mechanistic reason behind this observation using a multi-omic approach combining whole genome sequencing with transcriptomics, proteomics and metabolomics (Figures 2 and 3).

Hereditary Syndromes and Prostate Cancer

Prostate cancer tumours that harbour BRCA2 mutations for example, are more likely to respond to PARP inhibitors or to Cisplatin compared with non-BRCA mutated tumours. Although mutations in MMR genes are rare in PCa, the presence of mutations in one of the MMR genes

Figure 1.

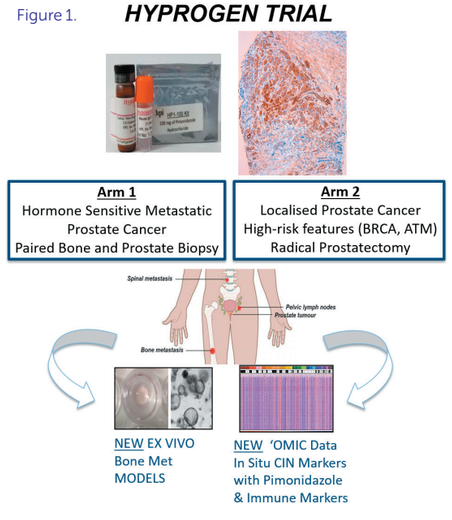


Figure 2.

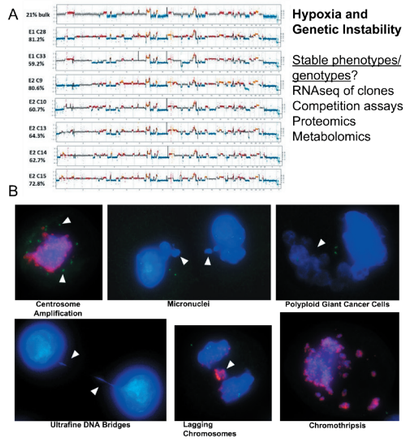


Figure 3.

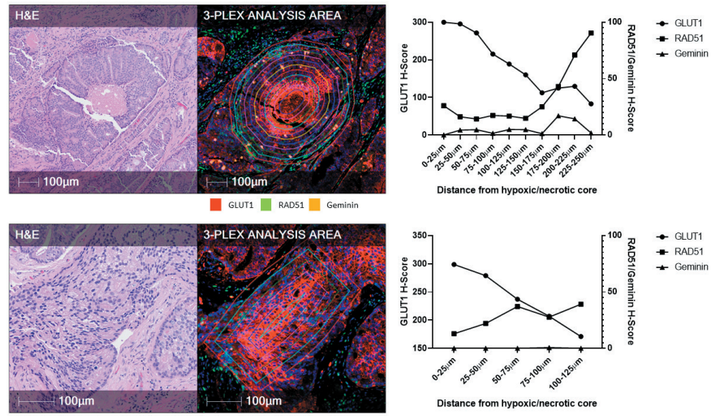


Figure 2.
A. Next-generation sequencing has revealed increased levels of genome instability in cells exposed to hypoxia. Clones with high rates of genomic alteration are being assessed for tumourigenicity and invasive phenotypes. B. Studies for mechanisms of chromosomal instability in hypoxic in vitro models.

Figure 3.
Hypoxia and HR Gene Expression.
Gradients of hypoxia can be detected in tumour biopsy material by monitoring the expression of GLUT-1, which is increased in hypoxic areas. The hypoxic phenotype can then be further explored by monitoring the expression of other genes in these areas –for example the DNA repair gene Rad51 as shown here. This technique can also be combined with the latest spatial transcriptomic techniques to provide detailed insights into the hypoxic tumour environment.

(MSH2, MSH6, EPCAM, MLH1 or PMS2) has been correlated with MSI and adverse pathology in PCa – and overall, patients with Lynch syndrome are at two-fold higher risk of developing prostate cancer. Our aim is to comprehensively characterise clinical material sampled from patients with germline DNA repair defects, and to develop matching pre-clinical models that allow experimental approaches. By working with research collaborators at Manchester University NHS Foundation Trust and in Melbourne, we have assembled a unique collection of BRCA2 and MMR-deficient specimens for further study. We are carrying out a detailed interrogation of these samples including next-generation sequencing studies and spatial transcriptomics to define the relationship between LOH, secondary and tertiary genomic rearrangements, patterns of gene expression and risk status. In addition, we are developing models of hereditary prostate cancer ex vivo by hTERT-immortalisation of normal prostate epithelium from germline carriers that undergo radical prostatectomy for prostate cancer (Figure 4). As these models form prostaspheres, 2D culture and 3D organoid phenotypes can be interrogated following additional activation of prostate cancer-related oncogenes (e.g., cMYC) or CRISPR knock-out of tumour suppressor genes (e.g., TP53, PTEN).

Prostate Cancer Genomics and Chromosomal Gains

Somatic chromosomal imbalance instability leads to cancer initiation and progression and the gain of a single chromosomal unit can activate or inhibit cell proliferation, immune system activation and metastatic capacity. Up to 20% of prostate cancer cases present with Chr. 8q, which harbours the c-Myc oncogene with co-amplification of up to 30-40 other genes. We are currently using molecular pathology approaches (in situ FISH, chromosomal instability assays, genomics and spatial transcriptomics) to understand the intra-prostatic cell heterogeneity of chromosomal gains using spatial 'omics on tumour foci within individual patients' prostate glands removed at surgery. Functional genomic studies to validate pathology findings is an option using prostate epithelial cells (PrEC) transfected with engineered Human Artificial mini-Chromosomes (HACs). The work will be completed primarily in the Translational Oncogenomics lab in collaboration with Prof Patrick Cai (HAC systems; FSE-Manchester Institute of Biotechnology), Prof David Wedge and Dr Pedro Oliveira.

Publications listed on page 71

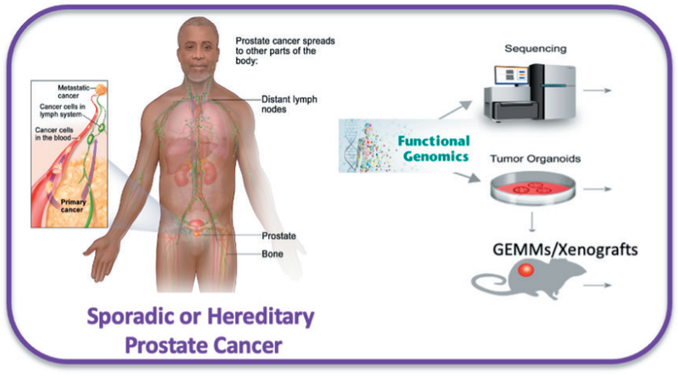


Figure 4.
Our approach involves working with clinical specimens isolated from patients with high-risk prostate cancers such as those arising in men with germline defects in DNA repair. We aim to identify the genetic drivers of high risk disease by undertaking a range of functional genomics approaches, and to explore novel treatment options for this group of patients.

TUMOUR SUPPRESSORS

Institute Fellow

Patricia Muller[1]

Postdoctoral Fellow

Yannick von Grabowiecki[1]

Graduate Students

Lobsang Dolma[1]

Callum Hall[1]

[1]Left in 2021

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 can lead to loss of p53 or expression of mutant versions of the p53 protein. These mutant p53 proteins often lose WT function, but can also gain novel functions in promoting invasion, metastasis and 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. Mutant p53 is known to promote chemoresistance. P-gp (P-glycoprotein) is one of the best studied proteins involved in chemotoxic drug efflux. We therefore decided to validate these findings. In various cell lines we could detect this interaction endogenously. Using CRISPR knockouts we determined that mutant p53 A431 cells were dependent on RCP and mutant p53 expression to promote resistance to cisplatin and etoposide. This resistance was also dependent on P-gp as loss of P-gp expression or inhibition with the third-generation P-gp inhibitor tariquidar restored sensitivity to chemotherapy. Loss of mutant p53 or RCP expression coincided with an increased expression of cleaved caspase 3 cells. In mice xenografts, loss of RCP in mutant p53 cells slowed down tumour growth and also showed an increase in cleaved caspase 3 upon cisplatin challenge.

Interestingly, restoration of RCP expression in RCP knockout cells restored resistance to cisplatin and etoposide, but expression of RCP in p53 KO cells did not. These data suggest that mutant p53 regulates RCP function, but not RCP expression to promote chemoresistance. We therefore decided to look at the location of P-gp in response to chemotherapeutic challenge. In mutant p53 cells, P-gp was rapidly detected on the plasma membrane in response to cisplatin, where it co-localised with RCP. Loss of RCP or loss of mutant p53 greatly reduced P-gp plasma membrane expression in response to cisplatin. 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 (Phatak et al Cell, Death and Disease 2021).

Interestingly, P-gp wasn't the only drug transporter discovered to interact with RCP. We also validated the transporter ATP7B as a bona fide RCP interaction protein (Von Grabowiecki et al, Frontiers in Oncology, in press). In response to copper, ATP7B translocates from the Golgi to the plasma membrane to facilitate copper efflux. The binding sites that allow for copper export can however also bind cisplatin and ATP7B has been shown important for cisplatin efflux. Similar to P-gp, we could show that ATP7B relocated to the plasma membrane in response to ATP7B in a mutant p53 dependent manner. Remarkably, ATP7B membrane translocation in response to copper was mutant p53 independent. Mutant p53 cells were also equally sensitive as p53 KO cells to copper-induced cell death, suggesting that the external stimulus dictates how RCP reacts. Together these data uncover a novel role for RCP in mutant p53 induced chemoresistance. Our data support a model in which RCP and drug transporters are localised in the same intracellular vesicles in mutant p53 cells that can rapidly be moved to the plasma membrane to increase transporter membrane expression in response to chemotherapeutic challenge.

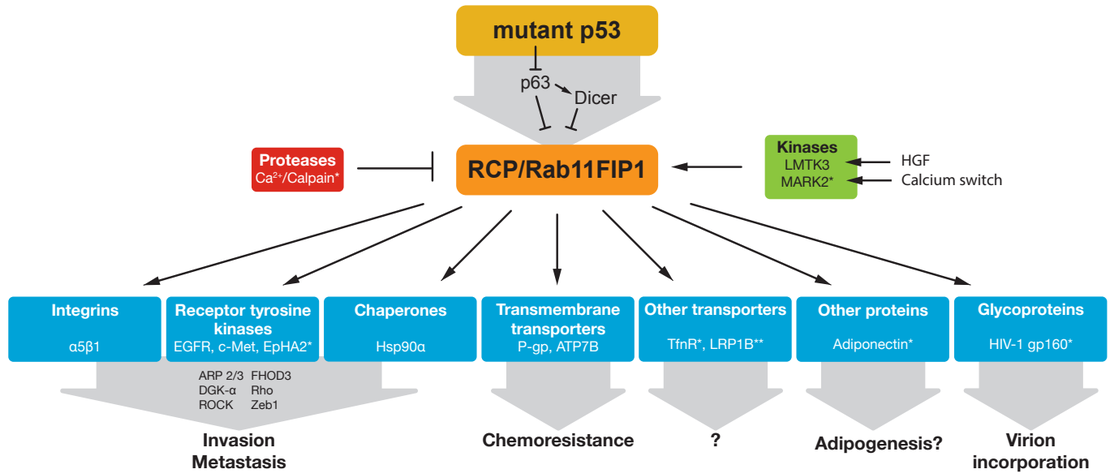


Figure 1.
Mutant p53 regulates RAB11-FIP1-dependent re-localisation of a variety of proteins.
Mutant p53 can regulate RAB11-FIP1 by inhibiting the p53 family member p63 and/ or the downstream target Dicer. Rab11-FIP1 enhances the re-localisation of a variety of proteins indicated in this figure. * indicates mutant p53 dependency. ** indicates colocalisation only with Rab11-FIP1. (Figure published in Von Grabowiecki et al Frontiers in Oncology).

Together our work shows that RCP can regulate various processes downstream of mutant p53 (Figure 1). These data raise the question how RCP regulates invasion, metastasis and chemoresistance at the same time. We speculate on this question in a recent perspective (Von Grabowiecki et al Frontiers in Cancer, in press) and we will be working on addressing this question in the future.

In order to determine tumour growth and invasion in vivo, we also published a pilot study determining the use of iRFP in vivo. We had three different instruments that would be able to detect fluorescence in the far red 700nm spectrum in our Institute. We validated that each instrument was able to detect iRFP and we then compared software and quantification tools using subcutaneous and tail vein injected xenograft growths of H1299 iRFP cells. All instruments were able to detect iRFP in subcutaneous growths and could measure increased growth of small tumours accurately. Most variations arose due to subjective gating of the researcher determining where the boundaries of the tumour in the image were. A new software tool to determine boundaries was developed and proved to generate less variability. Subtle differences between instruments became more apparent when imaging deep tissue tumours, with the Li-Cor pearl trilogy the most sensitive in detecting these tumours. (Hall et al Cancer Cell International 2021).

Publications listed on page 71

CANCER RESEARCH UK MANCHESTER INSTITUTE

RESEARCH SERVICES

RESEARCH SERVICES

Chief Laboratory Officer
Stuart Pepper

During 2020 the Institute's core facilities had to adapt to the challenges that the pandemic created. The managers were highly successful in leading their teams to adapt as necessary and by the end of the year many services were running at close to normal throughput of samples. Throughout 2021 demand for services has remained high and the core facilities were able to continue delivering the support needed by the Institute's research groups.

Chief Laboratory Officer
Stuart Pepper

The following articles demonstrate that core facilities have continued to develop and innovate as well as delivering well established services. A key theme is the successful interaction of facilities to support multi-disciplinary workflows. For example, interactions between Molecular Biology Core, Histology and VIA have continued to develop high number multiplex immunohistochemistry and spatial transcriptomic technologies. These techniques allow for spatial profiling of tissue from multiple angles and have now been applied to projects including work based on challenging archival samples. The development of the CODEX, which was introduced last year, has continued to provide an alternate approach for multiplex immunohistochemistry.

Traditionally the Computational Biology Support team have predominantly focussed on nucleic acid analysis, however over the past year there has been a renewed focus on the analysis of protein data sets generated by mass spectrometry. CBS have built a pipeline to extract site specific ions to allow semi-automated analysis, alongside the use of established software MaxQuant for quantitative proteomic data.

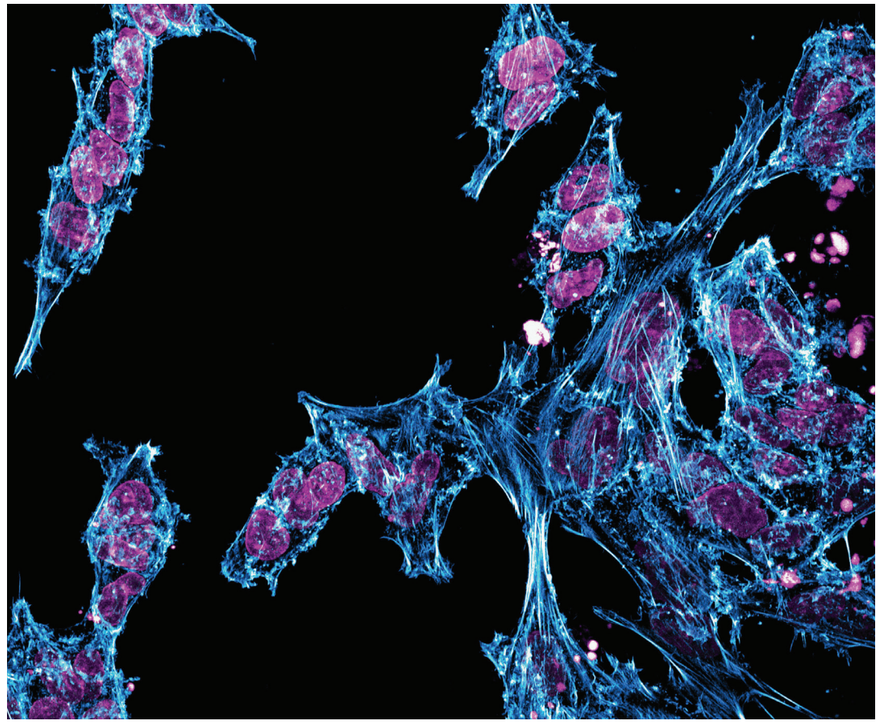
In close cooperation with the Bioinformatics and Biostatistics team in the Cancer Biomarker Centre, Scientific Computing set up a cBioPortal platform for the Institute that allows easy access and visualisation of cancer genomics data produced here. Various new technologies and skillsets were introduced this year, with one of the most important being the creation and usage of Bioconda software containers, allowing us to run either Conda, Docker or Apptainer (formerly Singularity) containers on our Phoenix High-Throughput data analysis platform. As part of ongoing commitment to supporting the processing of large data sets, a significant procurement was carried out to provide new data storage which will come online during 2022.

COVID-19 restrictions impacted on throughput in the in vivo facilities and so each team has looked for different opportunities. The Transgenic Breeding Team have undertaken a refresh of the NSG breeding colony to deal with the risk of genetic drift, which has been potentially exacerbated by the move towards smaller breeding colonies during lockdown. The Genome Editing and Mouse Models team were able to contribute to two online meetings, helping to maintain the external profile of the Institute despite restrictions on travel. By moving online these meetings managed to attract a larger attendance than was originally planned for an in-person meeting. For the Experimental Team it has been a very busy year procuring equipment for the new building. There has been a series of tight deadlines to organise procurement and finish design work for a lot of the infrastructure for the new animal unit with great progress made.

2022 promises to be an exciting year as the new building nears completion; planning is already underway for the relocation but there is still much to do to ensure an efficient relocation of the core facilities once the building opens.

Image of non-small cell lung cancer cells embedded in a 3D matrix to replicate invasion from a primary tumour into the surrounding tissue. Super-resolution imaging reveals sub-cellular details of the shape of the nucleus (magenta) and individual actin fibres (cyan), both of which are controlled by the cells for optimal invasion.

Image supplied by Andrew Porter(Cell Signalling)



Biological Mass Spectrometry

Duncan Smith, Yvonne Connolly

This year has seen a significant increase in demand for proteomics applications in the areas of quantitative profiling and post-translational modification analysis in both global and targeted approaches.

There has been great progress in a collaboration between research group Cell Division and core facilities Biological Mass Spectrometry and Computational Biology, centred on developing a data analysis pipeline focussed on quantitative profiling of specific sites of phosphorylation. We utilise high performance data dependent analysis to first define sites of post-translational modification, followed by the design of targeted Parallel Reaction Monitoring (PRM) experiments to specifically quantify phosphorylation sites utilising site specific fragment ions. Computational Biology has built a pipeline to extract site specific ions from the PRM data file format and semi-automate the quantitative analysis to move this application towards its planned destination of routine analysis. The Mass Spectrometry facility was relocated from Alderley Park to the Christie site in late 2021. The main MS laboratory is now temporarily situated in the Wolfson Molecular Imaging Centre and the Biochemistry lab sited at the Oglesby Cancer Research Building. We plan to return the facility to normal operation in February 2022.

Biological Resources Unit
Transgenic Breeding

Team Leader: Jennifer Hughes
Daniel Bennett, Tim Bloor, Carl Conway, Edyta Kijak[1], Wesley Moore, Kerry O'Shea[1], Victoria Preston, Rose Storey, Natalie Varley[1], Martin Vincent

[1]Left in 2021

The BRU Transgenic Breeding team breeds mice for CRUK MI researchers under the authority of a central breeding project licence held by the team leader. 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 and genetic background monitoring services, translates and transfers genotyping results, checks lines with deleterious phenotypes for onset of symptoms and provides the Named Animal Care and Welfare Officer (NACWO) service for CRUK MI mice in the facility. In accordance with Home Office requirements, the mice are closely monitored to ensure high welfare standards.

The breeding facility is housed in a clean unit with a high health status and is kept free from common mouse pathogens. We regularly screen the mice to check that no infections have been introduced and in the last year we have moved over to using non-sacrificial sampling methods for this purpose. To protect this high health status, new transgenic lines coming from

external sources must be transferred in as either embryos or sperm by the GEMM team, and then thoroughly health screened to ensure that the resulting offspring are specific pathogen free. At present mice required by our 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 in experiments.

Nine staff members currently provide day-to-day care for 87 different transgenic mouse lines that are spread across approximately 1200 cages, in a facility located within the main university campus. Our rooms in the facility are covered directly by the CRUK MI Establishment Licence, meaning that we benefit from being able to use the same National Veterinary Services as the team at Alderley Park, allowing consistency of practice. In the last year, 24 new breeding lines have been started and 43 breeding lines were closed. The new breeding lines include some that have either been rederived in or produced by the GEMM team, and others that have been generated by crossing existing lines. Turnover of individual breeding lines has been quite fast in some cases; four new breeding lines were both started and closed in 2021 and these were used to either answer very focused research questions or as tightly controlled stages within a breeding strategy.

In another unusual year the team has continued to adapt to new challenges. Numbers of mice being produced and used are still fewer than they were two years ago but a higher proportion of the mice that are being produced are also being used, with fewer colonies being kept ticking over. The team has put additional effort in working closely with researchers to intentionally keep numbers low and reduce wastage. The number of live breeding lines has reduced slightly but we have also seen a small increase in average numbers of cages within breeding lines, as numbers are expanded to meet experimental demands. As well as breeding particular transgenic lines for specific researchers, we also batch breed immune compromised NSG mice, which the Experimental Team then allocate for use as required. This year we have refreshed our NSG breeding colony by bringing in completely new breeders and have also scaled up numbers to meet demand.

Experimental Services

Team Leader: Lisa Doar

Diane Beeston, Tom Bosley[1], Jacqui Clayton, Laura Dean, Lisa Dique, Lisa Flynn, Heather Joy[2], Amber Kelly[2], Suzanne Kelly[2], Jacek Kruza, Emma Playle, Jo Roberts, Eirini Symeon[1], Rachel Walker, Lewis Woolley

[1]Joined in 2021
[2]Left in 2021

It took a while for the workload to build back up to more normal levels in 2021, due to the lockdowns and ensuing restrictions in 2020. The year has been another difficult one for the team; as well as further COVID-19 restrictions causing disruptions, we also had three technicians leave the Institute in the middle of the year, resulting in a busy couple of months whilst we were recruiting replacement staff. We now have two new technicians; Eirini Symeon and Tom Bosley, who have settled in well and are both going to be a great asset to the team.

We have continued to organise lots of training for the quieter periods, both for our own staff and for the researchers. It is pleasing to see how much training the team has managed to accomplish despite having been short staffed for several months of the year.

Some key achievements this year have been fully developing the image guided injection of tumour cells into the liver – this has been more successful than the surgical route and is also considerably quicker and less invasive, so is now the default method for carrying out this procedure. We have also trained some of our technicians in photoacoustic imaging of subcutaneous tumours, castrations, and intra-cardiac cell injections. Some of these were already established in the research groups but not in our core BRU team. This means we can now offer these techniques to other groups allowing us to provide a more comprehensive service to our users.

There has been much focus this year on further developing the plans for our new BRU facility for when we move back to the Christie site, planned for early 2023. We have now ordered all the large equipment, which will start arriving ready for installation from May 2022. In December, our technicians had a tour of the building site as it has reached the developmental stage where our working space can be clearly visualised, which is really exciting. By Q4 of this coming year the BRU facility will be completed and fully functional – an incredible achievement.

Flow Cytometry

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

[1]Left in 2021

The Flow Cytometry facility provides researchers with state-of-the-art instrumentation, expert advice and technical training as well as providing access to a professional, operator based cell sorting service. Our remit is to provide researchers with essential tools and advice, enabling them to investigate both fundamental and translational cancer research questions. Key to this approach is developing close collaborations with group leaders and their researchers as well as continuing the drive for excellence within the facility itself. This year the Institute has strengthened those ties by appointing group leaders as scientific advisors to each facility, an initiative that aims to align research aims with application development and advances in technology.

While flow cytometry, mass cytometry and cell sorting can be seen as distinct elements within the facility, they are actually interconnected strands that complement one another. Mass cytometry is an outstanding tool for exploring the immune landscape, what we learn here can be refined and adapted for analysis on the facility's flow analysers. Once novel populations of cells are identified, these can then be sorted and purified for downstream applications such as RNASeq using the facility's advanced suite of cell sorters.

Under the direction of Toni Banyard, mass cytometry has made significant contributions to a number of research projects this year. A collaboration with the Division of Cancer Sciences Targeted Therapy group saw the mass cytometer used in a prostate cancer murine model study. This study sought to determine, at the site of the tumour, the immune cell composition of the micro-environment under two conditions: radio therapy and radio therapy in combination with checkpoint inhibitors. From prior immunohistochemistry knowledge of this model, Toni developed a 37-marker panel to deep profile the immune landscape to decipher the effect that each therapy had on the tumour. This work was completed within 3 months and was expedited by use of the facility's extensive antibody library. This was a fine example of the smooth cooperation between the facility, group scientists and Scientific Computing, all of whom worked together to develop a robust workflow that efficiently analysed these complex datasets, producing quality and publishable data in record time.

Another study involving collaboration with Molecular Oncology, resulted in the identification of the immune signatures associated with UV exposure in skin tissue of specific mouse genotypes. These immune signatures may indicate underlying genetic susceptibility to UV radiation and melanoma development. This was technically challenging; skin tissue is a notoriously difficult tissue to work with and this project required optimising skin dissociation methods as well as the optimisation of a new panel. This novel dataset has created interest from the wider scientific community. A barcoding system that uses commercially available CD45 antibodies conjugated to different cadmium metal isotopes has been validated by the facility. This system allows researchers to barcode up to 30 human samples simultaneously, which minimises batch variation and reveals rare biological variation. This is currently being used for a human pilot study to determine T cell status after chemotherapy alone or in combination with Atezolizumab, and in another human study to determine T cell exhaustion using a 43-marker panel derived from our developing human antibody library.

The flow and cell sorting services worked alongside Cancer Inflammation and Immunity to investigate and characterise a unique population of intra-tumoural dendritic cells. By using several immune panels the group has been able to examine the role of these cells within the immune tumour microenvironment, answering fundamental questions, such as what stimuli drives this particular phenotype and what precisely are their function. Conventional dendritic cells (cDCs) present antigens to T cells, thereby orchestrating cytokine production and potent migratory responses. The importance and functionality of different cDC subsets present in the tumour niche are currently ill defined. Working with Maria Koufaki from the Cancer Inflammation and Immunity group, the facility has isolated populations of dendritic cells for use in further phenotypic delineation studies. In addition, we have identified and sorted distinct populations from bone marrow-derived cultures for analysis via downstream scRNAseq. Profiling mouse tumours through single-cell RNA sequencing has revealed a distinct, previously unknown, intra-tumoural cDC cluster and based on their transcriptional profile, these cells could be key effectors of anti-tumour immunity.

The facility's cell sorting service also played a pivotal part in the Stem Cell Biology group's study of the endothelial-to-haematopoietic transition (EHT) during the developmental stages of mouse embryos. This is an extremely rare transformational process and the group relies on the service to sort these exceptionally rare cells. The service is able to provide these rare populations either as bulk, highly enriched populations or as single-cells for use in downstream functional assays and transcriptomic analysis. The results from these experiments provide the group with information that sheds light on the underlying molecular mechanisms involved in the endothelial-to-haematopoietic transition.

Often research projects involve co-operation and co-ordination between facilities and researchers. The very nature of obtaining biological material, sample processing, sorting

and downstream applications can be time consuming and difficult to timetable. A flexible approach is often required. In order to extend access to the facility's sorters, selected users are trained to operate the facility's Aria III cell sorter. Trained and validated "super-users" are able to carry out sorts outside normal facility hours, allowing them to progress their studies in a timely fashion. Both Stem Cell Biology and Cancer Inflammation and Immunity have benefitted from this approach.

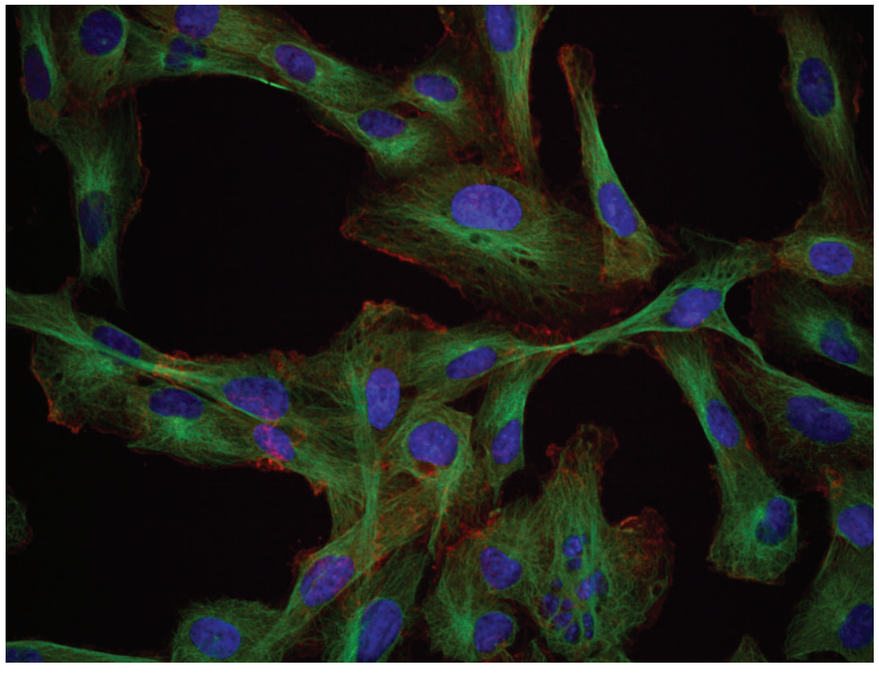
In the forthcoming year the facility will continue its collaboration with Scientific Computing to develop a faster, more robust workflow for the analysis of information rich datasets. Using open source R based scripts, the facility will be implementing its own workflows to ensure that the analysis pipeline is efficient, rigorous and transparent. We are also preparing for the move to the new building that will eventually house the CRUK Manchester Institute back at the Christie site in Withington, Manchester.

Genome Editing and Mouse Models

Natalia Moncaut, Athina Papaemmanouil and Lauren Street

The Genome Editing and Mouse Models (GEMM) facility is responsible for the generation of new genetically engineered mouse models using CRISPR-mediated gene editing approach. Working together with researchers at CRUK MI, GEMM provides strategic advice to generate forefront cancer mouse models to study mechanisms of tumour initiation, progression, and response to therapy.

By providing a much higher resolution of the transcriptional state of individual cells, single cell RNA sequencing (scRNAseq) of tumour-infiltrating cells has led, among other things, to the identification of rare populations that have putative fundamental roles within the tumour microenvironment. Current efforts to study these newly identified rare populations in cancer are hampered by both their scarcity and the lack of tools to specifically image, and alter these cells in normal and transformed tissue. Leveraging deep transcriptome information obtained with scRNA profiling, we are generating bespoke mouse models to specifically modify these cells and understand their function within tumours. Using Cre-lox technology combined with diphtheria toxin- or fluorescent marker-based approaches, the new mouse models will allow researchers to specifically ablate or label these cells. The advent of scRNAseq, and other multiplexing technologies, combined with the unprecedented possibility of introducing precisely and efficiently modifications into the mouse genome, is allowing us to design new experimental models to deepen our understanding of the biology of cancer in ways which were impossible to think about a few years back.



Intracellular localisation of lamellipodin (red channel) in RAS-transformed cells as evidenced by confocal microscopy. α-tubulin (green channel) is shown to delineate the cytoskeleton and 4',6-diamidino-2-phenylindole (DAPI) (blue channel) staining denotes the cellular nucleus.

Image supplied by Lisa Shlyakthina (Cell Plasticity & Epigenetics)

The challenges of the last 18 months of COVID-19-related lockdowns and restrictions led us to reach out to other transgenic facilities around the UK and resulted in the preparation of several initiatives. As part of the LASA Animal Science Transgenic Section, we organised a 3-day online meeting about *"Cryopreservation and Assisted Reproductive Technologies"*, and at the LASA Annual Conference, we led a section about "Colony management: moving forward from Covid-19" and ran an interactive workshop about cryopreservation, colony management and genetic drift. We also published an article, together with Sarah Hart-Johnson (The Francis Crick Institute), about the impact of lockdowns on mouse facilities. In this article, we proposed different approaches to maintain the genetic integrity of mouse colonies (Lab Animal 50(11):301-302, 2021). As part of the NC3Rs Breeding and Archiving Working Group, we also produced the new updated document about *"Sharing & Archiving of Genetically Altered Mice. Opportunities for Reduction and Refinement."*

Histology

Garry Ashton, Caron Abbey, Usman Mahmood[1], Katherine Lally[1], David Millard, Nicola Tonge[2], Deepti Wilks[3].

[1]Left in 2021
[2]Joined in 2021
[3]Haematological Malignancy Biobank

The Histology facility continues to be an important service, accessed by the majority of both basic and translational research groups within the CRUK MI, underpinning their research, allowing for the adoption and development of tissue-based experimental approaches. The Alderley Park facility together with the satellite laboratory housed within the OCRB allow the unit to offer a full range of both routine and advanced histological services for oncology research.

The unit's advanced services range from the extraction of both RNA and DNA, giving sufficient quantity and quality for NGS together with laser capture microdissection if required from relatively small amounts of material to tissue microarray construction. A number of new TMA's have been constructed, all of which are of the highest quality from several disease groups.

The high throughput, routine immunohistochemistry service, troubleshooting and antibody validation services continue to see exceptional demand. Multiplex immunofluorescence is now routine, allowing further interrogation of tissue sections. Multiplexing using both mRNA in situ hybridisation and protein immunohistochemistry on single tissue sections is also in demand.

In routine practice, both human and mouse tissue, in addition to organotypic assays, spheroids, agar plugs and cell pellets, have been processed for various research groups together with fresh vibratome tissue sections (50–250µm) for ex vivo cultures of tumours to evaluate and develop three dimensional studies. Requests for special stains have seen an increase, with Masson Trichrome, PAS and reticulin stains all requested. The unit continues to process tissue samples for the MCRC Biobank. Once approved, projects requiring access to histological samples from the biobank, liaise with the unit to ensure their specific requirements are met.

Over the year, the facility has seen two staff members move to new posts whilst we have successfully recruited a new scientific officer, with recruitment continuing in 2022. Focus remains on the training and continued professional development of staff, ensuring the unit continues to be at the forefront with technological developments, whilst also offering a comprehensive and flexible service relevant across all research themes.

In collaboration with the Drug Discovery Unit, the Histology facility has used both IHC and IF to study several proteins of interest to aid biomarker discovery of the LOX project. The targets include LOX, LOXL2, collagen 1, collagen 3 and fibronectin, with the data generated helping to understand the role of these proteins in both oncology and fibrosis. In addition, routine histology, H&E, Masson Trichrome and Picrosirius Red have been used for further analysis.

The Molecular Oncology group have been able to use mouse models to study the role of the tumour microenvironment in breast cancer metastasis. H&E evaluation in addition to standard IHC and IF have been used to study the impact of metastatic spread on various tissues. In addition to RNA extraction, multiplexed immunofluorescent labelling of tumour cells and stroma cells has been employed to identify potential interesting interactions. Tumour (pan-Cytokeratin, E-cadherin) cells, different stromal compartments, such as immune cells (CD45, CD3, CD8, CD4, F4/80), extracellular matrix (Sirius Red, Masson Trichrome), fibroblasts (vimentin) and other types of cells (Tomato and/or GFP positive) have all been identified using multiplex IF .

RESEARCH SERVICES (CONTINUED)

In collaboration with the Division of Cancer Sciences Targeted Therapy Group, the facility has helped develop four 5-plex multiplex IHC panels for use on human tissue. The panels have been developed to investigate the immune contexture of the tumour immune microenvironment and detect T-cells (CD4, CD8, PD-1, FoxP3), macrophages (CD68, CD163, PD-L1, pan-cytokeratin), myeloid cells (CD11b, CD14, CD15, HLA-DR) and a combination panel (CD4, CD8, CD68, pan-cytokeratin). The panels will be used to investigate what effects radiotherapy has on the tumour immune microenvironment. In addition, a cohort of 17 rectal cancer biopsies and 3 lung cancer biopsies, taken pre- and post-radiotherapy, have been stained using the Ultivue Immuno-8 panel to investigate the effect of radiotherapy on the tumour immune microenvironment (CD3, CD4, CD8, CD68, PD-1, FoxP3, PD-L1, pan cytokeratin). High quality training and the use of both Roche and Leica platforms have been required for this project's success.

In collaboration with both the Molecular Biology and Visualisation, Irradiation & Analysis Core Facilities, high number multiplex immunohistochemistry and spatial transcriptomic technologies continue to be evaluated and developed. These techniques allow for spatial profiling of tissue from multiple angles and both of which have produced exciting results. Currently, a 14-plex antibody panel is being used by the Skin Cancer and Ageing group to understand the immune cell types that contribute to the immune response in melanoma onset, progression and the response to immunotherapy. The core facility has supported the Translational Oncogenomics group in developing a multiplex immunofluorescence stain for RAD51, GLUT1 and Geminin. Through this they have been able to visualise expressional changes in RAD51 across dynamic gradients of hypoxia for the first time in primary prostate tumour samples. The group are now correlating these proteomic findings to transcriptome alterations across hypoxic gradients using the Visium 10x platform.

Molecular Biology Core and Computational Biology Support

Wolfgang Breitwieser, Christopher Clark, Lucy Goodman[1], Rachel Horner, Dave Lee, Amy Priestman[2], Andzhela Abu Rashed, Sudhakar Sahoo, Robert Sellers, Yannick von Grabowiecki[1], John Weightman

[1]Joined in 2021
[2]Left in 2021

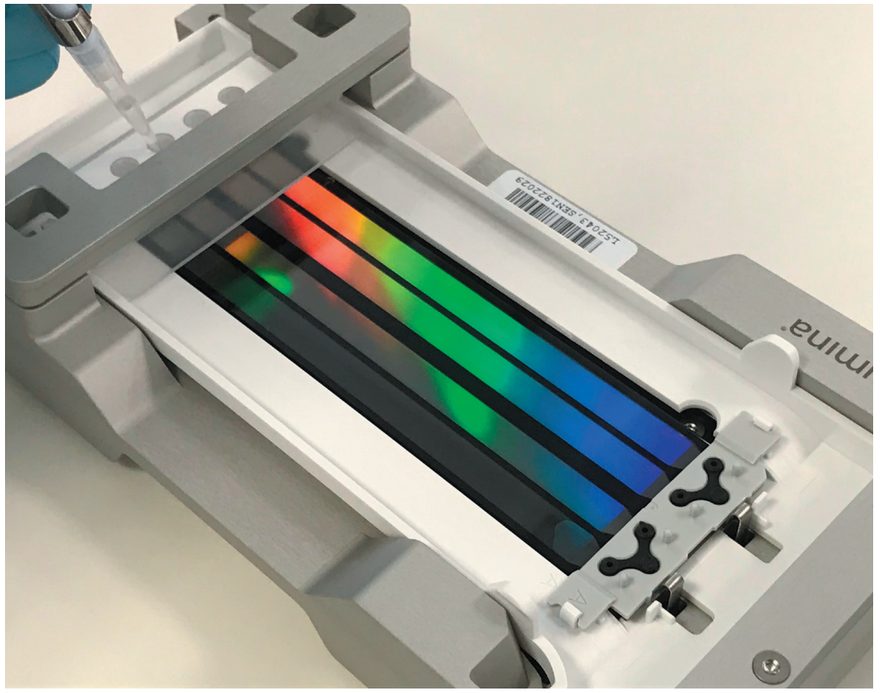
Over the last year, next generation sequencing has again been at the centre of the service's activities, with well over a hundred separate projects processed. For the vast majority of these the sequencing was carried out on our recently acquired Illumina Novaseq 6000 platform. This is Illumina's most powerful instrument and has the highest system specifications of any NGS instruments on the market. The platform enables a flexible set up allowing for the running of small to medium sized projects that were previously processed on the service's Nextseq 500 instrument, as well as very large projects that the service has up to now not been able to run cost effectively.

Alongside the validation of run performances on the Novaseq, we undertook a number of successful validation projects for NGS library preparation. Among these were methods for processing genomes and transcriptomes of very low quality and yields. We now have robust methods for whole exome or panel sequencing, as well as for RNA sequencing derived from biopsy samples or FFPE material. In addition, we have validated methods for transcriptome sequencing from samples with very low RNA yields down to single cell level.

Following the trend from previous years, single cell RNA and chromatin sequencing and bioinformatic analysis has continued to take centre stage of the service's activities. The predominant technology employed was 10X Genomics using their Chromium platform for droplet emulsion generation. In addition to 3' RNA sequencing applications, we validated single cell multi-omics methods, combining transcriptome with ATAC type chromatin analysis. Further applications using this technology included T cell receptor sequencing as well as multiplexing applications using TotalSeq and MultiSeq protocols. Additionally, we routinely processed single cell projects employing CITE-Seq protocols, enabling the simultaneous analysis of transcriptome and lineage marker proteins in single cells. This proves to be a powerful tool for annotation and refinement of cell types in complex tissues.

A further focus was the testing of methodologies for Spatial Transcriptomics (ST). This multi-omic technology integrates histological information with gene expression profiles of the same biological tissue. In a collaboration involving Institute research groups as well as Histology and Visualisation, Irradiation and Analysis (VIA) core facilities, we undertook a study using the 10x Genomics Visium technology to capture and analyse cellular transcriptomes of FFPE tumour sections in a spatial context.

Loading Novaseq 6000 S4 flow cell



Integrating spatial data with transcriptomics paves the way for the discovery of biological patterns without the need for *a priori* markers. To perform these analyses a bioinformatic toolbox has been developed making use of well established, single cell techniques along novel pattern-building and image analysis tools. For example, in collaboration with VIA, incorporating HALO software into our novel analysis workflow has allowed us to harness its powerful facets, such as immunofluorescence quantification and image classification, which now can be combined with Visium data. As a result, validated tissue protein markers can be viewed alongside their unbiased transcriptomic background. As a complement, using HALO, we have also developed a utility for users to define specific image regions for further analysis. Through this period, we have developed a high degree of experience with ST data, which will benefit all service users and allow us to maximise the exploration of the platform in future projects.

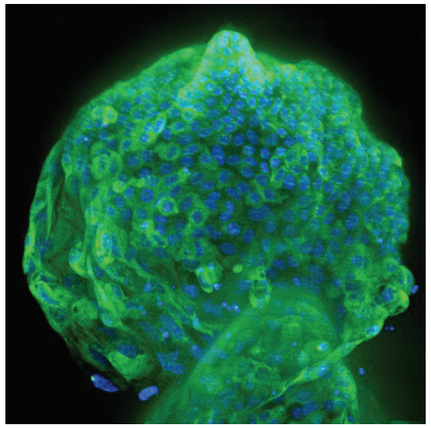
In further validation work, the Computational Support team explored MaxQuant, a widely used software in the field of quantitative proteomics, owing to its accuracy and flexibility in processing different types of quantitative proteomic methods, e.g. SILAC, Isobaric-based tagging, and label-free. Originally a Windows-only software, the increasing size of proteomics experiments and data available in the literature has resulted in the need for higher-throughput at the processing level. MaxQuant has now been installed on the Institute's High Performance Cluster, enabling us to meet large-scale informatics processing requirements. The programme has now been successfully applied in the analysis of a number of proteomics projects.

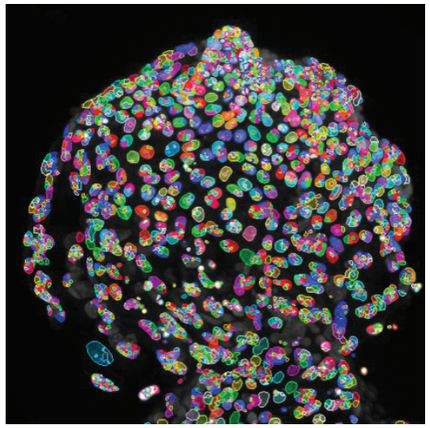
Scientific Computing

Marek Dynowski, John Campion, Kevin Doyle, Anoop Sanalkumar[1], Stephen Kitcatt, ZhiCheng Wang

[1]Joined in 2021

Support for Scientific Computing's High Performance Computing service has been strengthened by welcoming Anoop Sanalkumar into the team in early 2021. He is an HPC (High Performance Computing) System Engineer and will work with the team to run, support, and improve the Phoenix High Throughput data analysis platform. At the heart of this platform is the IBM Spectrum Scale based Central Research Data storage with a storage capacity of 2PByte. This system reached end of life in support terms and will be replaced with a new 2.5PByte IBM Elastic Storage system (ESS) 5000 SLx that is currently being installed. The new IBM storage platform allows Scientific Computing to provide a resilient modern storage system with high access speeds for CRUK Manchester Institute's important research data. The latest version of the IBM Spectrum Scale file system delivered with the ESS system enables flexible configuration for future optimisation of data life cycle management. This combination of software and hardware is particularly suited to meeting the





3D organoid acquired on the Opera Phenix using plastic bottomed 96-well plate with the 20x water objective in confocal mode. The organoid is stained with DAPI in blue and a membrane marker in green. 3D nuclear detection using PerkinElmer Harmony is demonstrated.

Images supplied by Steve Bagley (VIA). Data from Christopher Below and Claus Jorgensen (Systems Oncology)

needs of demanding AI workloads and automated high throughput bioinformatic workflows. Several improvements to the data life cycle in 2021 have already resulted in more efficient storage usage, increased capacity, and FAIRness (www.go-fair.org/fair-principles/) on the platform.

In 2021 it became clear that the infrastructure hosting the VIZ Columbus High Content Analysis software had neither the performance nor the capacity to keep up with increased user demand. Scientific Computing then designed and built a performance optimised platform on which to operate the Columbus server. The resulting improvements in performance, capacity, and network bandwidth enable the high-content analysis service to meet not only current demand, but even greater demand in the future.

Sharing large scientific datasets with external collaborators using secure technologies had been challenging in the past. Scientific Computing provided a solution by setting up a new sFTP server in cooperation with MI's Core IT team and Central IT at The University of Manchester. The new service allows users to create temporary One-Time accounts for sharing large research datasets (up to 2TB) with external users, using secure connections. Upload tools were developed by the team to simplify the usage of the service.

Various new technologies and skillsets were introduced by the team. One of the most important is the creation and usage of Bioconda software containers, allowing us to run either Conda, Docker or Apptainer (formerly Singularity) containers on our Phoenix High-Throughput data analysis platform. It reduces the effort of installing and speeds up the deployment of new software, as different types of software containers can be deployed using the same build recipe. It also ensures reproducibility and maintainability of software by enforcing proper documentation and build standards.

In close cooperation with the Bioinformatics and Biostatistics team in the Cancer Biomarker Centre, Scientific Computing setup a cBioPortal platform for the Institute that allows easy access and visualisation of cancer genomics data produced here. cBioPortal is a web interface that allows the interactive exploration of multidimensional genomics data sets and is an effective tool to lower the barriers between genomics data analysis and researchers. It provides easy access to molecular profiles and clinical data from large-scale cancer genomics projects, and therefore enables researchers to translate these datasets into biological insights and clinical applications.

Significant work this year has gone into designing and equipping the state-of-the-art data centre that will sit in the new replacement building, which will house the Cancer Research UK Manchester Institute and will host the Institute's IT, High-Performance Computing, and storage hardware. The installation of redundant uninterruptible power supplies and cooling systems ensures the high availability of the system operated in the centre. Access control and camera surveillance ensure high security in the data centre. Modern water-cooled rear door heat exchangers allow efficient operation of the systems even at full capacity. The new data centre meets high standards, including those required by clinical research.

Visualisation, Irradiation & Analysis

Steve Bagley, Alex Baker, Jianhua Tang, Kang Zeng

The facility has responded to COVID-19 by enabling most of the equipment and processing software to be operated remotely. Training has been undertaken both in-person and remotely, whilst support has taken a blended approach. To the credit of the VIA team, over fifty researchers have received training on the instruments, with around the same number receiving training for software application. During the year, 12 talks (internal and external) have been given by facility staff.

Collaboration with other core facilities has played a key role this year. Working with the Histology facility to support high-plex CODEX imaging, and providing imaging support for both the Histology and Molecular Biology Facilities for spatial transcriptomics, has enabled the implementation of new technologies. New initiatives have been supported by the research groups, such as assisting in the development of imaging 'cleared tissues', mIHC and the application of multiple modalities to tissue imaging. Support from Scientific Computing has enabled the IT infrastructure for high content screening to be improved, which in turn enables the researchers to process complex 3D/4D screening data much faster.

This year the facility was involved with the Royal Society Summer Science Exhibition, which was held virtually for the first time to the public and schools across the UK. A team of staff and students from the Institute were responsible for digital activities and presentations on the complexity of the tumour microenvironment.

The coming year will see our techniques becoming formalised so they can be applied by other research groups; the expansion of instrument standardisation in line with QUAREP guidelines to aid research integrity; and the major task will be preparing for the relocation of the VIA laboratory to the new building on the Christie site.

CANCER RESEARCH UK MANCHESTER INSTITUTE

PUBLICATIONS AND ADMINISTRATION

RESEARCH PUBLICATIONS

Cancer Biomarker Centre

(page 16)
Caroline Dive

Refereed research publications

Lee RJ, Wysocki O, Bhogal T, Shotton R, Tivey A, Angelakas A, Aung T, Banfill K, Baxter M, Boyce H, Brearton G, Copson E, Dickens E, Eastlake L, Gomes F, Hague C, Harrison M, Horsley L, Huddar P, Hudson Z, Khan S, Khan UT, Maynard A, McKenzie H, Palmer D, Robinson T, Rowe M, Thomas A, Tweedy J, Sheehan R, Stockdale A, Weaver J, Williams S, Wilson C, Zhou C, Dive C, Cooksley T, Palmieri C, Freitas A, Armstrong AC. (2021)
Longitudinal characterisation of haematological and biochemical parameters in cancer patients prior to and during COVID-19 reveals features associated with outcome.
ESMO Open 6(1):100005.

Payapilly A, Guilbert R, Descamps T, White G, Magee P, Zhou C, Kerr A, Simpson KL, Blackhall F, Dive C, Malliri A. (2021)
TIAM1-RAC1 promote small-cell lung cancer cell survival through antagonizing Nur77-induced BCL2 conformational change.
Cell Reports 37(6):109979.

Foy V, Lindsay CR, Carmel A, Fernandez-Gutierrez F, Krebs MG, Priest L, Carter M, Groen HJM, Hiltermann TJN, de Luca A, Farace F, Besse B, Terstappen L, Rossi E, Morabito A, Perrone F, Renehan A, Faivre-Finn C, Normanno N, Dive C, Blackhall F, Michiels S. (2021)
EPAC-lung: European pooled analysis of the prognostic value of circulating tumour cells in small cell lung cancer.
Translational Lung Cancer Research 10(4):1653-1665.

Hall C, von Grabowiecki Y, Pearce SP, Dive C, Bagley S, Muller PAJ. (2021)
iRFP (near-infrared fluorescent protein) imaging of subcutaneous and deep tissue tumours in mice highlights differences between imaging platforms.
Cancer Cell International 21(1):247.

Mahmood RD, Shaw D, Descamps T, Zhou C, Morgan RD, Mullamitha S, Saunders M, Mescallado N, Backen A, Morris K, Little RA, Cheung S, Watson Y, O'Connor JPB, Jackson A, Parker GJM, Dive C, Jayson GC. (2021)
Effect of oxaliplatin plus 5-fluorouracil or capecitabine on circulating and imaging biomarkers in patients with metastatic colorectal cancer: a prospective biomarker study.
BMC Cancer 21(1):354.

Bentham R, Litchfield K, Watkins TBK, Lim EL, Rosenthal R, Martínez-Ruiz C, Hiley CT, Bakir MA, Salgado R, Moore DA, Jamal-Hanjani M; TRACERx Consortium, Swanton C, McGranahan N. (2021)
Using DNA sequencing data to quantify T cell fraction and therapy response.
Nature 597(7877):555-560.

Au L, Hatipoglu E, Robert de Massy M, Litchfield K, Beattie G, Rowan A, Schnidrig D, Thompson R, Byrne F, Horswell S, Fotiadis N, Hazell S, Nicol D, Shepherd STC, Fendler A, Mason R, Del Rosario L, Edmonds K, Lingard K, Sarker S, Mangwende M, Carlyle E, Attig J, Joshi K, Uddin I, Becker PD, Sunderland MW, Akarca A, Puccio I, Yang WW, Lund T, Dhillon K, Vasquez MD, Ghorani E, Xu H, Spencer C, López JI, Green A, Mahadeva U, Borg E, Mitchison M, Moore DA, Proctor I, Falzon M, Pickering L, Furness AJS, Reading JL, Salgado R, Marafioti T, Jamal-Hanjani M; PEACE Consortium, Kassiotis G, Chain B, Larkin J, Swanton C, Quezada SA, Turajlic S; TRACERx Renal Consortium. (2021)
Determinants of anti-PD-1 response and resistance in clear cell renal cell carcinoma.
Cancer Cell 39(11):1497-1518.e11

Schenk MW, Humphrey S, Hossain ASMM, Revill M, Pearsall S, Lallo A, Brown S, Bratt S, Galvin M, Descamps T, Zhou C, Pearce SP, Priest L, Greenhalgh M, Chaturvedi A, Kerr A, Blackhall F, Dive C, Frese KK. (2021)
Soluble guanylate cyclase signalling mediates etoposide resistance in progressing small cell lung cancer.
Nature Communications 12(1):6652.

Cani AK, Dolce EM, Darga EP, Hu K, Liu CJ, Pierce J, Bradbury K, Kilgour E, Aung K, Schiavon G, Carroll D, Carr TH, Klinowska T, Lindemann J, Marshall G, Rowlands V, Harrington EA, Barrett JC, Sathiyayogan N, Morrow C, Sero V, Armstrong AC, Baird R, Hamilton E, Im SA, Jhaveri K, Patel MR, Dive C, Tomlins SA, Udager AM, Hayes DF, Paoletti C.
Serial monitoring of genomic alterations in circulating tumor cells of ER-positive/HER2-negative advanced breast cancer: feasibility of precision oncology biomarker detection.
Molecular Oncology [Epub 6 December 2021]

Burke H, Freeman A, Dushianthan A, Celinski M, Batchelor J, Phan H, Borca F, Kipps C, Thomas GJ, Faust SN, Sheard N, Williams S, Fitzpatrick P, Landers D, Wilkinson T. (2021)
Research Evaluation Alongside Clinical Treatment in COVID-19 (REACT COVID-19): an observational and biobanking study.
BMJ Open 11(1):e043012.

Royle JK, Hughes A, Stephenson L, Landers D.
Technology clinical trials: Turning innovation into patient benefit.
Digital Health [Epub 30 April 2021]

Powles T, Carroll D, Chowdhury S, Gravis G, Joly F, Carles J, Fléchon A, Maroto P, Petrylak D, Rolland F, Cook N, Balar AV, Sridhar SS, Galsky MD, Grivas P, Ravaud A, Jones R, Cosaert J, Hodgson D, Kozarewa I, Mather R, McEwen R, Mercier F, Landers D. (2021)
An adaptive, biomarker-directed platform study of durvalumab in combination with targeted therapies in advanced urothelial cancer.
Nature Medicine 27(5):793-801.

Freeman A, Watson A, O'Regan P, Wysocki O, Burke H, Freitas A, Livingstone R, Dushianthan A, Celinski M, Batchelor J, Phan H, Borca F, Fitzpatrick P, Landers D, Wilkinson TM. (2021)
Wave comparisons of clinical characteristics and outcomes of COVID-19 admissions - Exploring the impact of treatment and strain dynamics.
Journal of Clinical Virology 146:105031.

Other publications

Hynds RE, Frese KK, Pearce DR, Grönroos E, Dive C, Swanton C. (2021)
Progress towards non-small-cell lung cancer models that represent clinical evolutionary trajectories.
Open Biology 11(1):200247.

Chemi F, Mohan S, Guevara T, Clipson A, Rothwell DG, Dive C. (2021)
Early Dissemination of Circulating Tumor Cells: Biological and Clinical Insights.
Frontiers in Oncology 11:672195.

Frese KK, Simpson KL, Dive C. (2021)
Small cell lung cancer enters the era of precision medicine.
Cancer Cell 39(3):297-299.

Rolfo C, Mack P, Scagliotti GV, Aggarwal C, Arcila ME, Barlesi F, Bivona T, Diehn M, Dive C, Dziadziuszko R, Leighl N, Malapelle U, Mok T, Peled N, Raez LE, Sequist L, Sholl L, Swanton C, Abbosh C, Tan D, Wakelee H, Wistuba I, Bunn R, Freeman-Daily J, Wynes M, Belani C, Mitsudomi T, Gandara D. (2021)
Liquid Biopsy for Advanced NSCLC: A Consensus Statement From the International Association for the Study of Lung Cancer.
Journal of Thoracic Oncology 16(10):1647-1662.

Wheatley RC, Kilgour E, Jacobs T, Lamarca A, Hubner RA, Valle JW, McNamara MG.
Potential influence of the microbiome environment in patients with biliary tract cancer and implications for therapy.
British Journal of Cancer [Epub 18 October 2021]

Cancer Inflammation and Immunity

(page 20)
Santiago Zelenay

Refereed research publications

Canton J, Blees H, Henry CM, Buck MD, Schulz O, Rogers NC, Childs E, Zelenay S, Rhys H, Domart MC, Collinson L, Alloatti A, Ellison CJ, Amigorena S, Papayannopoulos V, Thomas DC, Randow F, Reis E Sousa C. (2021)
The receptor DNGR-1 signals for phagosomal rupture to promote cross-presentation of dead-cell-associated antigens.
Nature Immunology 22(2):140-153.

Giampazolias E, Schulz O, Lim KHJ, Rogers NC, Chakravarty P, Srinivasan N, Gordon O, Cardoso A, Buck MD, Poirier EZ, Canton J, Zelenay S, Sammicheli S, Moncaut N, Varsani-Brown S, Rosewell I, Reis E Sousa C. (2021)
Secreted gelsolin inhibits DNGR-1-dependent cross-presentation and cancer immunity.
Cell 184(15):4016-4031.e22.

Hutton C, Heider F, Blanco-Gomez A, Banyard A, Kononov A, Zhang X, Karim S, Paulus-Hock V, Watt D, Steele N, Kemp S, Hogg EKJ, Kelly J, Jackstadt RF, Lopes F, Menotti M, Chisholm L, Lamarca A, Valle J,

Sansom OJ, Springer C, Malliri A, Marais R, Pasca di Magliano M, Zelenay S, Morton JP, Jørgensen C. (2021)
Single-cell analysis defines a pancreatic fibroblast lineage that supports anti-tumor immunity.
Cancer Cell 39(9):1227-1244.e20.

Cueto FJ, Del Fresno C, Brandi P, Combes AJ, Hernández-García E, Sánchez-Paulete AR, Enamorado M, Bromley CP, Gomez MJ, Conde-Garrosa R, Mañes S, Zelenay S, Melero I, Iborra S, Krummel MF, Sancho D. (2021)
DNGR-1 limits Flt3L-mediated antitumor immunity by restraining tumor-infiltrating type I conventional dendritic cells.
Journal for Immunotherapy of Cancer 9(5):e002054.

Pelly VS, Moeini A, Roelofsen LM, Bonavita E, Bell CR, Hutton C, Blanco-Gomez A, Banyard A, Bromley CP, Flanagan E, Chiang SC, Jørgensen C, Schumacher TN, Thommen DS, Zelenay S. (2021)
Anti-Inflammatory Drugs Remodel the Tumor Immune Environment to Enhance Immune Checkpoint Blockade Efficacy.
Cancer Discovery 11(10):2602-2619.

Cell Division
(page 22)
Iain Hagan

Refereed research publications
Halova L, Cobley D, Franz-Wachtel M, Wang T, Morrison KR, Krug K, Nalpas N, Maček B, Hagan IM, Humphrey SJ, Petersen J. (2021)
A TOR (target of rapamycin) and nutritional phosphoproteome of fission yeast reveals novel targets in networks conserved in humans.
Open Biology 11(4):200405.

Other publications
Singh P, Halova L, Hagan IM. (2021)
Highly Synchronous Mitotic Progression in Schizosaccharomyces pombe Upon Relief of Transient Cdc2-asM17 Inhibition.
Methods in Molecular Biology 2329:123-142.

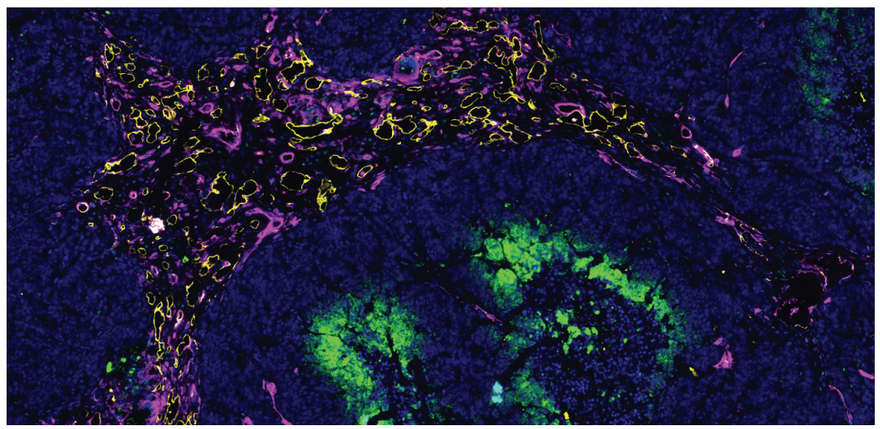
Cell Plasticity & Epigenetics
(page 24)
Maximiliano Portal

Other publications
Moran KL, Shlyakhtina Y, Portal MM. (2021)
The role of non-genetic information in evolutionary frameworks.
Critical Reviews in Biochemistry and Molecular Biology 56(3):255-283.

Shlyakhtina Y, Moran KL, Portal MM. (2021)
Genetic and Non-Genetic Mechanisms Underlying Cancer Evolution.
Cancers (Basel) 13(6):1380.

Cell Signalling
(page 26)
Angeliki Malliri

Refereed research publications
Pickering KA, Gilroy K, Cassidy JW, Fey SK, Najumudeen AK, Zeiger LB, Vincent DF, Gay DM, Johansson J, Fordham RP, Miller B, Clark W, Hedley A, Unal EB, Kiel C, McGhee E,



Representative immunofluorescence image of murine endothelial vessels (mCD31+, yellow), perfused blood vessels marked by intravenous (i.v) tomato lectin (pink), hypoxic tumour regions marked by pimonidazole (pimo, green) and DAPI (blue) in a CDX tumour with i.v tomato lectin and i.p pimo injection.

Image supplied by Sarah Pearsall (former PhD student and Scientific Officer in the Cancer Biomarker Centre)

Machesky LM, Nixon C, Johnsson AE, Bain M, Strathdee D, van Hoof SR, Medema JP, Anderson KI, Brachmann SM, Stucke VM, Malliri A, Drysdale M, Turner M, Serrano L, Myant K, Campbell AD, Sansom OJ. (2021)
A RAC-GEF network critical for early intestinal tumourigenesis.
Nature Communications 12(1):56.

Hutton C, Heider F, Blanco-Gomez A, Banyard A, Kononov A, Zhang X, Karim S, Paulus-Hock V, Watt D, Steele N, Kemp S, Hogg EKJ, Kelly J, Jackstadt RF, Lopes F, Menotti M, Chisholm L, Lamarca A, Valle J, Sansom OJ, Springer C, Malliri A, Marais R, Pasca di Magliano M, Zelenay S, Morton JP, Jørgensen C. (2021)
Single-cell analysis defines a pancreatic fibroblast lineage that supports anti-tumor immunity.
Cancer Cell 39(9):1227-1244.e20.

Budden T, Gaudy-Marqueste C, Porter A, Kay E, Gurung S, Earnshaw CH, Roeck K, Craig S, Traves V, Krutmann J, Muller P, Motta L, Zanivan S, Malliri A, Furney SJ, Nagore E, Virós A. (2021)
Ultraviolet light-induced collagen degradation inhibits melanoma invasion.
Nature Communications 12(1):2742.

Porter AP, Reed H, White GRM, Ogg EL, Whalley HJ, Malliri A. (2021)
The RAC1 activator Tiam1 regulates centriole duplication through controlling PLK4 levels.
Journal of Cell Science 134(7):jcs252502.

Payapilly A, Guilbert R, Descamps T, White G, Magee P, Zhou C, Kerr A, Simpson KL, Blackhall F, Dive C, Malliri A. (2021)
TIAM1-RAC1 promote small-cell lung cancer cell survival through antagonizing Nur77-induced BCL2 conformational change.
Cell Reports 37(6):109979.

Drug Discovery
(page 28)
Caroline Springer

Refereed research publications
Hutton C, Heider F, Blanco-Gomez A, Banyard A, Kononov A, Zhang X, Karim S, Paulus-Hock V, Watt D, Steele N, Kemp S, Hogg EKJ, Kelly J, Jackstadt RF, Lopes F, Menotti M, Chisholm L, Lamarca A, Valle J, Sansom OJ, Springer C, Malliri A, Marais R, Pasca di Magliano M, Zelenay S, Morton JP, Jørgensen C. (2021)
Single-cell analysis defines a pancreatic fibroblast lineage that supports anti-tumor immunity.
Cancer Cell 39(9):1227-1244.e20.

Brown M, Aljarah M, Asiki H, Leung LMH, Smithen DA, Miller N, Nemeth G, Davies L, Niculescu-Duvaz D, Zambon A, Springer C. (2021)
Toward the Scale-Up of a Bicyclic Homopiperazine via Schmidt Rearrangement and Photochemical Oxaziridine Rearrangement in Continuous-Flow.
Organic Process Research & Development 25(1):148-156.

Leukaemia Biology
(page 32)
Tim Somervaille

Refereed research publications
D'Souza N, Somervaille TCP, Mowatt DJ. (2021)
Chronic myeloid leukemia presenting as compartment syndrome with acute loss of upper limb function.
American Journal of Emergency Medicine 49:273-275.

Salisbury RA, Curto-Garcia N, O'Sullivan J, Chen F, Polzella P, Godfrey AL, Russell J, Knapper S, Willan J, Frewin R, Joshi S, Arami S, Burns S, Teh CH, Wadelin F, Dhanapal J, Neelakantan P, Milojkovic D, Psaila B, Szydlo R, Francis S, Cargo C, Jain M, McGregor A, Wallis L, Duncombe A, Hussein H, Dyer P, Munro L, Bond L, McMullin MF, Somervaille TCP, Garg M, Sekhar M, Harrison C, Mead AJ, Innes AJ. (2021)
Results of a national UK physician reported survey of COVID-19 infection in patients with a myeloproliferative neoplasm.
Leukemia 35(8):2424-2430.

Batta K, Bossenbroek HM, Pemmaraju N, Wilks DP, Chasty R, Dennis M, Milne P, Collin M, Beird HC, Taylor J, Patnaik MM, Cargo CA, Somervaille TCP, Wiseman DH. (2021)
Divergent clonal evolution of blastic plasmacytoid dendritic cell neoplasm and chronic myelomonocytic leukemia from a shared TET2-mutated origin.
Leukemia 35(11):3299-3303.

Williams MS, Basma NJ, Amaral FMR, Wiseman DH, Somervaille TCP. (2021)
Blast cells surviving acute myeloid leukemia induction therapy are in cycle with a signature of FOXM1 activity.
BMC Cancer 21(1):1153.

Simeoni F, Romero-Camarero I, Camera F, Amaral FMR, Sinclair OJ, Papachristou EK, Spencer GJ, Lie-A-Ling M, Lacaud G, Wiseman DH, Carroll JS, Somervaille TCP. (2021)
Enhancer recruitment of transcription

repressors RUNX1 and TLE3 by mis-expressed FOXC1 blocks differentiation in acute myeloid leukemia.
Cell Reports 36(12):109725.

Nicosia L, Boffo FL, Ceccacci E, Conforti F, Pallavicini I, Bedin F, Ravasio R, Massignani E, Somervaille TCP, Minucci S, Bonaldi T.
Pharmacological inhibition of LSD1 triggers myeloid differentiation by targeting GSE1 oncogenic functions in AML.
Oncogene [Epub 3 December 2021]

Molecular Oncology

(page 34)
Richard Marais

Refereed research publications

Mundra PA, Dhomen N, Rodrigues M, Mikkelsen LH, Cassoux N, Brooks K, Valpione S, Reis-Filho JS, Heegaard S, Stern MH, Roman-Roman S, Marais R. (2021)
Ultraviolet radiation drives mutations in a subset of mucosal melanomas.
Nature Communications 12(1):259.

Gaudy-Marqueste C, Macagno N, Loundou A, Pellegrino E, Ouafik L, Budden T, Mundra P, Gremel G, Akhras V, Lin L, Cook M, Kumar R, Grob JJ, Nagore E, Marais R, Virós A.
Molecular characterization of fast-growing melanomas.
Journal of the American Academy of ***Dermatology*** [Epub 16 July 2021]

Hutton C, Heider F, Blanco-Gomez A, Banyard A, Kononov A, Zhang X, Karim S, Paulus-Hock V, Watt D, Steele N, Kemp S, Hogg EKJ, Kelly J, Jackstadt RF, Lopes F, Menotti M, Chisholm L, Lamarca A, Valle J, Sansom OJ, Springer C, Malliri A, Marais R, Pasca di Magliano M, Zelenay S, Morton JP, Jørgensen C. (2021)
Single-cell analysis defines a pancreatic fibroblast lineage that supports anti-tumor immunity.
Cancer Cell 39(9):1227-1244.e20.

Valpione S, Mundra PA, Galvani E, Campana LG, Lorigan P, De Rosa F, Gupta A, Weightman J, Mills S, Dhomen N, Marais R. (2021)
The T cell receptor repertoire of tumor infiltrating T cells is predictive and prognostic for cancer survival.
Nature Communications 12(1):4098.

Gardrat S, Houy A, Brooks K, Cassoux N, Barnhill R, Dayot S, Bièche I, Raynal V, Baulande S, Marais R, Roman-Roman S, Stern MH, Rodrigues M. (2021)
Definition of Biologically Distinct Groups of Conjunctival Melanomas According to Etiological Factors and Implications for Precision Medicine.
Cancers (Basel) 13(15):3836.

Salih Z, Banyard A, Tweedy J, Galvani E, Middlehurst P, Mills S, Weightman J, Gupta A, Lorigan PC, Zhou C, Dhomen N, Valpione S, Marais R. (2021)
T cell immune awakening in response to immunotherapy is age-dependent.
European Journal of Cancer 162:11-21.

Au L, Hatipoglu E, Robert de Massy M, Litchfield K, Beattie G, Rowan A, Schnidrig D, Thompson R, Byrne F, Horswell S, Fotiadis N, Hazell S, Nicol D, Shepherd STC, Fendler A, Mason R, Del Rosario L, Edmonds K, Lingard K, Sarker S, Mangwende M, Carlyle E, Attig J, Joshi K, Uddin I, Becker PD, Sunderland MW, Akarca A, Puccio I, Yang WW, Lund T, Dhillon K, Vasquez MD, Ghorani E, Xu H, Spencer C, López JI, Green A, Mahadeva U, Borg E, Mitchison M, Moore DA, Proctor I, Falzon M, Pickering L, Furness AJS, Reading JL, Salgado R, Marafioti T, Jamal-Hanjani M; PEACE Consortium, Kassiotis G, Chain B, Larkin J, Swanton C, Quezada SA, Turajlic S; TRACERx Renal Consortium. (2021)
Determinants of anti-PD-1 response and resistance in clear cell renal cell carcinoma.
Cancer Cell 39(11):1497-1518.e11.

Other publications

Patton EE, Mueller KL, Adams DJ, Anandasabapathy N, Aplin AE, Bertolotto C, Bosenberg M, Ceol CJ, Burd CE, Chi P, Herlyn M, Holmen SL, Karreth FA, Kaufman CK, Khan S, Kobold S, Leucci E, Levy C, Lombard DB, Lund AW, Marie KL, Marine JC, Marais R, McMahon M, Robles-Espinoza CD, Ronai ZA, Samuels Y, Soengas MS, Villanueva J, Weeraratna AT, White RM, Yeh I, Zhu J, Zon LI, Hurlbert MS, Merlino G. (2021)
Melanoma models for the next generation of therapies.
Cancer Cell 39(5):610-631.

Dhomen N, Mundra PA, Marais R. (2021)
Sunglasses to hide behind may also prevent melanoma of the eyes.
British Journal of Cancer 125(4):470-472.

Prostate Oncobiology

(page 36)
Esther Baena

Other publications

Ali A, Du Feu A, Oliveira P, Choudhury A, Bristow RG, Baena E.
Prostate zones and cancer: lost in transition?
Nature Reviews Urology [Epub 19 October 2021]

Flores-Téllez TDNJ, Baena E.
Experimental challenges to modeling prostate cancer heterogeneity.
Cancer Letters [Epub 21 October 2021]

Finch AJ, Baena E. (2021)
Spatiofunctional Dynamics of NKX3.1 to Safeguard the Prostate from Cancer.
Cancer Discovery 11(9):2132-2134.

Skin Cancer and Ageing

(page 38)
Amaya Virós

Refereed research publications

Gaudy-Marqueste C, Macagno N, Loundou A, Pellegrino E, Ouafik L, Budden T, Mundra P, Gremel G, Akhras V, Lin L, Cook M, Kumar R, Grob JJ, Nagore E, Marais R, Virós A.
Molecular characterization of fast-growing melanomas.
Journal of the American Academy of Dermatology [Epub 16 July 2021]

Millán-Esteban D, Peña-Chilet M, García-Casado Z, Manrique-Silva E, Requena C, Bañuls J, López-Guerrero JA, Rodríguez-Hernández A, Traves V, Dopazo J, Virós A, Kumar R, Nagore E. (2021)
Mutational Characterization of Cutaneous Melanoma Supports Divergent Pathways Model for Melanoma Development.
Cancers (Basel) 13(20):5219.

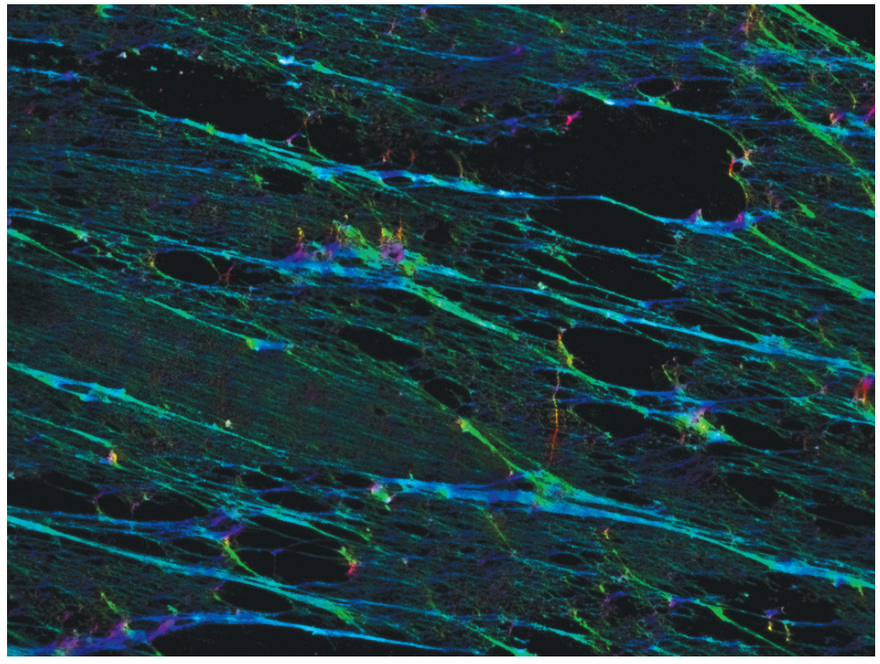
Budden T, Gaudy-Marqueste C, Porter A, Kay E, Gurung S, Earnshaw CH, Roeck K, Craig S, Traves V, Krutmann J, Muller P, Motta L, Zanivan S, Malliri A, Furney SJ, Nagore E, Virós A. (2021)
Ultraviolet light-induced collagen degradation inhibits melanoma invasion.
Nature Communications 12(1):2742.

Millán-Esteban D, García-Casado Z, Manrique-Silva E, Virós A, Kumar R, Furney S, López-Guerrero JA, Requena C, Bañuls J, Traves V, Nagore E. (2021)
Distribution and clinical role of KIT gene mutations in melanoma according to subtype: a study of 492 Spanish patients.
European Journal of Dermatology 31(6):830-838.

Budden T, Gaudy-Marqueste C, Craig S, Hu Y, Earnshaw CH, Gurung S, Ra A, Akhras V, Shenjere P, Green R, Jamieson L, Lear J, Motta L, Caulín C, Oudit D, Furney SJ, Virós A. (2021)
Female Immunity Protects from Cutaneous Squamous Cell Carcinoma.
Clinical Cancer Research 27(11):3215-3223.

Inhibition of collagen-cleaving matrix metalloprotein-1 (MMP1) restores melanoma invasion. Immunofluorescence of fibronectin fibres without UV radiation.

Image supplied by Tim Budden and Amaya Viros (Skin Cancer and Ageing)



Other publications

Nagore E, Virós A, Kumar R.
Positive Attributes of Anti-TERT CD4 T-Helper Type 1 Immune Responses in Melanoma.
Journal of Investigative Dermatology [Epub 16 October 2021]

Stem Cell Biology

(page 40)
Georges Lacaud

Refereed research publications

Menegatti S, Potts B, Garcia-Alegria E, Paredes R, Lie-A-Ling M, Lacaud G, Kouskoff V.
The RUNX1b Isoform Defines Hemogenic Competency in Developing Human Endothelial Cells.
Frontiers in Cell & Developmental Biology [Epub 16 December 2021]

Fadlullah MZ, Neo WH, Lie-A-Ling M, Thambyrajah R, Patel R, Mevel R, Aksoy I, Do Khoa N, Savatier P, Fontenille L, Baker SM, Rattray M, Kouskoff V, Lacaud G.
Murine AGM single-cell profiling identifies a continuum of hemogenic endothelium differentiation marked by ACE.
Blood [Epub 13 September 2021]

Villa F, Fujisawa R, Ainsworth J, Nishimura K, Lie-A-Ling M, Lacaud G, Labib KP. (2021)
CUL2LRR1, TRAIP and p97 control CMG helicase disassembly in the mammalian cell cycle.
EMBO Reports 22(3):e52164.

Jakobczyk H, Debaize L, Soubise B, Avner S, Rouger-Gaudichon J, Commet S, Jiang Y, Sérandour AA, Rio AG, Carroll JS, Wichmann C, Lie-A-Ling M, Lacaud G, Corcos L, Salbert G, Galibert MD, Gandemer V, Troadec MB. (2021)
Reduction of RUNX1 transcription factor activity by a CBFA2T3-mimicking peptide: application to B cell precursor acute lymphoblastic leukemia.
Journal of Hematology & Oncology 14(1):47.

Simeoni F, Romero-Camarero I, Camera F, Amaral FMR, Sinclair OJ, Papachristou EK, Spencer GJ, Lie-A-Ling M, Lacaud G, Wiseman DH, Carroll JS, Somervaille TCP. (2021)
Enhancer recruitment of transcription repressors RUNX1 and TLE3 by mis-expressed FOXC1 blocks differentiation in acute myeloid leukemia.
Cell Reports 36(12):109725.

Neo WH, Meng Y, Rodriguez-Meira A, Fadlullah MZH, Booth CAG, Azzoni E, Thongjuea S, de Bruijn MFTR, Jacobsen SEW, Mead AJ, Lacaud G. (2021)
Ezh2 is essential for the generation of functional yolk sac derived erythro-myeloid progenitors.
Nature Communications 12(1):7019.

Other publications

Neo WH, Lie-A-Ling M, Fadlullah MZH, Lacaud G. (2021)
Contributions of Embryonic HSC-Independent Hematopoiesis to Organogenesis and the Adult Hematopoietic System.
Frontiers in Cell & Developmental Biology 9:631699.

Jaffredo T, Balduini A, Bigas A, Bernardi R, Bonnet D, Canque B, Charbord P, Cumano A, Delwel R, Durand C, Fibbe W, Forrester L, de Franceschi L, Ghevaert C, Gjertsen B, Gottgens B, Graf T, Heidenreich O, Hermine O, Higgs D, Kleanthous M, Klump H, Kouskoff V, Krause D, Lacaud G, Celso CL, Martens JHA, Méndez-Ferrer S, Menendez P, Oostendorp R, Philipsen S, Porse B, Raaijmakers M, Robin C, Stunnenberg H, Theilgaard-Mönch K, Touw I, Vainchenker W, Corrons JV, Yvernogeau L, Schuringa JJ. (2021)
The EHA Research Roadmap: Normal Hematopoiesis.
Hemasphere 5(12):e669.

Systems Oncology

(page 42)
Claus Jørgensen

Refereed research publications

Lee BY, Hogg EKJ, Below CR, Kononov A, Blanco-Gomez A, Heider F, Xu J, Hutton C, Zhang X, Scheidt T, Beattie K, Lamarca A, McNamara M, Valle JW, Jørgensen C. (2021)
Heterocellular OSM-OSMR signalling reprograms fibroblasts to promote pancreatic cancer growth and metastasis.
Nature Communications 12(1):7336.

Below CR, Kelly J, Brown A, Humphries JD, Hutton C, Xu J, Lee BY, Cintas C, Zhang X, Hernandez-Gordillo V, Stockdale L, Goldsworthy MA, Geraghty J, Foster L, O'Reilly DA, Schedding B, Askari J, Burns J, Hodson N, Smith DL, Lally C, Ashton G, Knight D, Mironov A, Banyard A, Eble JA, Morton JP, Humphries MJ, Griffith LG, Jørgensen C.
A microenvironment-inspired synthetic three-dimensional model for pancreatic ductal adenocarcinoma organoids.
Nature Materials [Epub 13 September 2021]

Hutton C, Heider F, Blanco-Gomez A, Banyard A, Kononov A, Zhang X, Karim S, Paulus-Hock V, Watt D, Steele N, Kemp S, Hogg EKJ, Kelly J, Jackstadt RF, Lopes F, Menotti M, Chisholm L, Lamarca A, Valle J, Sansom OJ, Springer C, Malliri A, Marais R, Pasca di Magliano M, Zelenay S, Morton JP, Jørgensen C. (2021)
Single-cell analysis defines a pancreatic fibroblast lineage that supports anti-tumor immunity.
Cancer Cell 39(9):1227-1244.e20.

Pelly VS, Moeini A, Roelofsen LM, Bonavita E, Bell CR, Hutton C, Blanco-Gomez A, Banyard A, Bromley CP, Flanagan E, Chiang SC, Jørgensen C, Schumacher TN, Thommen DS, Zelenay S. (2021)
Anti-Inflammatory Drugs Remodel the Tumor Immune Environment to Enhance Immune Checkpoint Blockade Efficacy.
Cancer Discovery 11(10):2602-2619.

Gough RE, Jones MC, Zacharchenko T, Le S, Yu M, Jacquemet G, Muench SP, Yan J, Humphries JD, Jørgensen C, Humphries MJ, Goult BT. (2021)
Talin mechanosensitivity is modulated by a direct interaction with cyclin-dependent kinase-1.
Journal of Biological Chemistry 297(1):100837.

Translational Oncogenomics

(page 46)
Rob Bristow

Refereed research publications

Bibby BAS, Thiruthaneeswaran N, Yang L, Pereira RR, More E, McArt DG, O'Reilly P, Bristow RG, Williams KJ, Choudhury A, West CML. (2021)
Repurposing FDA approved drugs as radiosensitizers for treating hypoxic prostate cancer.
BMC Urology 21(1):96.

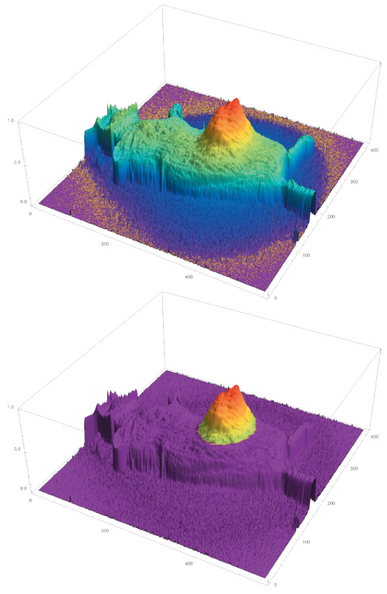
Other publications

Rebello RJ, Oing C, Knudsen KE, Loeb S, Johnson DC, Reiter RE, Gillessen S, Van der Kwast T, Bristow RG. (2021)
Prostate cancer.
Nature Reviews Disease Primers 7(1):9.

Ali A, Du Feu A, Oliveira P, Choudhury A, Bristow RG, Baena E.
Prostate zones and cancer: lost in transition?
Nature Reviews Urology [Epub 19 October 2021]

New software – MouseTensity – quantifies fluorescent images of subcutaneous tumours in mice.
By displaying the intensity of the whole mouse in 3D (top), the overall intensity (flat and homogenous over the whole mouse) can clearly be seen compared to the intensity of the tumour (appearing as a mountain). Using a sliding scale, the 'gate' can be set and the intensity of the mountain calculated (bottom).
Cancer Cell Int 21, 247 (2021).

Image supplied by Simon Pearce (Cancer Biomarker Centre) and Callum Hall (Tumour Suppressors)



Tumour Supressors

(page 48)
Patricia Muller

Refereed research publications

Budden T, Gaudy-Marqueste C, Porter A, Kay E, Gurung S, Earnshaw CH, Roeck K, Craig S, Traves V, Krutmann J, Muller P, Motta L, Zanivan S, Malliri A, Furney SJ, Nagore E, Virós A. (2021)
Ultraviolet light-induced collagen degradation inhibits melanoma invasion.
Nature Communications 12(1):2742.

Hall C, von Grabowiecki Y, Pearce SP, Dive C, Bagley S, Muller PAJ. (2021)
iRFP (near-infrared fluorescent protein) imaging of subcutaneous and deep tissue tumours in mice highlights differences between imaging platforms.
Cancer Cell International 21(1):247.

Alhebshi H, Tian K, Patnaik L, Taylor R, Bezecny P, Hall C, Muller PAJ, Safari N, Creamer DPM, Demonacos C, Mutti L, Bittar MN, Krstic-Demonacos M. (2021)
Evaluation of the Role of p53 Tumour Suppressor Posttranslational Modifications and TTC5 Cofactor in Lung Cancer.
International Journal of Molecular Sciences 22(24):13198.

Phatak V, von Grabowiecki Y, Janus J, Officer L, Behan C, Aschauer L, Pinon L, Mackay H, Zanivan S, Norman JC, Kelly M, Le Quesne J, Muller PAJ. (2021)
Mutant p53 promotes RCP-dependent chemoresistance coinciding with increased delivery of P-glycoprotein to the plasma membrane.
Cell Death & Disease 12(2):207.

von Grabowiecki Y, Phatak V, Aschauer L, Muller PAJ. (2021)
Rab11-FIP1/RCP functions as a major signalling hub in the oncogenic roles of mutant p53 in cancer.
Frontiers in Oncology 11:804107.

Select additional publications

La Montagna M, Shi L, Magee P, Sahoo S, Fassan M, Garofalo M. (2021)
AMPKα loss promotes KRAS-mediated lung tumorigenesis.
Cell Death & Differentiation 28(9):2673-2689.

Monteverde T, Sahoo S, La Montagna M, Magee P, Shi L, Lee D, Sellers R, Baker AR, Leong HS, Fassan M, Garofalo M. (2021)
CKAP2L Promotes Non-Small Cell Lung Cancer Progression through Regulation of Transcription Elongation.
Cancer Research 81(7):1719-1731.

Shi L, Magee P, Fassan M, Sahoo S, Leong HS, Lee D, Sellers R, Brullé-Soumaré L, Cairo S, Monteverde T, Volinia S, Smith DD, Di Leva G, Galuppini F, Paliouras AR, Zeng K, O'Keefe R, Garofalo M. (2021)
A KRAS-responsive long non-coding RNA controls microRNA processing.
Nature Communications 12(1):2038.

Boehm U, Nelson G, Brown CM, Bagley S, Bajcsy P, Bischof J, Dauphin A, Dobbie IM, Eriksson JE, Faklaris O, Fernandez-Rodriguez J, Ferrand A, Gelman L, Gheisari A, Hartmann H, Kukat C, Laude A, Mitkovski M, Munck S, North AJ, Rasse TM, Resch-Genger U, Schuetz LC, Seitz A, Strambio-De-Castillia C, Swedlow JR, Nitschke R. (2021)
QUAREP-LiMi: a community endeavor to advance quality assessment and reproducibility in light microscopy.
Nature Methods 18(12):1423-1426.

Nelson G, Boehm U, Bagley S, Bajcsy P, Bischof J, Brown CM, Dauphin A, Dobbie IM, Eriksson JE, Faklaris O, Fernandez-Rodriguez J, Ferrand A, Gelman L, Gheisari A, Hartmann H, Kukat C, Laude A, Mitkovski M, Munck S, North AJ, Rasse TM, Resch-Genger U, Schuetz LC, Seitz A, Strambio-De-Castillia C, Swedlow JR, Alexopoulos I, Aumayr K, Avilov S, Bakker GJ, Bammann RR, Bassi A, Beckert H, Beer S, Belyaev Y, Bierwagen J, Birngruber KA, Bosch M, Breitlow J, Cameron LA, Chalfoun J, Chambers JJ, Chen CL, Conde-Sousa E, Corbett AD, Cordelieres FP, Nery ED, Dietzel R, Eismann F, Fazeli E, Felscher A, Fried H, Gaudreault N, Goh WI, Guilbert T, Hadleigh R, Hemmerich P, Holst GA, Itano MS, Jaffe CB, Jambor HK, Jarvis SC, Keppler A, Kirchenbuechler D, Kirchner M, Kobayashi N, Krens G, Kunis S, Lacoste J, Marcello M, Martins GG, Metcalf DJ, Mitchell CA, Moore J, Mueller T, Nelson MS, Ogg S, Onami S, Palmer AL, Paul-Gilloteaux P, Pimentel JA, Plantard L, Podder S, Rexhepaj E, Royon A, Saari MA, Schapman D, Schoonderwoert V, Schroth-Diez B, Schwartz S, Shaw M, Spitaler M, Stoeckl MT, Sudar D, Teillon J, Terjung S, Thuenauer R, Wilms CD, Wright GD, Nitschke R. (2021)
QUAREP-LiMi: A community-driven initiative to establish guidelines for quality assessment and reproducibility for instruments and images in light microscopy.
Journal of Microscopy 284(1):56-73.

Naqvi A, MacKintosh ML, Derbyshire AE, Tsakiroglou AM, Walker TDJ, McVey RJ, Bolton J, Fergie M, Bagley S, Ashton G, Pemberton PW, Syed AA, Ammori BJ, Byers R, Crosbie EJ.
The impact of obesity and bariatric surgery on the immune microenvironment of the endometrium.
International Journal of Obesity (Lond) [Epub 2 December 2021]

Moncaut N, Hart-Johnson S. (2021)
The impact of COVID-19 lockdowns on the genetic integrity of your mouse colonies.
Lab Animal 50, 301–302.

Pappalardi MB, Keenan K, Cockerill M, Kellner WA, Stowell A, Sherk C, Wong K, Pathuri S, Briand J, Steidel M, Chapman P, Groy A, Wiseman AK, McHugh CF, Campobasso N, Graves AP, Fairweather E, Werner T, Raoof A, Butlin RJ, Rueda L, Horton JR, Fosbenner DT, Zhang C, Handler JL, Muliaditan M, Mebrahtu M, Jaworski JP, McNulty DE, Burt C, Eberl HC, Taylor AN, Ho T, Merrihew S, Foley SW, Rutkowska A, Li M, Romeril SP, Goldberg K, Zhang X, Kershaw CS, Bantscheff M, Jurewicz AJ, Minthorn E, Grandi P, Patel M, Benowitz AB, Mohammad HP, Gilmartin AG, Prinjha RK, Ogilvie D, Carpenter C, Heerding D, Baylin SB, Jones PA, Cheng X, King BW, Luengo JI, Jordan AM, Waddell I, Kruger RG, McCabe MT. (2021)
Discovery of a first-in-class reversible DNMT1-selective inhibitor with improved tolerability and efficacy in acute myeloid leukemia.
Nature Cancer 2(10):1002-1017.

Kitagawa A, Jacob C, Jordan A, Waddell I, McMurtry IF, Gupte SA. (2021)
Inhibition of glucose-6-phosphate dehydrogenase activity attenuates right ventricle pressure and hypertrophy elicited by VEGFR inhibitor + hypoxia.
Journal of Pharmacology and Experimental Therapeutics 377(2):284-292.

Khan MT, Irlam-Jones JJ, Pereira RR, Lane B, Valentine HR, Aragaki K, Dyrskjøt L, McConkey DJ, Hoskin PJ, Choudhury A, West CML. (2021)
A miRNA signature predicts benefit from addition of hypoxia-modifying therapy to radiation treatment in invasive bladder cancer.
British Journal of Cancer 125(1):85-93.

Khan MT, Yang L, More E, Irlam-Jones JJ, Valentine HR, Hoskin P, Choudhury A, West CML. (2021) Developing Tumor Radiosensitivity Signatures Using LncRNAs.
Radiation Research 195(4):324-333.

EXTERNAL SEMINAR SPEAKERS 2021

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

Patrick Caswell
University of Manchester

Tobias Zech
University of Liverpool

Christian Frezza
MRC Cancer Unit, University of Cambridge

Helfrid Hochegger
University of Sussex

Jordan Raff
Sir William Dunn School of Pathology, University of Oxford

Michael Speicher
Medical University of Graz, Austria

David Pellman
Blavatnik Institute, Harvard Medical School

Gunnel Halldén
Cancer Research UK Barts Centre

Seth Coffelt
University of Glasgow

Steven Pollard
University of Edinburgh

Daniela Thommen
Netherlands Cancer Institute

Ian Hardcastle
Newcastle University

Eric Miska
The Gurdon Institute

Constanze Bonifer
Institute of Cancer and Genomic Sciences

Anna Obenauf
Research Institute of Molecular Pathology

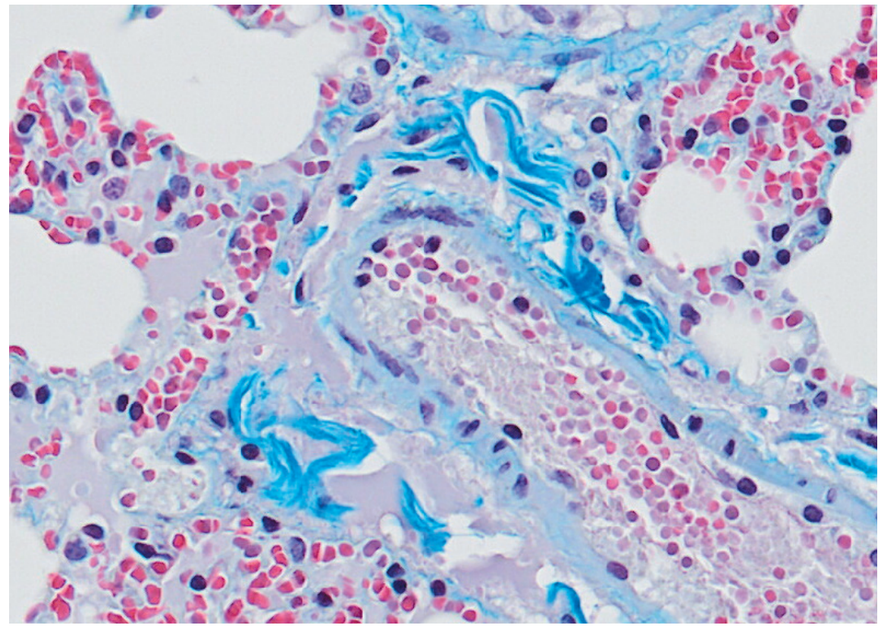
Mark Dawson
Peter MacCallum Cancer Centre, Australia

Vicky Sanz-Moreno
Cancer Research UK Barts Centre

Claire Eyers
Institute of Systems, Molecular and Integrative Biology

Histological section of lung tissue using Masson's Trichrome staining to identify lung fibrosis. Fibrosis is characterised by excessive formation of fibrous connective tissue, of which collagen is the main component. MT staining of increased collagen accumulation in tissue is an accurate indicator of fibrosis. Light blue – collagen; dark blue – nuclei; and red – muscle, erythrocytes and cytoplasm.

Image supplied by Leo Man Ho Leung (Drug Discovery Unit)



Ashani Weeraratna
John Hopkins Bloomberg School of Public Health

Denes Hnisz
Max Planck Institute for Molecular Genetics

Channing Der
UNC School of Medicine

Muzlifah Haniffa
University of Newcastle

Adrian Hayday
The Francis Crick Institute

Christian Zierhut
The Institute of Cancer Research

Greg Wang
UNC School of Medicine

Rosalind Eeles
The Institute of Cancer Research

Stephen Elledge
Harvard Medical School

OPERATIONS

Chief Operating Officer
Caroline Wilkinson

Chief Laboratory Officer
Stuart Pepper

Chief Finance Officer
Mike Berne

Chief Human Resources Officer
Rachel Powell

Our response to the ongoing COVID-19 pandemic continued to dominate much of 2021. As case rates remained high in the northwest for much of the year, we continued to adopt various restrictions to ensure the safety of the Institute's staff and students. These arrangements remained under the purview of the Institute's Emergency Response and Business Continuity Committee. A Hybrid working pilot scheme was introduced for certain teams as we emerged from the latest work from home requirements, and we look forward to reviewing the results in early 2023.

In the summer we said farewell to Steve Morgan who retired from his post as the Institute's receptionist. Steve had been at the Institute for 12 years and was a friendly and welcoming presence on the front desk. After the Paterson Building fire, he manned reception at the Oglesby Cancer Research Building where he continued to support the local cancer research community. He will be greatly missed, and we wish him all the best for an enjoyable and well-deserved retirement. Belen Conti took maternity leave in the autumn with Karen Lee joining to provide support to the Senior Management Team. Another new arrival was Andrew Porter who took on the newly created role of Research Integrity and Training Adviser. Andrew's remit is to support the scientific community in maintaining the highest standards of research integrity and to oversee additional training opportunities, particularly for our Early Career Researchers.

Other new initiatives include the development of an Equality, Diversity and Inclusion strategy, which we look forward to implementing over the coming year; the broadening of our Health and Safety Committee to include well-being with an associated focus group to feed into the discussions specifically on this topic; and an initiative to send STEM ambassadors into local schools to engage with students regarding careers in science. For this latter endeavour, we have chosen schools within range of the new research facility that is being built on the site of our old home, the Paterson Building, at the site of the Christie NHS Foundation Trust.

Many of the wider Operations team have been involved in the ongoing design work and discussions on operational arrangements for the new facility, which is set to be completed by the end of 2022, with our relocation due in the early part of 2023.

Institute Administration Team

Ruth Cox, Soraya Francis, Maria Belen Conti, Karen Lee[1]

[1]Maternity cover

Ruth Cox is Executive Assistant to the Institute Director. Belen Conti is Executive Assistant to the Senior Management Team and her role is being covered by Karen Lee during her maternity leave. Soraya Francis is our Administration Services Coordinator.

This year the Administration Team have ensured that the Institute remained connected, engaged and productive while our staff were working from home and socially distanced. Since government guidance permitted, they have facilitated the return to working back in offices. This has included implementing COVID-19-safe arrangements for day-to-day working, meetings and other activities, which has allowed the Institute to work and interact with each other in a secure environment.

They have organised many meetings, seminars and workshops in a virtual and hybrid format. The regular Director's updates publicise the Institute's good news and keep staff informed with regards to COVID-19 guidance. As a team they support the Director and the Institute Faculty day-to-day and have helped to organise the second virtual Institute Colloquium and a range of education and engagement events for staff and students throughout the year. We were delighted to be able to end the year with an outdoor Christmas party to reward staff for their hard work during a challenging year.

We have hosted a varied programme of national and international speakers in our External Seminar Series, almost all of which were conducted online, and are grateful to all our invited speakers for committing their time to give talks. Details can be found at cruk.manchester.ac.uk/seminars.

Finance and Purchasing

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

2021 led us into the second year of the COVID-19 pandemic, and while many restrictions had been lifted it still brought more challenges for the Institute Finance and Purchasing team to face. The continued impact of COVID-19 on research funding resulted in significant planning and forecasting being required to minimise the impact on the scientific output of the Institute. The UK's exit from the European Union also provided additional complications with a direct impact on funding and procurement and delivery.

Despite these challenges, the team continue to support the Institute Director and the management of the £23.8m 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 allow the maximum scientific output per pound. Despite global financial pressures, we have been successful in a number of new awards this year, with several million-pound flowing to the Institute in relation to research applications and agreements.

Over the course of the next year, the Institute will finalise the planning and preparation for moving into the new replacement Paterson Building in Withington, which while being an exciting prospect will bring forward new challenges to the Institute finances and the team.

Human Resources

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

[1]Joint with Administration

Over the past year, the HR department has continued to deliver a high-quality proactive service to the Institute, despite the restrictions and challenges caused by the pandemic. Human Resources provide 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, we have created, developed and adapted to new ways of working to ensure our proactive service continued to support staff and the Institute.

During 2021, we successfully recruited 34 individuals into the Institute. In addition, we are delighted to have recruited a new Junior Group Leader, Evangelos Giampazolias, who will join the Institute in January 2023.

As an Institute we are committed to developing our staff and ensuring that Personal Development Reviews (Contribution Reviews) are undertaken, and in 2021 we had a 99.6% completion rate. We have also continued our commitment to joint partnership working with the union, which has resulted in the revision of several HR policies and procedures throughout the year.

The Human Resources team has been heavily involved in supporting the Institute and staff throughout the COVID-19 pandemic with the management of the furlough scheme, staff engagement, supporting mental health and wellbeing, and the adoption of hybrid working for some teams and departments. We are

incredibly proud of how our staff have coped with the disruption and continued to be productive despite the difficulties that pandemic has presented.

During 2021, the department has been involved in developing the Institute's Equality, Diversity and Inclusion Strategy with a view to launching this in early 2022. Our vision is to create a diverse and inclusive culture which develops, attracts, and maintains a positive environment for staff and students whilst achieving the Institute's aim to deliver world class cancer research.

Next year, the HR department will continue its focus on Equality Diversity and Inclusion and wellbeing, in addition to the relocation to the new building and a review of the Personal Development Review process.

Information Technology

Steve Royle, Matthew Young, Brian Poole, Krar Haider

The CRUK Manchester Institute Core IT team is a group of four experienced IT professionals. We provide a wide range of IT support services to over 400 research and support staff, currently spread across several sites. We manage IT service desks on our two main sites at Alderley Park and the Oglesby Cancer Research Building.

2021 was a challenging year. A great deal of effort has been spent responding to staff working from home due to the ongoing pandemic and we are still learning and adapting to an ever-changing 'new normal'.

With most of our staff working from home, ourselves included, and after prolonged restrictions and less frequent visits back to the office or lab, we have continued to provide the same high level of IT support using a selection of new and existing remote support tools. Likewise, our researchers are now almost self-sufficient using their new set of remote/home working tools (VPN, Zoom, MS Teams, O365 etc). MS Teams and Zoom continue to be our (virtual) meeting rooms of choice presently and our staff have adapted well to these video conferencing platforms.

We currently manage over 600 desktop computers, comprising a mixture of Windows 10 PCs and laptops, Apple iMacs and Mac Books, plus a growing number of tablet devices, mainly Apple iPads and iPhones. All these devices are centrally authenticated, with access to cloud-based services plus a central file store, a server farm and network printing. The Core IT infrastructure comprises a 400Tb enterprise-class file storage facility for our research data. This is based on a replicated design and is hosted in two geographically separate datacentres to provide a resilient, high availability, redundant, and fit for purpose storage facility. They are connected by a dedicated CRUK MI resilient wired and wireless network infrastructure across all CRUK MI research facilities at Alderley Park and the OCRB.

Supporting multi-site operation and remote/home 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 and upgrade others. 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.

Over the year, planning work has accelerated as we plan our return to our former 'home' in Withington, based on The Christie NHS Foundation Trust site, in a new custom-built cancer research building to be shared between CRUK MI, The Christie and The University of Manchester.

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 ongoing Coronavirus pandemic. The Institute's COVID-19 strategy group continued to meet and develop appropriate responses to ensure that the Institute provides and operates a COVID-19 safe workplace. Staff were kept informed of constantly evolving and changing workplace arrangements via email and regular staff updates, delivered via Zoom. We continued to monitor COVID-19 infections of work colleagues, but we have seen no evidence of any workplace transmission, demonstrating that our COVID-19-safe workplace arrangements were effective with a high level of compliance by our staff in implementing them.

As the situation improved, we enabled an increase in laboratory occupancy with laboratory research taking on a more familiar look and feel. We have also enabled increases in office occupancy. Whilst at the same time the Institute has developed plans for hybrid working where this is practicable, such as office-based staff. Taken together, the Institute is resembling a more normal pre-pandemic workplace.

The Institute has established a Wellbeing Focus Group, which aims to improve the wellbeing of our staff. We hope this will help to counteract, at least in part, some of the negative aspects the pandemic has had on people's wellbeing and mental health. Accordingly, the Institute is funding the training of some mental health first-aiders.

Other areas in which the Health and Safety Team has been active includes contributing to the late design stages of the new building. It is anticipated that this work will increase markedly over the coming months. We are also undertaking an audit of our health and safety arrangements and feeding back our findings to University colleagues. We have also continued to undertake more normal activities such as implementing an inspection programme, advising staff when and where required and running our general and biosafety committees. We expect the coming year to be busy with the exciting completion of our new building and planning and implementing our move back.

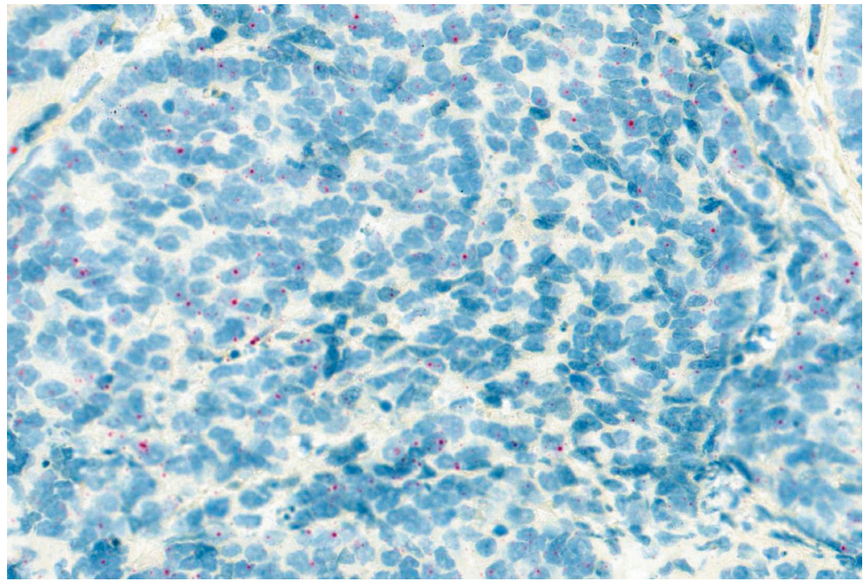
Electronics

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.

Prostate tissue from prostate cancer patient. In-situ hybridisation for transcription factors Runx1 and Runx2 mRNA. Red probe: Runx1 mRNA; brown probe: Runx2 mRNA. Scanned at 40X on Leica Versa. Tissue provided by Noel Clarke (Professor of Urological Oncology, Christie NHS Foundation Trust).

Image supplied by Esther Baena (Prostate Oncobiology) and Renaud Mevel, Rahima Patel, Georges Lacaud (Stem Cell Biology)



Laboratory Services

Mark Craven, Busola Atuegbe, Tony Dawson, Corinne Hand, Petra Kubinova, Christine Whitehurst (and support from Domestic Assistant, Garfield Banton)

Despite the ongoing COVID-19 related disruption, the Laboratory Services department based at the Oglesby Cancer Research Building has remained open under safe working practices and continued to supply the various sites with their required items. We supply sterile glassware, plastics and bespoke microbiological media to the scientists at OCRB, alongside the satellite lab sites at the Kay Kendall labs, WMIC Building, Incubator Building and Proton Beam Centre. Whilst the Institute is based at Alderley Park, we continue to support the scientists there, alongside the on-site Avantor team. Here we also provide sterile plastics and bespoke microbiological media and are making plans for the transition when the Avantor team leave site later in 2022.

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

- maintain and service two photographic dark rooms, one in OCRB and one at Alderley Park
- provide a drop-in monthly Pipette Clinic at both OCRB and Alderley Park
- organise the delivery of clean, general and tissue culture lab coats across the site

In addition, we have been supporting the design of the Lab Services department that will be housed within the new cancer research facility at the Withington site, which will be operational in early 2023.

Logistics

Andrew Lloyd, Michael Alcock, Edward Fitzroy, Nigel Fletcher, Sedia Fofana, Jonathan Lloyd, Wayne Howarth, Robin Sherratt, (with support from: Domestic Assistant, Garfield Banton; and Tony Dawson from Laboratory Services)

The role of the Logistics team is to deliver an efficient and effective service, providing on the ground and behind the scenes support for the research activities carried out across the Institute's various locations.

The team's role is varied and covers many responsibilities. These include:

- Receipting and distribution of goods
- Waste management support
- Cryogenics, dry ice delivery and compressed gases
- Central stores
- Daily sample transport service
- Facilitating the movement of equipment

Receipting and distribution

The team operate a back of house service and take direct delivery of consumables from the couriers. Items are receipted and distributed accordingly. Items that cannot be delivered immediately are stored in the department at the appropriate temperature pending their delivery

Waste management support

The team have continued to support waste management across the Institute and its other locations. We have also supported new recycling initiatives this year. We are well supported by Alderley Park and the University waste teams as well as the CRUK Manchester Institute Lab Services staff. This has enabled us to recycle large volumes of Institute waste. Between Alderley Park and OCRB we are currently recycling cardboard, plastic bottles, tin-coated steel cans, wooden pallets, polystyrene boxes, ink toners, glass, used tip boxes and plastic media bottles as well as scrap metal.

Cryogenics, dry ice delivery and compressed gases

The team facilitate the delivery of dry ice. Gas cylinders are also monitored and replaced, as necessary. The team look after the Institute's cell lines and other key biological samples by monitoring the liquid nitrogen levels in the cell storage tanks and will top up when required. During these unprecedented times, we have increased our gas stocks and increased the levels of nitrogen in the cell storage tanks to provide additional resilience.

Central stores

Researchers can order stock items from central stores via the intranet, which can be collected or distributed by the Logistics team. We currently stock over 100 items, from tissue culture essentials to cleaning products. Included in this system are the enzymes and media stored in the Institute's freezers (Sigma, Life tech, Promega, New England Bio labs, and Qiagen). Because of the COVID-19 pandemic supply chains have struggled, and we worked hard to source alternatives. We continue to buy in bulk, producing savings for the Institute.

Daily sample transport service

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

Facilitating the movement of equipment

The team also aid with moving heavy equipment or furniture and helping facilitate internal rearrangements. This past year we have supported the handing back of two separate lab spaces at Alderley Park. We have also relocated the Digital ECMT from Alderley Park to OCRB.

Scientific Administration

Caroline Wilkinson, Gillian Campbell, Julie Edwards, Christopher McCauley, Steve Morgan[1], Andrew Porter[2], David Stanier[3]

[1]Retired in 2021
[2]Joined in 2021
[3]Joint with HR

The Scientific Administration team provides a variety of services to aid the smooth running of the Institute, including oversight of our Postgraduate Education Programme (by Julie Edwards our Postgraduate Education Manager– see the Education section of this report). Gill Campbell assists our scientists in sourcing external funding opportunities and in preparing applications. This allows us to expand the breadth of the research we undertake. There was success this year in applications to the Melanoma Research Alliance, the Rosetrees Trust and Target Ovarian Cancer amongst others. The grant application process is overseen by our Grants Committee, chaired by Iain Hagan, who provide critical input for all our applications and provide feedback for practice interviews related to funding awards.

Andrew Porter joined the team from the Cell Signalling group, in the newly created post of Research Integrity and Training Adviser. The role is aimed at supporting the research community in maintaining the highest research integrity. A key part of the role is to review and undertake quality control checks on all CRUK MI manuscripts prior to final submission and to assist Group Leaders to maintain a complete and appropriate archive of the original data (including digital data) used in submissions for manuscripts. Andrew has developed some research integrity induction sessions for new starters and helped source other bespoke workshops on research topics such as statistical analysis and the ARRIVE guidelines for reporting of in vivo research. He has also established a network with his counterparts in other Institutes to drive further discussions and share best practice.

Andrew and Gill also form part of the Institute's communications team together with Belen Conti and overseen by Caroline Wilkinson. They manage all forms of communications for the Institute including our newsletters, this report, content for social media accounts, our external website, liaison with CRUK and The University of Manchester over press releases and approve any other external communications involving the Institute's staff and students. This year they have introduced more video content to our social media platforms and have started planning a refresh of our external website. A new streamlined process for engaging with the press and science media teams at CRUK and The University of Manchester has been developed to ensure that we take every opportunity to promote our research. Both Gill and Andrew were part of the team that put together the highly successful research engagement exhibit at the Royal Society Summer Science Exhibition.

David Stanier is the Institute's Information Governance Coordinator and Administrative Officer supporting the Institute's Information Governance Guardian, Caroline Wilkinson, with the management of information security, data protection and record management to ensure information governance disciplines are embedded within working practice across the Institute. To facilitate this, David regularly liaises with the University's Data Protection Officer and Information Governance Office over best practice. This year the Institute established its own Information Governance Committee to ensure best practice is being followed across the Institute. Both David and Andrew are undertaking training in Microsoft Teams as part of The University of Manchester's Digital Champions' scheme so will seek to aid the

implementation and best use of these tools across the Institute.

David also contributed to the organisation of our second online colloquium together with Gill, Andrew, Belen and Soraya Francis. The online platform Gather Town was used to promote greater interaction. After two years online we are hoping to return to an in-person format later in 2022.

Chris McCauley provides support for our various online platforms including our intranet, external website as well as the recruitment and PhD application portals. He has continued to refine our staff recruitment portal, JobMarker, and the PhD portal to optimise the experience for applicants and recruiting managers as well as exploring platform upgrades for the various sites and adapting them according to accessibility criteria.

Steve Morgan continued with his reception duties at the Oglesby Cancer Research Building alongside staff from the University's Faculty of Biology, Medicine and Health running the reception service and the Institute's switchboard. He retired in September after twelve years at the Institute where he had become a friendly and welcoming presence to all. He will be much missed and we wish him all the best for a long and happy retirement.

Animal Welfare

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

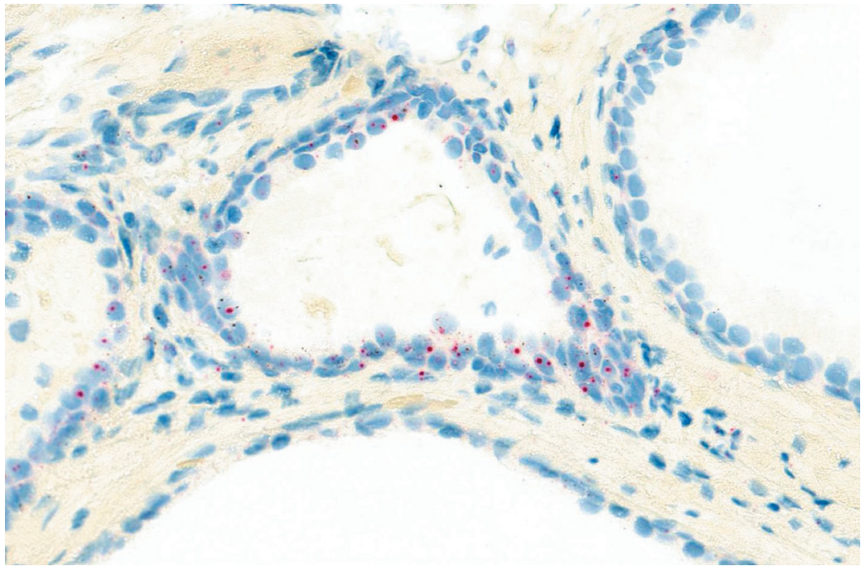
The Institute upholds the highest standards of welfare for the laboratory mice used in our research. All animal research activities are conducted in full compliance with the Animals (Scientific Procedures) Act 1986 (ASPA) and are scrutinised by the Institute's Animal Welfare and Ethical Review Body (AWERB). The AWERB supports all staff involved with animal research, promotes a 'culture of care' for staff and animals, ensures 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 principles (replacement, reduction and refinement of the use of animals). It also reviews the ethics of proposed collaborations and grant applications involving animal research.

The arrival of the pandemic and lockdowns throughout 2020 and into 2021 provided challenges in ensuring the safety of our staff and care for our animals whilst preserving our research activity as far as possible. Animal studies were limited in 2020 to the most critical or ongoing long-term, and breeding stocks of transgenic mice were kept to minimal numbers to preserve stock. We have been able to take on more studies in 2021 as COVID-19 restrictions were relaxed. Veterinary inspections of the animal unit continued online with in-person visits returning as work-attendance restrictions began to be lifted, allowing our Named Veterinary Surgeon to see the animals and continue advising scientists. Similarly, the activities of the AWERB and the twice-yearly meetings for all licensees continued virtually. We also held a joint virtual 3Rs' poster session with AstraZeneca including a total of five presentations from Institute scientists with the best poster prize awarded to CRUK MI PhD student Bianca Blochl from the Cell Plasticity & Epigenetics group (entitled, *Tackling the molecular mechanisms of cancer initiation: a high-throughput in vitro approach using single cell transcriptomics*). Applications and amendments to Project Licences continued uninterrupted. Overall, there was a reduction of 15% in the numbers of mice used in regulated procedures under the Act in 2021 (a total of 19,352) compared to 2020. The Institute continues to uphold high standards of regulatory compliance, promptly reporting any unexpected findings or incidents to the Home Office's Animals in Science Regulation Unit. All incidents were satisfactorily resolved with the ASRU inspectorate with suitable adjustments made where relevant.

We have adapted well to the new working practices introduced by the Home Office since July. Caroline Wilkinson was invited by ASRU to be a member of the Regulated Community Change Team advising on the impact of their programme of regulatory reform. She also continues to be a member of the national Establishment Licence Holders Committee and participates in training new ASPA Establishment Licence Holders.

Prostate tissue from benign prostatic hyperplasia patient. In-situ hybridisation for transcription factors Runx1 and Runx2 mRNA. Red probe: Runx1 mRNA; brown probe: Runx2 mRNA. Scanned at 40X on Leica Versa. Tissue provided by Noel Clarke (Professor of Urological Oncology, Christie NHS Foundation Trust).

Image supplied by Esther Baena (Prostate Oncobiology) and Renaud Mevel, Rahima Patel, Georges Lacaud (Stem Cell Biology)



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, NC3Rs, RSPCA and LASA to further the sharing of knowledge and advice on laboratory animal use.

Much energy has been put into the construction of the new research facilities in the new, replacement Paterson Building. We anticipate our return to the original site in Withington by early 2023.

Cancer Research UK Commercial Partnerships

Martyn Bottomley

Cancer Research UK Commercial Partnerships (CP) Team (formerly Cancer Research Technology (CRT)) is a specialist oncology-focused development and commercialisation team, which is part of Cancer Research UK's Research and Innovation Directorate. The CP Team aims to maximise patient benefit from CRUK-funded research worldwide by advancing research discoveries into development with pharmaceutical and biotechnology parties. We aim to bridge the gap between cutting edge academic research and industrial development of cancer therapeutics, 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.

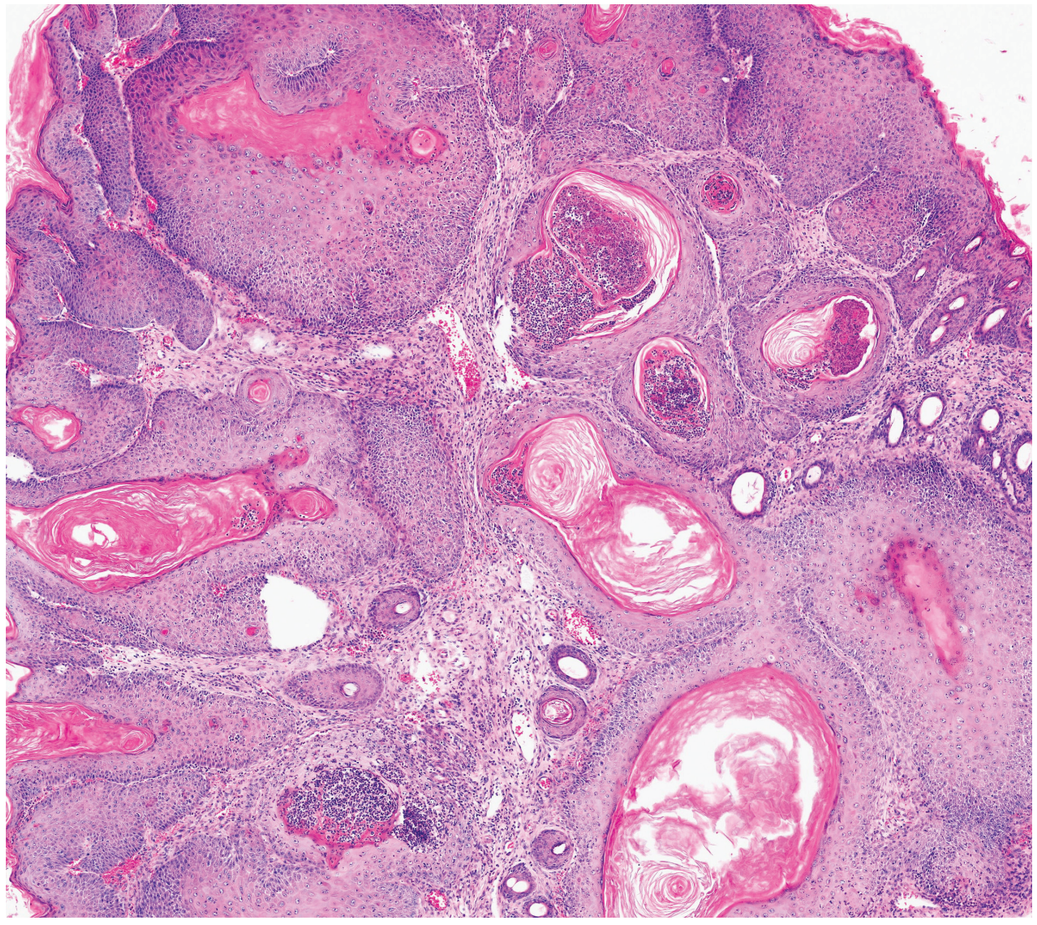
The CP Team continues to work in functionally distinct sub-teams to provide greater strength, depth and accountability in our core activities supporting translation and commercialisation, as well as providing clearer and more streamlined interfaces with other teams across R&I with whom we collaborate to achieve our joint goals of progressing CRUK science. This is enabling us to build deeper and more strategic relationships with our funded centres, Institutes and universities, as well as improving internal information flow and collaboration.

Translating new discoveries into patient benefit has not progressed at the same pace as discovery research. To address this disparity, the CRUK-PACE team was formed to Promote an Academic Culture of Entrepreneurship within our research community. As part of this initiative, we are a partner in the Alderley Park Oncology Development Programme launched in December 2020. This national Programme is designed to develop and progress start-up oncology projects. Funded by Innovate UK and Cancer Research UK, the programme brings together a unique collaboration of global pharmaceutical and healthcare companies, research institutions and public bodies to identify and progress exciting oncology innovations that will improve the diagnosis and treatment of cancer. Its goal is to bring forward viable

oncology projects much more quickly to significantly increase their likelihood of commercial success, and ultimately, patient benefit. In the first programme in 2021, two projects from the CRUK Manchester Institute, led by Professor Caroline Springer and Professor Richard Marais respectively, were successful in progressing through all stages of the programme to the final pitch to investors day. This resulted in the formation of two spin out companies to progress both opportunities.

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 have recruited a joint role starting in March 2022 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.

CP is also currently actively managing a broad portfolio of development programmes and exciting licensing opportunities originating from the Cancer Research UK Manchester Institute that continue to attract commercial partners. The projects include several drug discovery assets from the Drug Discovery Unit and a novel pan-cancer treatment response biomarker from Santiago Zelenay's group. 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.



Histological image showing the cutaneous papilloma from a female mouse following exposure to the carcinogen DMBA/TPA.

Image supplied by Amaya Viros and Tim Budden (Skin Cancer and Ageing group)

POSTGRADUATE EDUCATION

Postgraduate Education Manager
Julie Edwards

Postgraduate Tutor
Angeliki Malliri

Postgraduate Director and Chair of the Education Committee
Tim Somervaille

The Cancer Research UK Manchester Institute offers postgraduate degrees (PhD) for students interested in a career in 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 the potential and opportunity to study for a cancer-related PhD degree. This is achieved through a structured 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 continuing in academia, industry or healthcare, and securing positions in destinations across the UK, Europe and the USA. In 2021, 100% of our graduates found positions following PhD completion: academic (55%), scientific industry (36%) and return to clinical training (9%).

In 2021, we welcomed seven new graduate students and one clinical research fellow to our PhD programme, working in a variety of fields including leukaemia biology, cancer biomarkers, systems oncology, cell division, 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 first author papers in a variety of journals including *Nature Communications, Nature Materials, Cell Reports, Cancer Cell, Cancer Cell International and the European Journal of Cancer.* First author review articles were also accepted and published in *Critical Reviews in Biochemistry and Molecular Biology and Nature Reviews Urology.*

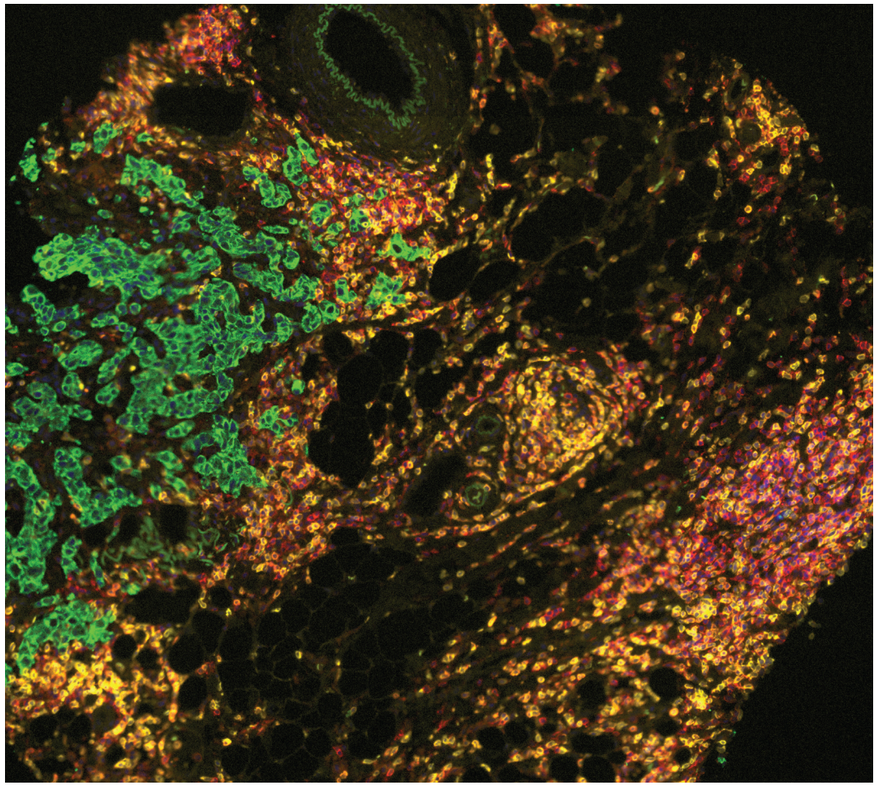
The Cancer Research UK Manchester Graduate Programme

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 of commencement and monitored with formal student assessments at key stages throughout the duration of the programme. Modes of assessment include annual written reports, oral presentations and progress meetings which are designed not only to provide formal points at which progress (of both the student and the project) can be monitored, but are beneficial in the development of presentation skills fundamental to the majority of academic careers in science and beyond.

Graduate training and student welfare is monitored by the Institute's Education Committee. A main supervisor and a second or co-supervisor are nominated for each student providing advice and support on both academic and non-academic matters. Students are assigned an advisor – similar to a personal tutor on an undergraduate programme – whose role is to provide impartial support and advice in a pastoral role.

The CRUK Manchester Institute has an established internal and external seminar series featuring talks from leading scientists in cancer research, and all our students benefit from these events. Speakers are internationally renowned scientists, and we consider it essential that our students are exposed to outstanding research from leaders in different disciplines, providing a broad understanding of many aspects of cancer research and basic biology. In addition, we hold a programme of internal research seminars given by our scientists and PhD student attendance 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, student forums and external conferences. Conferences and seminars play an essential and important role in connecting and networking with colleagues across the Institute, nationally and internationally and these have continued successfully virtually over the last 12 months. Staying connected with peers and colleagues has remained a key component for students over the last two years not only in terms of research progress, but for their mental health and wellbeing. A programme of in-house training events, external and internal seminars continued to be delivered online in 2021, encouraging connection and engagement of students during home/hybrid working patterns. The Institute and Education Committee recognises the impact of the working restrictions on mental health and wellbeing of students and has worked hard to identify areas where additional support is necessary.

Despite the adversities experienced in 2021, the CRUK Manchester Institute student research and activities have thrived virtually providing a continued and important basis for expanding knowledge. Our PhD students have adapted extremely well to the virtual/hybrid approach to giving their talks, PhD pre-viva seminars and PhD viva examinations.



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)

STAy (Science TakeAway) is a committee 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 over the last 12 months have included virtual pub quizzes, escape rooms, tabletopia, coffee mornings and in-person 'STAy for Lunch' gatherings when permitted – all encouraging students, scientific staff and post-docs to engage and keep well connected.

The CRUK Manchester Institute Colloquium is an annual event that normally takes place off-site at Lancaster University, however this September it was successfully held online using the interactive digital platform Gather Town. The event is an excellent opportunity for our new intake of students to meet other established PhD students, members of the Institute, including group leaders, postdoctoral fellows, and scientific officers. This forum communicates up to date science in the form of oral presentations given by group leaders and second year PhD students, as well as poster presentations from a range of scientists across the Institute covering all aspects of cancer research. We congratulated two students in 2021 who were awarded the Lizzy Hitchman Prize for the best poster - Charlotte Bell, from the Cancer Inflammation and Immunity group for her work on chemotherapy induced COX2, and Francesco Camera, from Leukaemia Biology for his studies on transcription factor IRX3 and acute myeloid leukaemia.

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: The Francis Crick, CRUK Manchester Institute, London Research Institute (LRI), Cambridge Institute (CI), Beatson Institute (BICR), Netherlands Cancer Institute (NKI), European School of Molecular Medicine, Milan (SEMM, IFOM & IFEO), and the German Cancer Research Centre (DKFZ).

The 14th IPSCC was organised by PhD students from The Beatson Institute, Glasgow and held virtually on 16-18 June 2021, having previously been postponed from 2020 due to the COVID-19 pandemic. The conference was well attended given there were no restrictions/ limitations on numbers due to the virtual online format, with 163 participants and 21 student talks scheduled over the two and half days. The conference used a combination of platforms - Gather Town and Zoom. Gather Town is an interactive platform with conference attendees making their own avatar to easily navigate around the different areas of the conference and interactive social spaces. There were 3 plenary lectures over the 2.5 days from Professor Laura Machesky, Director of the Institute of Cancer Science, University of Glasgow; Dr Christian Frezza, Programme Leader, MRC Cancer Unit, Cambridge Cancer Centre; and Professor Ross Cagan, Professor of Precision Medicine, University of Glasgow.

CRUK Manchester Institute was represented by 18 students from years 1 to 4+. There were 21 talks in total over the 2.5 days, and three excellent talks were given by our students:

- Ryan Guilbert, Cell Signalling – *Investigating the role of cell-cell junctions in neuroendocrine small cell lung cancer*
- Melissa Frizziero, Cancer Biomarker Centre – *Generation of a Circulating Tumour Cell (CTC)-Derived eXplant of a NeuroEndocrine Carcinoma of unknown origin*
- Charlotte Bell, Cancer Inflammation and Immunity – *Cytotoxic therapy-induced activation of the COX-2/PGE2 pathway by dying tumour cells reduces the efficacy of chemotherapy and immunotherapy combinations*

Posters were scored and ranked with prizes awarded at the end of the conference for the best oral presentation and the top two posters from ~90 posters. We congratulated CRUK Manchester Institute final year student, Eimear Flanagan from the Cancer Inflammation and Immunity group, who won one of the best poster prizes showcasing her work on *Pan-cancer cell line screen identifies Prostaglandin E2 as a dominant modulator of the tumour microenvironment.*

We are looking forward to joining the German Cancer Research Centre (DKFZ) students in person at the next IPSCC in June 2022.

PhD studentship recruitment

PhD recruitment to our core funded studentships is highly competitive, with 300-500 applicants competing for between four and eight places each year. CRUK core funded studentships are full time for 4 years with an approved research project to be undertaken 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. Interviews are typically conducted annually over a two-day period in January/February; these were held virtually in February 2021 with successful recruitment to the available six core funded studentships commencing in autumn 2021.

Additional PhD studentships and clinical fellowship positions were awarded to the CRUK Manchester Institute core groups in 2021 funded via Cancer Research UK Manchester Centre Clinical Training Fellowship (ACED Alliance) and Clinical Academic Training Award (CAT), Christie charitable funding and the Faculty of Biology, Medicine and Health, University of Manchester split PhD programme collaborations between Manchester-Melbourne (Global Doctoral Research Network (GOLDEN) Dual Award) and Manchester-Israel (Weizmann Institute of Science).

Our students benefit from access to advanced state-of-the-art facilities, including advanced imaging, mass spectrometry, flow cytometry, histology and next generation sequencing. CRUK Manchester Institute research groups offering PhD studentships and projects covering a wide breath of cancer research are currently based over two sites: Alderley Park, Cheshire and the Oglesby Cancer Research Building, Manchester.

Education Committee 2021

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

Our goal is for every student to have a project that is both achievable and intellectually stimulating and demanding. Projects and students are monitored by the Education Committee ensuring that the proposed plan of research is achievable, and that progress is made consistently throughout the course of the studentship. Various assessments at key stages throughout a CRUK Manchester Institute PhD studentship are a vital component in ensuring successful PhD completion and graduation of our students. Such assessments are not only crucial in the development of students throughout their postgraduate programme, but importantly enhance future employability and academic careers.

Education Committee Members

Tim Somervaille
Angeliki Malliri
Caroline Dive, Ex-Officio Member
Wolfgang Breitwieser[2]
Julie Edwards
Carlos Lopez Garcia[1]
Claus Jørgensen
Elaine Kilgour
Georges Lacaud
Amaya Viros
Caroline Wilkinson

Student Representatives

Ryan Guilbert[2]
Melissa Frizziero
Victoria Fife (nee Gernedl)[1]

[1]Joined in 2021
[2]Left in 2021

THESES

Christopher Below
Systems Oncology

Analysing stiffness-mediated signalling in pancreatic ductal adenocarcinoma

Eimear Flanagan
Cancer Inflammation and Immunity

Identification and characterisation of tumour cell-derived modulators of inflammation

Matthew Howell
Cancer Biomarker Centre

The utility of circulating free DNA analysis in patients presenting to the Phase I clinical trials clinic

Manuela La Montagna
Cancer Biomarker Centre/Translational Networks in Lung Cancer

Dissecting the role of AMPK in non-small cell lung cancer

Sarah Pearsall
Cancer Biomarker Centre

Molecular characterisation of vasculogenic mimicry in pre-clinical models of small cell lung cancer

Ron Rodrigues Pereira
Translational Oncogenomics

Data-driven identification of genomic aggression in localised prostate cancer

Maximilian Schenk
Cancer Biomarker Centre

Dissecting chemoresistance in small cell lung cancer

Fabrizio Simeoni
Leukaemia Biology

The role of the forkhead transcription factor FOXC1 in AML

Ivana Steiner
Prostate Oncobiology

Dissecting the role of LY6D+ castration-resistant progenitors in prostate tumourigenesis

CANCER RESEARCH UK'S RESEARCH ENGAGEMENT

Research Engagement Manager

Iqra Choudhry[1]

Tim Hudson[2]

[1]Joined in 2021
[2]Left in 2021

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.

CRUK research engagement activities have changed significantly during the COVID-19 pandemic. With restrictions in place for the majority of 2021, we have shared our science with the public in new and varied ways this year.

During the summer, several scientists from the Manchester Institute took their science communication to a national audience as part of the annual Royal Society Summer Science Exhibition. Due to the pandemic, the programme was delivered online, as a digital Summer Showcase reaching a far wider audience. Researchers from the groups of Claus Jørgensen and Santiago Zelenay delivered a fascinating digital experience to show what makes up a tumour to an audience of hundreds. Postdoc Joanna Kelly delivered a lightning lecture on mapping tumours, which has been watched online over 13,000 times. PhD student Charlotte Bell also featured in the Showcase, putting together the introductory video that highlighted our research, as well as helping the participants to make their very own cardboard tumour models at home, using nothing but a cereal box, a pen and a pair of scissors!

CRUK fundraising events were once again held in person in September 2021, and several of our researchers not only took part in our local Relay For Life event in Stockport, but also addressed CRUK supporters at the virtual Ultimate Relay summit. Scientists Steve Lyons, Denis Alferez, John Castle and Samantha Littler connected the fundraisers to the science that Relay For Life helps to fund at the CRUK Manchester Institute. Event leader Steve made such a lasting impression on his fellow Relayers that he was invited back to speak at the launch of the 2022 Relay For Life series.

The United Nations Climate Change COP26 Summit took place in Glasgow this year, with the city hosting over 25,000 delegates from more than 200 countries. Several CRUK scientists addressed the attendees at the climate event through a video on lung cancer research which featured Professor Caroline Dive, alongside other CRUK researchers, talking about her work on the tumour microenvironment.

Around one third of all CRUK funding comes from donations left to the charity in wills, and our annual Legacy campaigns raise public awareness, showcasing the science these donations fund. This year, Denis Alferez and Alexandra Hendry and their research were featured in the regional media for the 2021 CRUK Legacy campaign.

Caroline Dive addresses COP26 attendees in her video on lung cancer research and the tumour microenvironment.

The Cancer Research UK Manchester Institute contributed to the cancer exhibition at the Science and Industry Museum, Manchester: 'Cancer Revolution: Science, Innovation and Hope'. The exhibition features work from the Cancer Biomarker Centre and Biological Resources Unit.

Photo credit: Science Museum Group © The Board of Trustees of the Science Museum, London

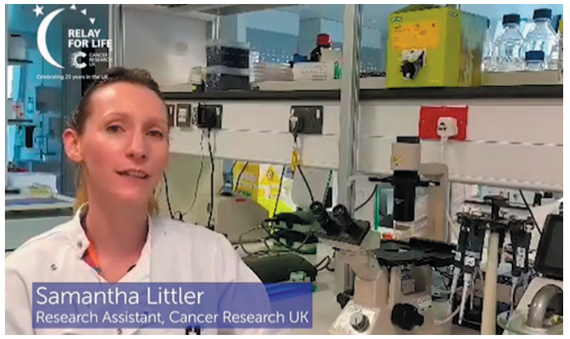
CRUK partnered with the Science Museum Group to bring important conversations about the experience of a cancer diagnosis and treatment, as well as the future of cancer research, to the public. In October 2021, the Science Museum Group launched 'Cancer Revolution: Science, Innovation and Hope' at the Science and Industry Museum in Manchester. The exhibition places patient experiences alongside the work of CRUK scientists, to show visitors the impact our science is having on the lives and experiences of patients and what we hope to achieve in the future. Our own Chief Laboratory Officer Stuart Pepper was involved in helping to craft the exhibition, and research from across the Manchester Institute and other CRUK Centres has been featured in the exhibition itself, including Professor Caroline Dive's work on liquid biopsies.

The exhibition launch was a huge success, with 250 guests participating in engagement activities communicating some of the science featured in the exhibition. The most popular of these activities was one which explained how a 'liquid biopsy' is taken from a patient, bringing the work of the Cancer Biomarker Centre to life for the visitors. Since the launch of the exhibition, the museum has welcomed thousands of visitors. Despite changing COVID-19-related restrictions, the exhibition has been more successful than we could have anticipated, sparking important conversations about the research we do and the impact it has on a patient's cancer journey. At the end of March 2022, the exhibition will move to the Science Museum in London, where it will be open for even more visitors to experience.

The work of researchers from the CRUK Manchester Institute has also been featured in *The Guardian*, in a series of articles that looks at the past, present and future of cancer research. Caroline Dive's work on liquid biopsies has been highlighted as one of the five most exciting pieces of current cancer research. Professor Karen Kirkby's (University of Manchester's Division of Cancer Sciences) research with CRUK and The Christie on proton beam therapy was also featured in an article that puts Manchester, and its unique place in the history of radiography and radiotherapy treatments, on the map. These articles have been read by thousands and are in the top 7% of articles viewed on *The Guardian* website.

As always, our scientists have taken part in raising money for Cancer Research UK – from getting involved in online fundraising to participating in Race For Life and Relay For Life events, there have been some truly inspiring efforts to raise much-needed funds.

Looking ahead to 2022, we are eagerly awaiting the opportunity to welcome CRUK supporters and fundraisers into our labs again, and to bring the science at the CRUK Manchester Institute to people in person once more.



Steve (CRUK MI) and Sam (Division of Cancer Sciences) address CRUK supporters at the virtual Ultimate Relay summit

ACKNOWLEDGEMENT FOR FUNDING FOR THE CANCER RESEARCH UK MANCHESTER INSTITUTE

The total funding of the CRUK Manchester Institute for 2021 was £23.8m. The major source of this funding was awarded by Cancer Research UK (CRUK) via a core grant of £10.7m plus additional strategic funding of £2.7m. 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 Research England generated income at a cost of £2.1m.

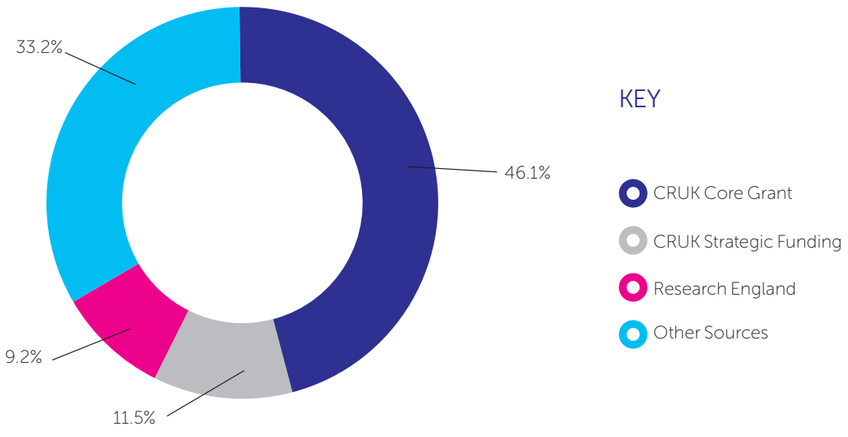
The balance of the Institute's funding is received from a number of additional sources. The research carried out through these additional projects enhances and supports the research undertaken by the core funding.

These sources are as follows:

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

We are immensely grateful to all our sponsors.

CRUK MANCHESTER INSTITUTE FUNDING 2021



CAREER OPPORTUNITIES AT THE CANCER RESEARCH UK MANCHESTER INSTITUTE

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

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

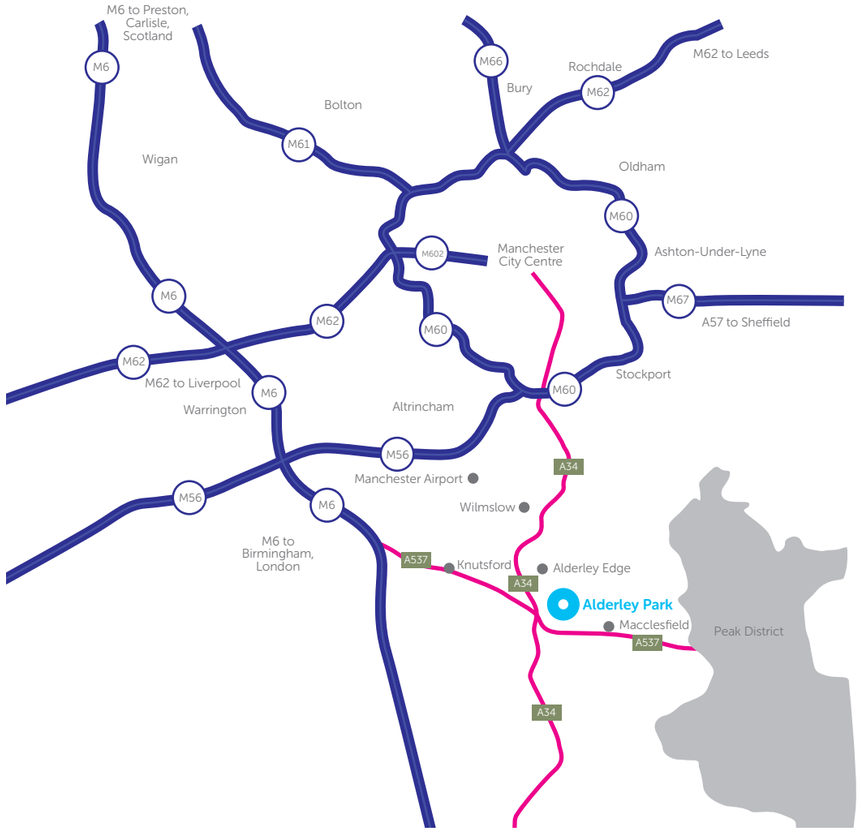
Opportunities exist at a number of levels in the Institute. We have a well-established programme of degrees by research which is described in the section on Postgraduate Education. We encourage applications from suitably qualified graduates to apply to join either the PhD or MD programmes. Graduates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

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

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

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

CONTACT DETAILS



Cancer Research UK Manchester Institute
Director: Professor Caroline Dive

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

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

Tel +44(0) 161 306 0871

Cancer Research UK
Cancer Research UK is a registered charity in England and Wales (1089464), Scotland (SC041666) and the Isle of Man (1103). Registered address: 2 Redman Place, London, E20 1JQ.

Tel 0300 1231022
www.cruk.org

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

ISSN 1479-0378

Edited by: Caroline Wilkinson
Gillian Campbell

CANCER RESEARCH UK
MANCHESTER INSTITUTE

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

www.cruk.manchester.ac.uk