OXFORD CANCER

DPhil in Cancer Science

University of Oxford

2022 Intake Project Book

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DPhil in Cancer Science
2022 Intake Project Book

Introduction

This handbook provides an overview for prospective students looking to study for a DPhil in Cancer Science starting in 2022 at Oxford University. The Programme provides research based doctoral training for cancer researchers from clinical, biological, engineering, mathematics and statistics background. Students will receive a world-leading research training experience that integrates an education initiative spanning cancer patient care, tumour biology and research impact; on- and post-programme mentorship; and a specialised, fundamental, subject-specific training tailored to individual research needs. Students participating in the scheme will be offered:

- a choice of interdisciplinary cutting-edge cancer research projects.
- the ability to gain a working in-depth knowledge of the fundamentals of cancer biology and cancer patient care through advanced level seminars.
- a world-renowned research environment that encourages the student's originality and creativity in their research.
- opportunities to develop skills in making and testing hypotheses, in developing new theories, and in planning and conducting experiments.
- an environment in which to develop skills in written work, oral presentation and publishing the results of their research in high-profile scientific journals, through constructive feedback of written work and oral presentations.

At the end of their DPhil course, students should:

- have a thorough knowledge of the basic principles of cancer research including the relevant literature and a comprehensive understanding of scientific methods and techniques applicable to their research.
- be able to demonstrate originality in the application of knowledge, together with a practical understanding of how research and enquiry are used to create and interpret knowledge in their field.
- have developed the ability to critically evaluate current research and research techniques and methodologies.
- be able to act autonomously in the planning and implementation of research.
- have the grounding for becoming an influential cancer researcher of the future.

Selection Criteria & Eligibility

There are four tracks in the programme as described below, meaning that non-clinicians, undergraduate medical students and post-graduate medical trainees are all eligible to apply for the fully funded (at home rate) studentships.

Application Track 1 – Clinical Trainees. Qualified doctors at all stages of training from the foundation training to higher specialist training.

Application Track 2 – Medical Undergraduates. Medical students who are currently undertaking a primary medical qualification (MBBS, MBChB or equivalent)

Application Track 3 – Non-Clinical/Fundamental Scientist. Science graduates that hold (or be predicted to achieve) the equivalent of a first-class or strong upper second-class undergraduate degree with honours in a biological, medical, or chemical science, as appropriate for the projects offered.

Application Track 4 – Non-Clinical/Fundamental Scientist. Science graduates that hold (or be predicted to achieve) the equivalent of a first-class or strong upper second-class undergraduate degree with honours in a engineering, mathematical/data, ***or*** physical science, as appropriate for the projects offered.

All applicants will be judged on the following:

- commitment and passion to a career in cancer research
- evidence of motivation for and understanding of the proposed area of study
- commitment to the subject, beyond the requirements of the degree course
- preliminary knowledge of relevant research techniques
- capacity for sustained and intense work
- reasoning ability and academic curiosity.

Funding

All offered places are fully funded at the home rate. This includes salary/stipend, University/College fees, and a research consumables budget of £13k p.a.. Salary and stipend provisions are summarised below:

- Application Track 1 – 3 years of salary at Grade E63 or E64 Clinical Researcher rate.
- Application Track 2 – 3 years of stipend at the flat rate of £19,000 per annum.
- Application Track 3 & 4 – 4 years of stipend at the flat rate of £19,000 per annum.

International applicants are eligible, however funding is limited to the Home level for this programme and therefore international applicants would need to either source further funding or support themselves financially for the remaining fees.

Notable Scholarships

Black Academic Futures Scholarships
These awards offer UK Black and Mixed-Black students scholarship funding to pursue graduate study at Oxford, alongside a programme of on course mentoring and support. The Medical Sciences Division has guaranteed places across its DPhil courses (including the DPhil in Cancer Science). For more information, visit the Black Academic Futures website.
To receive a Black Academic Futures Scholarship, submit your application to the DPhil in Cancer Science Programme by the December deadline. All those that include eligible ethnicity will automatically be considered. You do not need to submit any additional documents and there is no separate scholarship application form for these awards.

How to Apply

A detailed summary on how to apply can be found here. In brief, prospective students apply with a prioritised list of three projects selected from this booklet by Friday 3rd December 2021. Shortlisted students will be invited to interview in January/February. If successful, students will be allocated a project on the basis of their ranking during the review process. It is strongly suggested that students contact supervisors of projects they are interested in applying for prior to application.

Projects

Projects are listed below in the following structure "Title [Eligible Application Tracks] – Primary Supervisor Page number."
Clicking on the project title below will take you to the project page.

1. ARH3/ADPRHL2 as a biomarker for PARP inhibitor sensitivity/resistance [1,2,3,4] - Prof. Ahel

Primary Supervisor: Prof. Ivan Ahel
Additional Supervisors: Prof. Ahmed Ahmed
Eligibility: Track 1, 2, 3 and 4 students are eligible to apply for this project.

Project Summary

To protect the genome from damage organisms have evolved a cellular defence mechanisms termed the DNA damage response (DDR). The DDR includes a diverse set of signal transduction pathways and effector proteins that act to sense DNA lesions and effectively repair the damage, limiting the propagation of genomic instability. Exploiting DDR pathways to specifically target and kill cancer cells has become an attractive therapeutic avenue within cancer research. This is exemplified by the synthetic lethal interaction between PARP inhibition and BRCA1 or BRCA2-deficient tumours1. Ivan Ahel (co-supervisor on this project) laboratory recently identified HPF1 protein as a novel interactor and critical regulator of PARP1 ADP-ribosylation activity upon DNA damage2. Functionally, HPF1 suppresses DNA damage-induced hyper auto-modification of PARP1 and promotes in trans ADP-ribosylation of histones and many other proteins involved in regulation of genome stability. They further demonstrated that HPF1 is a critical specificity factor that allows modification of target proteins by PARP1 on serine residues (Ser-ADPr)3,4. Crucially, the work also identified ARH3 as a hydrolase which specifically removes Ser-ADPr5 and further showed that Ser-ADPr is the major form of ADP-ribosylation following DNA damage6. Taken together, the insights surrounding Ser-ADPr open a large, exciting, and novel area of research into the fundamental understanding of the pathways regulated by this modification. Strikingly, our recent data show that ARH3 knockout in model cell lines associates with PARP inhibitor (PARPi) resistance, while ARH3 overexpression is associated with PARPi sensitivity7. Based on these results, we hypothesize that ARH3 activity and protein levels affect sensitivity to PARPi, thus representing; i) a predictor for the success of these therapies and, ii) a novel target for further drug development. Currently, PARP inhibitors are used to treat ovarian cancer and several other cancers, and we therefore propose to test the hypothesis that ARH3 expression might be a useful diagnostic tool with which to stratify cancer patients into sub-groups that will be sensitive/resistant to PARPi treatment with a particular focus on ovarian cancer. The mechanism of sensitivity/resistance of cells with deregulated ARH3 expression cells to PARPi is unknown, and elucidating this mechanism will be another goal of this proposed work.

Research Objectives

Objective 1

Characterise the effect of ARH3 under- and overexpression in a series of model and primary cancer cell lines on PARP inhibitor sensitivity/resistance. We will collect and test a variety of ovarian cancer cell lines, profiling them for ARH3 protein expression levels and then treating with several different PARPi of varying PARP-trapping capabilities (olaparib, talazoparib, veliparib). To determine the impact of ARH3 protein levels on PARPi vulnerability, we will not only assess drug sensitivity and levels of PARP1, PARG, and ARH3 across a panel of ovarian cancer cell lines, but also assess the impact of systemically varying ARH3 by knockdown, knock out and inducible overexpression in HGSOC lines of defined genotype, including Ovcar8 (BRCA1/2 wt, PARPi resistant), PE01 (BRCA2-mutant, PARPi sensitive), Kuramochi (BRCA2-mutant, PARPi partially sensitive) and COV362 (BRCA1-mutant, PARPi sensitive). Rescue experiments with wild type vs. catalytically inactive ARH3 will assess the suitability of ARH3 as a target for the development of inhibitors.

Objective 2

To determine the frequency of ARH3 gene alterations in a larger set of HGSOC samples, we will: i) interrogate data of an ongoing whole exome sequencing study of 504 ovarian cancers searching for ARH3 and PARG copy number alterations and mutations; and ii) perform semi-quantitative detection of ARH3, as well as of PARG, PARP1 and PAR, by immunohistochemistry (IHC) on two independent sets of tissue microarrays (TMAs) containing a total of 1200 ovarian cancers. To augment these analyses, which will be limited by the small number of tumors treated with PARPi, we will also evaluate levels of ARH3, PARG, PARP1 and PAR in patient-

derived xenograft (PDX) models that have been assayed for response to single-agent PARPi, including ones that have a high HRD score but did not respond.

Objective 3
Elucidating the mechanistic basis for the sensitivity/resistance of cells with deregulated ARH3 expression cells to PARPi (modulation of the PARP-trapping, regulation of DNA repair pathway choice, regulation of the chromatin structure/epigenetic marks). For these studies we will use largely cell biology/biochemical and genomics approaches. This objective will be performed in co-supervisor (Dr Ivan Ahel) laboratory at the Sir William Dunn School of Pathology, University of Oxford.

Translational Potential
Our data suggest that ARH3 protein expression levels in cancer patients might be a marker that confers sensitivity/resistance of the tumour to PARPi, providing a rationale for using PARPi for certain patients. In longer term, understanding the mechanisms of DNA repair and PARPi resistance through studies of ARH3 protein, may reveal new, unexpected avenues for treatments in the future.

References
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2. Bioengineered organoid models to investigate the interaction between driver mutations and cancer microenvironment in colorectal cancer progression [2,3,4] - Prof. Bayley

Primary Supervisor: Prof. Hagan Bayley
Additional Supervisors: Prof. Xin Lu
Eligibility: Track 2, 3 and 4 students are eligible to apply for this project.

Project Summary

Colorectal cancer (CRC) is one of the major causes of cancer death. The adenoma-carcinoma sequence model for CRC reports the sequential alteration in the Adenomatous Polyposis Coli (APC), Kirsten Rat Sarcoma (KRAS), Mothers Against Decapentaplegic 4 (SMAD4) and Tumour Protein p53 (TP53) genes, which initiate CRC development. However, contradictory data from genetic characterisation of a large cohort of CRC patients suggesting complex alternative pathways. Further, it has been increasingly recognised that the interactions between cancer cells and the neighbouring microenvironment, including fibroblasts, immune cells and neurons, are important in cancer initiation, progression and metastasis. For example, metastatic cancer cells are frequently observed to migrate along nerves in clinic. Previously, we have established a droplet-based 3D printing technique to construct functional 3D neural tissues, and a versatile technique for bioengineering tubular GI tissues. Here, we propose the bioengineering of organoid models which will carry different combinations of driver mutations and surrounding microenvironment. These engineered organoids will be used to test the competition and cooperation between different driver mutations and also the interactions with between CRC epithelial cells and cancer microenvironment. We will use confocal microscopy, cell motion sensing, and single cell RNA sequencing techniques to quantify these interactions in vitro, followed by implantation in mice to confirm the tumorigenesis potential of the engineered organoids. Understanding the impact of different driver mutations and cancer microenvironment in cancer progression will aid the development of new strategies for cancer treatment.

Research objectives

Colorectal cancer (CRC) is considered as a result of accumulation of key genetic mutations which set colorectal epithelial cells on the path to carcinogenesis.[1] The sequential acquisition of the driver mutations, APC, KRAS, SMAD4 and p53, is proposed to correlate with specific CRC stages from adenoma to carcinoma. Although this well-established paradigm has been supported by many following research, the exact process which leads to the oncogenic transformation of healthy colorectal tissue to metastatic tumour remains unclear. Further, the genetic characterisation of a large cohort of CRC patients found that while most tumours contain one or two driver mutations, less than 7% of tumours contain mutations in all three genes, APC, KRAS and p53, suggesting alternative genetic pathways to CRC.[2] Moreover, during tumour growth, intratumoral heterogeneity arises through the complex evolutionary trajectories of genetically diverse clones.[3] Both clonal selection and cooperation have been indicated to drive tumorigenesis.[3,4] Besides genetic alternations, the tumour microenvironment has been increasingly recognised as an important factor for cancer progression, which serves to maintain the cancer stem cell niche and promote metastasis.[5] For example, In the clinic, the migration of metastatic cancer cells along nerves is frequently observed, but remains poorly understood.[6]

Project plan and previous work

Here we propose to use engineered organoids carrying different combinations of driver mutations to investigate the clonal competition and cooperation. We also propose to bioengineer patterned organoids with cancer organoids surrounded by distinct cell types existing in CRC microenvironment, including fibroblasts, immune cells and neurons, to recapitulate the interactions between CRC epithelial cells and their microenvironment during cancer progression. Both the bioengineering and the organoid/neuron/fibroblast/immune cell culture techniques have been established in the Bayley Lab and Lu lab. Previously, the Bayley Lab established a droplet-based 3D printing technique for the construction of functional 3D neural tissues, using human induced pluripotent stem cell (iPSC)-derived neural stem cells (Figure 1A-C).[7] Moreover, our collaborative efforts (Bayley Lab and Lu Lab) have led to a versatile technique for bioengineering tubular gastrointestinal (GI) tissues, containing defined layers: an epithelial layer derived from GI organoids and a sub-epithelial layer from fibroblasts (Figure 1D).[8] The Lu lab has also established bio-banks for both mouse and human GI organoids and fibroblasts. With the proposed project, we will optimise culture conditions for the engineered organoids, especially the co-culture systems with fibroblasts, neurons and immune cells. Proliferation, differentiation, migration and invasion of the cancer epithelial cells will be monitored and analysed through confocal microscopy, immunohistology, and single cell RNA sequencing. We will use confocal microscopy combined with the Motion Sensing

Superpixels (MOSES) technique developed in the Lu lab to monitor cell growth and migration.[9] Single cell analysis will reveal the genes associated interactions between different driver mutations and the mutations with cancer microenvironment, which will provide a molecular understanding of the potential pathways involved. The in vitro results will be further tested through mouse implantation experiments to confirm the tumorigenesis potential of the engineered organoids.

Potential outcomes

We will use the engineered organoids to test the following hypotheses: 1) Organoids carrying different driver mutations influence each other on organoid growth, symmetry breaking and differentiation. 2) CRC epithelial cells interact with fibroblasts, neurons and immune cells, and shape the tumour microenvironment to facilitate the growth and migration of cancer cells. 3) The implanted engineered organoids carrying specific identified combinations of driver mutations have higher potential to form metastatic tumour in mice. 4) Based on the results, we will propose therapeutic strategies to block the interactions between tumour clones, and tumour and its microenvironment, and stop cancer progression. The project is based on existing technologies[7,8,10] and both the collaborating PIs have strong track records and will provide important guidance for the potential candidate.

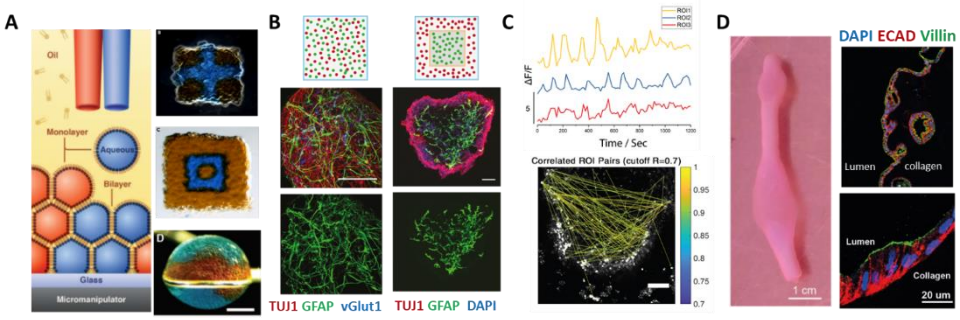

Figure 1: 3D droplet printing technique, and printed functional neural and gastrointestinal tissues. A, Droplet network printing mediated by lipid bilayer formation (left) and a gallery of printed 3D droplet networks with different patterns (right).[10] **B**, Neuron-glial tissues printed from designed patterns.[7] Cortical neurons were derived from human iPSCs. **C**, Printed human neural tissues showed spontaneous calcium oscillations (top) and formed a connected neural network (bottom) after 44 days of post-printing differentiation.[7] **D**, Engineered GI tract with a fibroblast-induced complex shape (left) and Engineered GI tissue with the epithelium layer derived from fused human duodenum organoids (left).[8]

Translational Potential

CRC is one of the major causes of cancer deaths worldwide.[11] The proposed project would elucidate the nature of tumour heterogeneity and the role of cancer microenvironment in cancer progression, which aligns with CRUK and the Oxford Centre's research priorities. Specifically, we will use engineered colon organoids to model colon cancer initiation and progression. New treatment strategies, such as targeting interaction between cancer cells and their microenvironment, might be discovered from the proposed project. The potential new treatments could also be used in combination with other therapies to provide a viable approach for the treatment of colon cancer in clinic.

Training opportunities

The potential DPhil student will be trained in the following fields: 1) Organoid culture derived from both mouse and human GI tissues; 2) Bioengineering techniques including 3D printing, hydrogel manipulation and microfabrication; 3) Microscopy (especially confocal microscopy) and general molecular biology techniques; 4) single cell sequencing and data analysis; 4) mouse implantation.

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3. Genetic engineering of the cell of origin of sonic hedgehog medulloblastoma in human pluripotent stem cell-derived cerebellar organoids [1,2,3] - Associate Prof. Becker

Primary Supervisor: Associate Prof. Esther Becker
Additional Supervisors: Dr. John Jacob
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Project Summary
Medulloblastoma is the commonest malignant brain cancer in children with a large unmet therapeutic need (1). Although cure rates of ~ 70% have been achieved, adverse effects of the disease and its treatment are debilitating or lethal and include neurocognitive effects, growth retardation and metastatic spread (2). There are four major subtypes of medulloblastoma that differ in terms of their molecular profiles, demographics, clinical characteristics and treatment (2). The failure of two-dimensional cell culture models or animal models to accurately recapitulate medulloblastoma in patients has resulted in a lack of innovative new treatments for over thirty years. Sonic hedgehog medulloblastoma (SHH-MB) is a major subtype whose developmental and cellular origins have been extensively characterised (3). Although granule cell progenitors of the cerebellum have been proposed as the most likely candidate cell of origin, the consequences of mutations arising in this specific cellular context remain to be elucidated. This is important as identical patterns of somatic mutations have distinct phenotypic effects that depend on the cell lineage or cell of origin (4). This project will bring together cutting-edge human induced pluripotent stem cell (hiPSC) and gene editing technologies to generate a sophisticated and novel in vitro organoid model of SHH-MB to investigate disease progression and as a platform to test new therapies.

Background
SHH-MB is a devastating disease that accounts for a third of cases of medulloblastoma (1). SHH is a signalling molecule that regulates cell proliferation, specifies cell identities in the developing central nervous system and is involved in stem cell maintenance (3). The incidence of SHH-MB is markedly increased in patients with Gorlin syndrome, which is caused by germline heterozygous mutations in the tumour suppressor gene, PTCH1 that encodes the SHH receptor (3). PTCH1 mutations constitutively activate SHH signalling within developing SHH-responsive granule progenitor cells, defined by the expression of the transcription factor, ATOH1 (5), triggering loss of the remaining PTCH1 allele and further genetic events of tumorigenesis (6). The effects of the PTCH1 mutation are manifested by uncontrolled proliferation of granule progenitor cells, but not of their multipotent ancestral cells (7). To model processes in the developing human cerebellum and SHH-MB, three-dimensional cerebellar organoids containing all major cell types of the developing cerebellum, have been generated from hiPSC by the Becker group (8).

Research objectives
Objective 1
Genetically engineer hiPSC lines harbouring an ATOH1 reporter. To facilitate human granule progenitor cell identification and enrichment, viral mediated delivery of an ATOH1 reporter transgene (9) or CRISPR knock-in of a reporter gene at the endogenous ATOH1 locus (10) in hiPSC will be performed. To assay the phenotype of presumptive human granule progenitor cells in culture, clonal lines with a normal karyotype and pluripotency characteristics will be expanded and differentiated to cerebellar organoids. After organoid dissociation and cell sorting by flow cytometry for the fluorescent reporter, tagged and untagged cells will be compared for the expression of a panel of markers of human granule progenitors by immunostaining and qPCR (8). Both groups of cells will be treated with SHH to test for a strong proliferative response of tagged cells (3). These experiments will enable the identification and isolation of the cell subtype that is implicated in SHH-MB.

Objective 2
Introduce SHH-MB-relevant mutations into an hiPSC reporter line. We will target PTCH1 that is mutated in SHH-MB in the generated ATOH1 reporter hiPSC line, to produce heterozygous and homozygous hiPSC clones using CRISPR. Successfully targeted clones will be identified by polymerase chain reaction and confirmed by Sanger sequencing. We will select hiPSC based on the above characteristics and differentiate these mutant lines to cerebellar organoids. The phenotype and growth properties of the tagged mutant cells will be assayed in situ in the

organoid and in tagged human granule progenitor cells isolated by flow cytometry, for the expression of cerebellar markers, SHH-MB markers and cell proliferation assays (11, 12). This aim will deliver and validate a well-defined set of tools with which to model SHH-MB.

Objective 3
Characterise co-culture models of mutant granule progenitor cells and cerebellar organoids. We will perform co-cultures of isolated tagged wild type or mutant human granule cell progenitors and wild type or mutant heterozygous cerebellar organoids to mimic tumour formation in vitro. The growth of the labelled granule progenitor cells will be tracked using fluorescence microscopy and time lapse imaging. The models will be analysed immunohistochemically and molecularly and compared to existing datasets of patient SHH-MB (13). Together, these experiments will provide unique insights into early tumour development and the role of specific genetic mutations in the cells of origin and their environment.

Collaborations
This is a novel collaboration that brings together three ongoing collaborators, Dr Jacob, Prof. Becker and Prof. Cowley, and a new collaborator, Dr Riepsaame. Dr Jacob is a clinician-scientist and neurologist, who is building expertise in the biology of medulloblastoma using organoids. Prof. Becker has established protocols for the generation of cerebellar organoids. Prof Cowley is Head of the James Martin Stem Cell facility and will provide expert guidance in stem cell culture, and the generation of stable transgenic cell lines. Dr Riepsaame is Head of Genome Engineering Oxford and will advise on vector design, the cloning of transgenes and reporter gene knock-in. Together, our broad expertise covers the full scope of this project.

Translational Potential
Isolation of the cell of origin or cell lineage that gives rise to medulloblastoma would make it possible to systematically identify the sequence of genetic events that initiate oncogenesis and its progression, potentially leading to the discovery of early disease biomarkers. By serving as a drug-testing platform, there could also be important therapeutic implications, based on the paradigm of colorectal cancer. In mice with this cancer, identification of the cell of origin led to trials of preventive chemotherapy in the premalignant phase (14). The novel co-culture system holds great potential to become the leading in vitro model because this technology uses human cells derived from iPSC, which can generate a limitless supply of three-dimensional cultures of cerebellar cells and isogenic controls. Furthermore, clinically relevant MB driver mutations can be engineered within granule progenitor-organoid co-cultures in a manner that recapitulates patient disease.

Training opportunities
Prof. Becker and co-investigators have extensive experience of training and mentoring students. There will be training in stem cell maintenance and differentiation, organoid culture, microscopy, flow cytometry, molecular biology and gene editing techniques. Participation in the drafting of manuscripts will be strongly supported. Students will be encouraged to present their findings at conferences.

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4. The role of RNA binding proteins in immune cell modulation in pre-invasive carcinoma [1,2,3] - Dr. Blagden

Primary Supervisor: Dr. Sarah Blagden
Additional Supervisors: Dr. Jelena Bezbradica Mirkovic
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Project Summary

Macrophages and T cells play a crucial role in immune surveillance to prevent invasive carcinoma.[1,2] Cancers must therefore evade the immune response to become established and will often modulate the immune response to prevent the activation of tumoricidal T cells and macrophages.[3] Our group and others have shown this immune modulation is already present in pre-invasive lesions that serve as precursors to invasive cancers.[4] Two such pre-invasive conditions are serous tubal intra-epithelial carcinoma (STIC), a precursor of ovarian cancer, and ductal carcinoma in situ (DCIS), a precursor of breast cancer. In both STIC and DCIS we observe greatly elevated expression of RNA-binding proteins (RBP) known to drive cancer progression by promoting anabolism and ribosome biogenesis.[5]

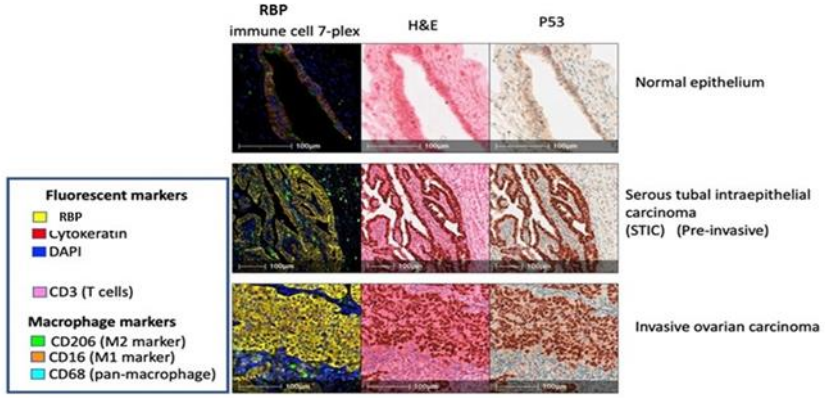

Figure 1. Expression of RBP (yellow) as part of an immune multiplex panel stain, alongside mP53 and H&E staining in normal epithelial tissue, STIC lesion, and ovarian carcinoma. Elevated RBP expression correlates with expression of mutant P53, a key indicator of the presence of a STIC. Also included in the immune multiplex panel are stains for the macrophage markers CD206, CD16 and CD68, and the T cell marker CD3.

However, we have also observed elevated levels of RBP in the macrophages and T cells surrounding STIC and DCIS (Figure 2a). Furthermore, we have shown in vitro that RBP expression is increased during macrophage polarisation suggesting a role in immune cell phenotype (Figure 2b).

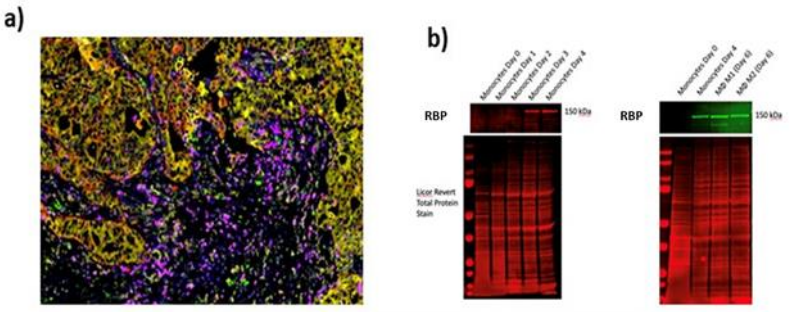

Figure 2: (a) Expression of RBP (yellow) as part of an immune multiplex panel stain for ovarian cancer, showing exclusion of T cells (CD3 purple) and M2-like macrophage (CD206 – green) at the tumour border. (b) RBP is expressed in late monocytes and M1 and M2 macrophages but not in early monocytes.

In this project, the candidate will use immunofluorescence multiplex microscopy to characterise the immune microenvironment of STIC and DCIS lesions and establish the role of RNA-binding proteins (RBPs) in transitioning a preinvasive lesion to its invasive counterpart. Furthermore, using fresh blood cones in vitro, they will characterise the role of RBPs in macrophages and T cells. Finally, using RBP-conditional knockout mice, they will validate the role of RBPs in tumour development and in the modulation of the tumour immune microenvironment. Collectively, these findings will determine how the immune system is modulated by pre-invasive cancer cells to allow their subsequent progression to invasive carcinoma.

Research Objectives

Objective 1

Using patient tissue samples of STIC and DCIS, as well as invasive ovarian and breast cancer, characterise the expression of RBPs in tumour cells and in immune cells to determine how they modulate the pre-invasive and invasive immune microenvironment.

Proposed outcome: Determine (a) if RBPs drives the formation of pre-invasive lesions and their progression to invasive cancer and (b) determine if they are associated with reduced immune cell infiltration and loss of immune surveillance

Objective 2

Characterise the role of RBPs in immune cells using macrophages and T cells from fresh blood cones by altering their expression through shRNA/siRNA-mediated knockdown

Proposed outcome: Determine the role of RBPs in immune cells through macrophage and T cell activity assays when the target is depleted or overexpressed

Objective 3

Validate the role of RBPs in immunity in an in vivo setting using RBP conditional knockout mice

Proposed outcome: Determine if RBP-null macrophages and T cells result in altered immune cell infiltration in tumours established in mice

Collaborations

Collaborations will be facilitated as the student will work jointly in the labs of Dr. Blagden and Dr. Bezbradica Mirkovic. The candidate will have regular meetings with both supervisors and will regularly assess how different branches of the project connect with each other.

Translational potential

In an ongoing parallel project, we are already exploring whether the high levels of RBP expression in pre-invasive lesions can be used as an early detection marker for ovarian and breast cancer. This project will provide evidence that preinvasive cancer is immune regulated and enable us to explore whether immune-targeted therapies could be used as cancer prevention in high-risk patients.

Training opportunities

Immunofluorescence microscopy, use of HALO software for multiplex fluorescence analysis, flow cytometry, handling of human blood and tissues, mouse work (candidate will be trained to receive a personal license)

References

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5. Modelling cancer stem cell dormancy using organoids and advanced 3D culture models [1,2,3,4] - Dr. Boccellato

Primary Supervisor: Dr. Francesco Boccellato
Additional Supervisors: Prof. Colin Goding
Eligibility: Track 1, 2, 3 and 4 students are eligible to apply for this project.

Research summary

Over recent years substantial advances have been made in our understanding of cancer and the development of a range of more effective therapies. Nevertheless, after apparently successful anti-cancer therapy, disease may recur even after many years owing to the presence of therapy-resistant cells. One of the principle causes of relapse is cancer cell dormancy. Some cancer cells stop dividing and enter a dormant state resembling that used by many physiological stem cells that divide to regenerate damaged tissue or replace cells that are naturally turned over. Why and how cells enter or emerge from a state of dormancy is unclear, but understanding how cells become dormant may offer opportunities for therapies designed to reduce relapse. Investigating human dormant cancer stem cells has been difficult due to the lack of appropriate in vitro models, and our current knowledge is extrapolated from experiments in mouse models or steady state analysis of human tumour masses. In this project we aim to use a newly identified biomarker for dormancy to detect and study this cell state ex vivo by using patient-derived biopsies. Further we will use organoids and other advanced cell culture models (the mucosoids) to identify and isolate dormant cells in vitro. The specific aims are to: 1. Characterise the hallmarks of dormant cells; 2. Decipher the signals regulating the generation, maintenance and elimination of dormant cancer cells. This study promises to identify potential therapeutic vulnerabilities in cancer dormancy. To achieve this we will use single cell RNA-seq, label retention and lineage tracing, 3D culture systems, and live cell fluorescent reporter assays to compare dormant cancer cells with their physiological counterparts.

Research objectives

Background. Tumours contain two kinds of non-proliferating cancer cells that are either quiescent or dormant. Most studies on dormancy do not distinguish between these two states. By examining physiological stem cells we have found that a hallmark of dormant, but not quiescent, cells is that they turn off expression of the majority of genes by shutting down transcription, evidenced by absence of an RNA polymerase II (PolII) modification associated with active transcription (Figure 1A). Since low transcription would reduce protein translation and suppress cellular metabolic activity, transcriptional silencing would allow cells to remain inactive for long periods. Importantly, such 'PolII-negative' cells are also found in human melanomas, mouse patient derived xenografts (PDX) (Figure 1B) and in 3D models of melanoma (not shown), but are not observed in 2D culture. The Goding lab has also developed a novel lentivirus-delivered ratiometric fluorescent reporter designed to enable detection of dormant PolII-low cells (Figure 1C). Using this reporter in intestinal organoids revealed that PolII-low cells at the base of the crypt (Figure 1C, open arrows) exhibited both red and green fluorescence, whereas PolII high cells (eg. white closed arrows) were red only. Although still to be tested widely, this reporter potentially enables live dormant cells to be isolated and characterised. The Boccellato lab has developed a homeostatic, long-lived (>1 year) stem cell based human primary cell culture, called mucosoids, for the stomach (Figure 2A,B) and is currently using this technology to cultivate cells from fallopian tubes (Figure 2C,D).

Objective A. **Characterise the hallmarks of dormant cells.** Since PolII-low dormant cells can readily be detected in organoids, 3D culture and in tumours we will use the PolII marker in co-immunofluorescence assays to identify characteristics of dormant cells together with antibodies directed against candidate markers of interest. These include epigenetic marks, cell surface markers associated with minimal residual disease (eg aquaporins, CD36), and markers of stem cell populations identified by lineage tracing experiments in gastric and other organoid systems. In parallel, we will undertake laser-capture microdissection of human and PDX model tumour sections followed by RNA-seq to identify gene expression patterns associated with the PolII-low population, and by using the live cell fluorescent reporter assay in organoids or mucosoids, isolate candidate dormant cells for single cell-RNA-seq. We anticipate that we will identify biomarkers of dormant cells that will be useful for monitoring their generation and response to microenvironmental cues and to therapies, and a gene expression program that will reveal mechanisms underpinning the generation and maintenance of dormant cells.

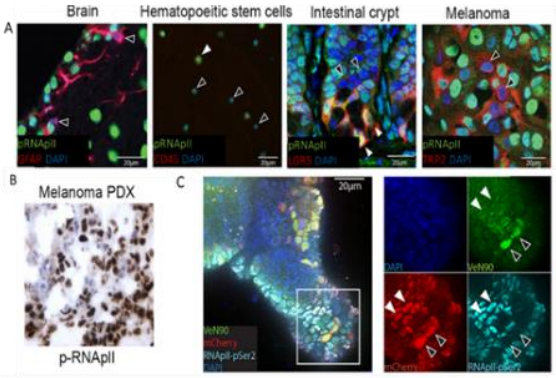

*Figure 1. Detecting PolII-low dormant cells. **A.** Immunofluorescence using PolII-specific antibody (Green) together with lineage markers (red) and DNA (DAPI, blue). **B.** Detection of PolII-low cells in a melanoma PDX mouse tumour. **C.** Activity of a ratiometric fluorescent reporter to detect dormant cells. PolII-Low cells (open arrows) marked by absence of blue fluorescence are Green and red, but PolII-High cells are red only.*

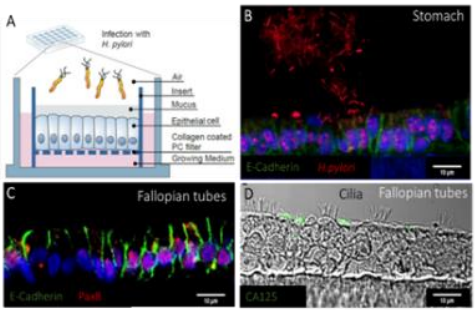

*Figure 2. **A.** Schematic of a mucosoid culture. **B.** Gastric mucosoid culture infected with H.pylori. **C.** A fallopian tube mucosoid culture stained for the stem cell marker Pax8. **D.** the same culture stained for CA125 and with visible cilia*

Objective B. **Decipher the signals regulating dormancy.** Using the biomarkers identified, including low PolII, we will then vary culture conditions for physiological and cancer organoids and 2D cultures to identify signalling pathways that increase or decrease the proportion of dormant cells. These conditions include hypoxia, nutrient limitation, infection and signalling molecules such as TGF or TNF that are important in generating physiological stem cells, as well as targeted and chemotherapies. RNA-seq data will be mined for clues to key signalling pathways that may represent targetable vulnerabilities in dormant cells. The results obtained will generate novel insights into the origins of cancer stem cells, their relationship to physiological stem cells and should identify potential therapeutic vulnerabilities in dormant cells.

The strength of this proposal comes from the shared interests and complementary expertise of each group in comparing physiological stem cells to those present within cancers, and especially in using the low-PolII status of dormant cells to identify and characterize dormant cells and their relationship to minimal residual disease.

Translational potential

Stomach cancer and ovarian have a higher rate of recurrence especially when diagnosed and treated at later stages (the recurrence rate is 60% and 75% respectively). We anticipate that the characterization of dormant cells may enable therapeutic vulnerabilities to be identified, with the potential to prevent and eradicate dormant cancer cells. We have developed a novel stem cell driven primary culture for the stomach and for fallopian tubes as a tool to investigate epithelial cell lifestyle. By studying the biology of the maintenance of dormancy in healthy cells *in-vitro*, we aim to understand the mechanisms that allow dormant cells to escape therapeutical targeting in cancer. The identification of further markers associated with features of cell dormancy can have a diagnostic value for the identification of gastric and ovarian cancer patients with higher probability of disease relapse. Moreover, by understanding how cells leave the dormancy state, we are exploring new options to reduce the number of insensitive cells and augment the efficacy of chemotherapy.

Training opportunities

Day-to-day supervision and training will be provided by Francesco Boccellato and from post-docs in the Goding and Ahmed labs who will meet on a weekly basis. As Francesco Boccellato is new to CRTF he will benefit from co-supervision by Colin Goding who has supervised over 17 PhD students/Clinical Research Fellows who all completed on time and have publications arising from their work. Also, the clinical supervisor Ahmed Ahmed has extensive experience in training CRTFs and DPhil students. The CRTF will formulate a structured research and development plan in consultation with the supervisors to ensure progress. Extensive training is provided in cutting edge technologies including organoid and mucosoid culture, single cell RNA-seq, and real-time imaging, and complementary skills.

References

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6. Multiscale computational modelling as a mechanistic tool to understand genotype-phenotype relationships in colorectal cancer[4] – Prof. Byrne

Primary Supervisor: Prof. Helen Byrne
Additional Supervisors: Prof. Xin Lu, Prof. Heather Harrington, Prof.Simon Leedham
Eligibility: Track 4 students are eligible to apply for this project.

Abstract
In vitro organoids derived from human colorectal cancer stem cells provide exquisitely detailed dynamic information about the growth patterns of colorectal cancer and their responses to treatments. At the same time, analysis of single-cell RNA sequencing (scRNA-seq) data enables identification and characterisation of different cell types. Machine learning techniques can establish relationships between the genotype and phenotype of cells within the organoids, however they lack a mechanistic basis. This project aims to bridge this mechanistic gap through computational modelling and, then, to exploit the new understanding to predict responses to existing treatments and to identify new therapeutic targets.

Research objectives
Background. Colorectal cancer is among the most prevalent human cancers, causing almost 1 million deaths per year globally and placing a significant socio-economic burden on public health. Improvements in its early detection and treatment are urgently needed to reduce incidence rates and improve patient outcomes. Organoids are 3-dimensional, self-organised multicellular structures that derive from stem cells and recapitulate features of a particular tissue or tumour. As such, patient-derived colorectal cancer organoids represent an ideal in vitro assay for studying colorectal cancer progression and its response to external perturbations [5]. In ongoing joint work, researchers in the Byrne, Harrington, Leedham and Lu labs are developing and applying statistical and topological methods to qualitatively and quantitatively describe the spatial patterns of organoids and how these change over time, under different culture conditions and for genetically distinct cancer stem cells. They are also using data science tools (eg U-MAP and Mapper) to analyse scRNA-seq data collected from the organoids in order to classify and quantify different organoid cell types and when they emerge. Integration of these data using machine learning techniques provides an exciting opportunity to relate the genetic and phenotypic profiles of cancer stem cells and their progeny. Without a mechanistic underpinning, it is unclear how these relationships can be exploited to improve patient treatment and outcomes. In this project, we aim to bridge this gap by developing hybrid cell-based computational models that describe the growth of organoids derived from colorectal cancer stem cells and using them to establish a mechanistic basis for observed genotype-phenotype relationships. These computational models will extend hybrid cell-based models developed in the Byrne lab to study homeostasis in the intestinal crypt and the early stages of colorectal cancer [1,2,3]. The existing models couple subcellular models of Wnt and Notch to cell proliferation, migration and differentiation and have been used to investigate monoclonal conversion of intestinal crypts, and the fixation and invasion of mutant cells in intestinal crypts.

Objectives. The main project objectives are first to develop a hybrid cell-based model that simulates the growth dynamics and spatial patterns of organoids derived from colorectal cancer stem cells, and then to use it to establish a mechanistic basis for observed genotype-phenotype relationships in colorectal cancer cells and to predict responses to different perturbations.

Approaches. We will develop a spatially-resolved, multiscale computational model that describes the in vitro growth of organoids derived from colorectal cancer stem cells and distinguishes individual cells. Within each cell, dynamic subcellular models of signalling pathways frequently mutated in colorectal cancer (eg Wnt, p53, Notch and KRAS) will be coupled to dynamic models of cell behaviours (eg cell proliferation, migration, differentiation and death). The cellular and subcellular dynamics will be regulated by cross-talk with neighbouring cells and external stimuli which represent specific organoid culture conditions. Model simulations will generate dynamic macroscale data about the size, morphology and spatial composition of the organoids and subcellular data about the genes and proteins expressed by each cell. By performing extensive parameter

sensitivity analyses in which we focus on varying parameters that are known to vary with specific mutations [1,2,6], we will use the computational model to investigate how mutations in specific pathways (eg p53, KRAS) affect organoid growth dynamics and their response to different culture conditions. In this way, we will learn how changes in the cells' genotype (and their culture conditions) affect the growth dynamics of the organoids. Comparison with in vitro data will be used to generate mechanistic explanations for genotype-phenotype relationships derived from the experimental data.

Proposed outcomes. The project will deliver a versatile multiscale computational model that simulates the growth of organoids derived from colorectal cancer cells in diverse culture conditions and that can be calibrated against experimental data. The model will serve as a valuable tool for testing experimentally-derived hypotheses, for guiding experimental design, and as a testbed for generating new predictions. By providing dynamic and spatially-resolved information about the cellular and subcellular heterogeneity of colorectal cancer organoids, the computational model will also be ideally suited for analysing and interpreting spatially-resolved, multiplexed imaging data.

Translational potential

The mechanistic relationships between cell genotype and phenotype that this project aims to deliver have significant translational potential for supporting clinical decision making and, in particular, the personalisation of treatment for patients with colorectal cancer. They could also lead to the identification of novel therapeutic targets.

This project aligns with three underpinning scientific themes of the Oxford Cancer Centre's research strategy: cancer big data, digestive cancers, and genes. By increasing understanding of the mechanisms by which colorectal cancers develop, it will also help to accelerate the discovery and development of new therapeutics, a key objective of CRUK strategy.

Training opportunities

Prof. Byrne will be responsible for overall project strategy and, with Prof. Harrington, provide day-to-day supervision of the DPhil student, drawing on her extensive experience of mentoring non-clinical DPhil students. Prof. Harrington brings expertise in data science, particularly topological data analysis. The student will attend relevant Masters-level lectures, as necessary, and receive technical training in mathematical and computational modelling, particularly hybrid multiscale models that couple agent-based models with ordinary and partial differential equations. Profs Lu and Leedham will join monthly team meetings. Prof. Lu has extensive mentoring experience and will provide expertise in cell and molecular biology techniques, including single cell sequencing and organoid culture technologies [4]. Prof. Leedham brings complementary clinical expertise. The student will benefit from expertise at the Mathematical Institute's Wolfson Centre for Mathematical Biology (WCMB) and the Ludwig Institute for Cancer Research in Oxford. They will integrate with the wider scientific and clinical communities in Oxford through established collaborative networks, and with national and international communities at conferences. The student will join WCMB's large mathematical oncology group, participate in its weekly group meetings and attend WCMB's formal seminar series. They will benefit from the Mathematical Institute's mentoring and career development programmes, the latter providing training in areas that include scientific writing, oral presentations and public engagement

References

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7. Understanding STING regulation in cancer and the crucial role of ubiquitination in the ER [2,3] – Associate Prof. Christianson

Primary Supervisor: Associate Prof. John Christianson
Additional Supervisors: Dr. Eileen Parkes
Eligibility: Track 2 and 3 students are eligible to apply for this project.

Project Summary
Cancers interact with their surrounding environment (the tumour microenvironment) by remodelling it to contain cells promoting tumour invasion and spread, and resistance to anti-cancer therapies. Innate immune pathways, typically used to defend cells from infection by viral and bacterial pathogens, are hijacked in cancer. The mechanisms by which cancer cells modify innate immunity are currently not well understood. A key pathway is the cGAS-STING pathway – the cytoplasmic sensor cGAS recognises non-self or mislocalised DNA and activates STING (the STimulator of Interferon Genes). STING is embedded in the endoplasmic reticulum (ER) – activation of the STING-mediated interferon response requires oligomerisation and efflux from the ER (1). Fine tuning of this response is paramount, and ubiquitination of STING has emerged as an important post-translational modification capable of modulating these signalling events. Importantly, evidence is emerging of important interferon-independent effects of cGAS-STING signalling which may drive tumour progression. Establishing how ubiquitination and its conjugating machinery impact the cGAS-STING pathway is key to understanding how cancers subvert this pathway to their own ends.
This DPhil project will biochemically and functionally characterise ER-resident ubiquitination machinery that modulates STING signalling in order to delineate its regulation of the interferon response.

Research objectives
Recently, our lab identified a multi-subunit complex organised around ER-resident ubiquitin ligase (E3) RNF26, whose constituents modulate signalling through STING to scale the magnitude of the interferon response (2). We are now investigating how each component of this RNF26 complex impacts STING to contribute to the response, focusing on defining protein-protein interactions, key functional domains, ubiquitin linkages, complex assembly, and its synergy (or competition) with other ubiquitin ligases. This is crucial as understanding STING regulation will identify mechanisms of resistance to immune targeting agents (immune checkpoint blockade and STING agonists) in advanced cancers.

Objective 1: Molecular dissection of ubiquitin conjugating machinery competing to modify STING in the ER. Genomic editing, gene silencing and dominant negatives will establish the individual and combinatorial contributions of ER-resident E3s (RNF26, RNF5, gp78) to STING properties including its; stability/degradation, ubiquitination profile, oligomerisation, trafficking, and activation of the downstream interferon response, in model cell lines. The diversity and dynamic nature of ubiquitin chain linkages modifying STING will then be explored using both mass spectrometry and sensitivity to linkage-specific deubiquitinases.
Outcome: Establishment of key ubiquitination events governing STING in the ER and consequently the magnitude of its downstream signalling cascade

Objective 2: Defining how cofactors contribute to ER-E3 recognition and/or ubiquitination of STING. Potentially important regulatory domains of E3 complex components identified bioinformatically will be evaluated functionally using truncations and site-directed mutagenesis. This will be complemented by proximity-labeling strategies coupled with proteomics to define the spatiotemporal organisation of E3 complexes and their interaction/s with STING. STING agonists and antagonists (currently being developed for clinical applications) will be used to pharmacologically probe for changes in E3-STING interaction.
Outcome: An understanding of the how ubiquitin multifaceted regulation of STING at the ER influences response to activating treatments.

Objective 3: Preclinical validation of STING modulating factors. Identified STING regulating factors will be modified using gene editing and CRISPR-cas9 approaches using an ex vivo platform (i.e. culturing cells from patient samples). These samples will be used to generate organoids with/without fibroblasts and patient-matched immune cells. This

near-patient system will be used to determine the effect of modulation of STING regulating factors on the tumour microenvironment. Using this platform, immunotherapeutic stimulants can be added to determine the role of STING-modulating E3s and co-factors in response to existing immunomodulating treatment will be investigated. Flow cytometry and T cell activity assays will be employed to measure the impact of novel targets on immune response in this near-patient model.
Outcome: Characterisation of targetable mechanisms of STING suppression determining response to cancer immunotherapy.

Collectively this research will develop insights into the fundamental cellular controls of immune signalling. Along with ongoing work in the lab, it will form part of our broad effort to explore and define ubiquitination events and mechanisms at the ER responsible for essential cellular homeostatic functions.

Translational potential
This project will address important fundamental and clinical questions relevant to personalising immunotherapy treatment in cancer. Tailoring immune targeting approaches and understanding resistance mechanisms (such as STING repression) has potential to improving clinical responses. In this study novel STING regulating mechanisms will be characterised as potential biomarkers and/or targets for further clinical study. Moreover, this proposal uses patient samples for 3D modelling, further supporting translation of this work to the clinical setting.

Training opportunities
There will be multiple training opportunities available during the project including; advanced cell biology and biochemistry, proteomic sample preparation and analysis, flow cytometry, and 2D and 3D cell culture modelling. There will also be opportunities to present findings at local, national and international conferences.

References:
1.Hopfner K and Hornung V (2020) Molecular mechanisms and cellular functions of cGAS–STING signalling. Nature Reviews Molecular Cell Biology. 197: 1-21 (LINK). **2.** Fenech EJ, Lari F, Charles PD, Fischer R, Thezenas ML, Bagola K, Paton AW, Paton JC, Gyrd-Hansen M, Kessler BM, Christianson JC (2020) Interaction mapping of endoplasmic reticulum ubiquitin ligases identifies modulators of innate immune signaling. eLife 2020;9:e57306 DOI: 10.7554/eLife.573 (LINK) **3.** Parkes EE, Walker SM, Taggart LE, et al. Activation of STING-Dependent Innate Immune Signaling By S-Phase-Specific DNA Damage in Breast Cancer. J Natl Cancer Inst . 2017;109(1). doi:10.1093/jnci/djw199.

8. Genetic and functional characterisation of novel immune escape mutations in DNA mismatch repair deficient cancer [1,2,3] – Dr. Church

Primary Supervisor: Dr. David Church
Additional Supervisors: Prof. Tim Elliot and Dr. Nicola Ternette
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Project Summary
Defects in DNA mismatch repair (dMMR) occur in many cancer types, where they cause increased tumour mutation burden (TMB) and instability at DNA microsatellites (MSI). dMMR colorectal cancers are characterised by an increased density of infiltrating immune cells, and in early stages, a better prognosis (presumed due to a T cell response against mutated (non-self) peptides)1. However, the fact that such cancers grow to be clinically detectable indicates that immune escape has occurred. Previous work has identified that this is often due to acquired defects in MHC class I antigen presentation; however current understanding of these is incomplete. Thus there is a pressing unmet need to identify and characterise additional mechanisms of immune escape, both for prognostication, and for prediction of benefit from immunotherapies which have become standard of care for metastatic dMMR tumours. Our unpublished analysis of 16,000 whole genome sequences from the Genomics England 100,000 Genomes Project (100KGP) has identified two novel candidate immune escape mutations which occur commonly in dMMR cancer of multiple types. Both act within the antigen processing and presentation pathways, providing a strong rationale for their further investigation. This proposal seeks to do this by combining genetic and immunological analysis of human cancer, with functional analysis of antigen presentation and the immunopeptidome in cell and animal models. The student will gain training and expertise in state-of-the-art experimental methods and bioinformatic analysis, and benefit from a highly collaborative project environment.

Research objectives
The objectives of this project are:
1. To help define the frequency, genetic, immunological and clinical correlates of novel candidate immune escape mutations in dMMR cancer, with focus on colorectal and endometrial cancers (CRC & EC)
2. To determine the impact of novel immune escape candidates on antigen processing and presentation in cell lines and human cancers
3. To define the impact of candidate immune escape mutations on the growth, immunopeptidome, immune infiltrate and sensitivity to immunotherapy of MMRd cancers in-vivo
Corresponding work packages and outcomes include:

WP1. *Characterisation of novel immune escape mutations and their correlates in MMRd cancer*
Preliminary data: Unpublished analysis of colorectal and endometrial cancers from the GEL 100KGP has identified recurrent frameshift mutations which occur with high frequency among dMMR cancers (25-50% cases). Further analysis of all 16,000 tumours indicates these genes are mutated across dMMR tumours of multiple types with variable prevalence (Fig. 1A). Preliminary analysis of the Cancer Cell Line Encyclopaedia, TCGA and a panel of endometrial cancer cell lines indicates these mutations are associated with reduced mRNA level and loss of protein expression. Interestingly, one of the novel genes operates in a ribosome-associated quality control pathway which has recently been implicated in MHC class I antigen presentation, while the second gene functions in the transport of MHC class I molecules. Thus, both are plausible immune escape variants in this hypermutated, immunogenic tumour subgroup.
Proposed work: The relationship between mutation of novel immune escape genes and other genomic factors (e.g. TMB, neoantigen burden, other immune escape mutations, clonality etc) and transcriptome will be defined in the Genomics England and TCGA (access approved) cohorts. In related work, the type, density, and localisation of Intratumoral immune infiltrate will be determined by multispectral co-IF (eg Vectra Polaris or GE Cell Dive) on FFPE tumour slides in CRCs and ECs from the Genomics England cohort. Digital pathological analysis of images will be performed by the group of Viktor Koelzer (Univ Zurich) in an extension of an existing collaboration2. Correlation of novel immune escape mutations with genomic factors and immune infiltrate will be performed by the student (after training) using unsupervised (e.g. random forests) and supervised methods with penalization given high-dimensionality of data. The student may also have the opportunity to travel to Leiden, Groningen or Zurich to contribute to this work or the corresponding analysis of the PORTEC3 trial (450 cases with tumour material).

Correlations with clinicopathological variables and clinical outcome (eg Cox PH models) will be performed by the student with all required training provided.
Outputs: Genomic, immunological and clinical correlates of novel immune escape mutations in MMRd cancer.
Academic value and collaborations: Definition of correlates and consequences of candidate immune escape mutations in common cancers. Collaborations with members of the GEL EC domain, Koelzer, Nijman (de Bruyn) and Bosse groups.

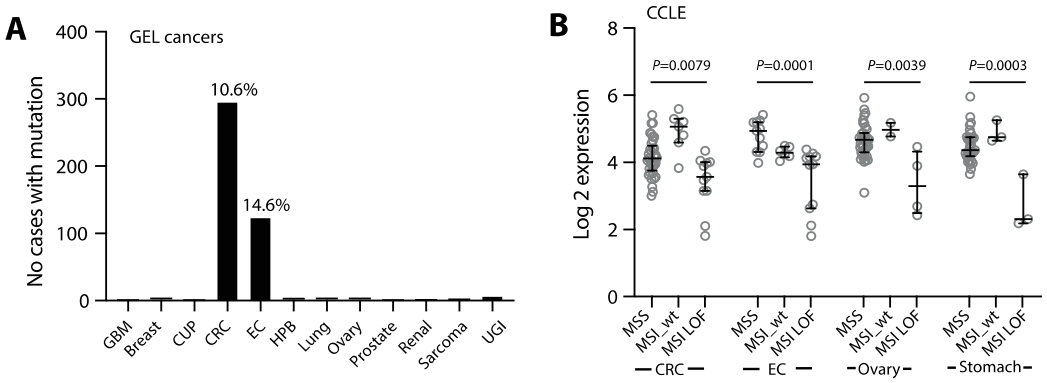

Figure 1. (A) Frequency of novel LOF immune escape mutation in Genomics England cancers by tumour type. (B) Association of MSI and immune escape gene LOF mutation status with expression in Cancer Cell Line Encyclopedia (CCLE)

WP2. *Impact of immune escape mutations on the immunopeptidome in cell lines and human cancers*
Preliminary data: The Ternette group have established reliable experimental workflows for the purification of MHC class I and II molecules from cells and the elution and characterisation of the immunopeptidome by mass spectrometry3. In unpublished work, they have extended this to characterise the immunopeptidome in renal cell carcinoma. The Elliott group have substantial expertise in the analysis of antigen processing. Exome sequencing of 25 EC cell lines in the Church laboratory reveals similar frequency of immune escape mutations to that found in the Genomics England cohort .
Proposed work: To define the impact of immune escape mutations on MHC class I presentation and the immunopeptidome we will perform both: (i) re-introduction of novel immune escape genes by stable re-expression (e.g. transduction) in EC/CRC cell lines with LOF mutations; (ii) CRISPR-Cas9 knockout in cells with normal expression of these genes. MHC class I pathway components will be interrogated by in-situ methods including live cell imaging where informative. Definition of the impact of such re-introduction/loss will be performed by the student under the supervision of a postdocs from the Elliott and Ternette labs. If successful, experiments will be extended to human cancers (~100 frozen ECs available at present; opportunity for prospective collection).
Outputs: Demonstration of the impact of novel immune escape mutations on the MHC class I processing and antigen presentation.

Translational potential
The widespread use of ICB for MMRd ECs and proven importance of antigen presentation in sensitivity to such agents provides immediate translational relevance. We will aim to rapidly transfer the findings of this work into the clinic through the TransPORTEC group and the UK AADSG.

Training opportunities
The student will join a recent, but well supported and highly collaborative research program. Genomic analysis of GEL cancers will be done under the supervision of Andreas Gruber, lead bioinformatician in the endometrial cancer GeCIP. AI-based image analysis will be led by the group of Viktor Koelzer. Functional work will be supported by dedicated postdoctoral scientist and research assistants in the Church, Elliott and Ternette laboratories.

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9. How does the tumour metabolic microenvironment affect macrophage function in germinal centre-derived lymphoma? [1,2,3] – Dr. Clarke

Primary Supervisor: Dr. Alex Clarke
Additional Supervisors: Prof. Irina Udalova
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Project Summary

Lymphoma is one of the most common types of cancer, with peaks in incidence in early adulthood and later life. Most lymphoma originates from B cells, and in particular those undergoing the germinal centre (GC) reaction[1]. The GC reaction occurs in secondary lymphoid tissue following infection or vaccination. The responding B cells entering the GC undergo an evolutionary process, in which they compete for help from T cells, and if successful, rapidly proliferate while at the same time mutating the DNA region which determines their specificity. This process then cycles until finally B cells with refined receptors exit to produce antibodies or as memory B cells. However, the DNA mutation which GC B cells undergo makes them vulnerable to genetic translocations that can induce lymphoma.
GC B cells have some of the highest proliferation rates of any cell in the body, and the overwhelming majority will die by apoptosis as they lose the competition for selection. The clearance of these dying cells is performed by a process known as efferocytosis, by GC resident macrophages called tingible body macrophages (TBM)[2]. Surprisingly little is known about TBM, and in particular how they maintain homeostasis whilst engulfing and digesting so many apoptotic cells. This question becomes even more important in lymphoma, as TBM become a prominent feature of the tumour landscape, giving rise to the 'starry sky' appearance classically seen in Burkitt's lymphoma, a GC-derived cancer typically seen in children in Africa or in association with AIDS.

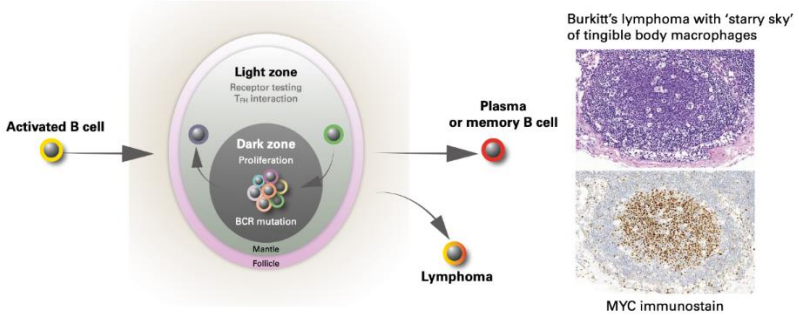

Figure.1. Germinal centre reaction, and example of tingible body macrophage dominance

Recent work has shown that as macrophages engulf dead cells their metabolism is altered, and they can secrete metabolites which have important biological effects[3,4]. How TBM interact metabolically with both the normal and tumour microenvironment (TME) is unknown. This important, because it is widely established that the disturbed metabolism in the TME profoundly affects the anti-cancer immune response[5]. Understanding how TBM contribute to this in the lymphoma TME would be a significant advance. In this project, you will characterise the metabolism of TBM in lymphoma, and understand how this interacts with tumour cells to influence disease.

Research objectives

Objective 1: Measure metabolism in TBM in situ in normal GCs and in lymphoma TME. We will use advanced imaging techniques to understand the metabolic profile of TBM in patient biopsies and murine lymphoma models, including mass spectrometry imaging, high dimensional imaging of metabolic proteins, and dynamic in vivo tracer systems. This aim will be facilitated by collaboration with the Oxford Lymphoid service (Dr Graham Collins and Dr Gareth Turner), and an existing partnership with the National Physical Laboratory.

Objective 2: Experimentally interfere with efferocytosis using genetically-engineered mouse models, and examine the effect on the progression of experimental lymphoma. We will both conditionally knock out Mertk and Cd47 in macrophages, to reduce and enhance efferocytosis respectively. We will then examine the effect on TBM metabolism, and lymphoma, and study how these interact.

Objective 3: Understand the effects of TBM-derived metabolites identified in objective 1 on lymphoma both in vitro and in vivo, aiming to develop novel therapeutic approaches.

Translational potential

Understanding how TME metabolism is controlled in lymphoma would be a significant advance, because it determines both tumour growth and progression, and also the outcome of the anti-tumoral immune response. Modification of efferocytosis is possible, and targeting the CD47 axis, thereby enhancing phagocytosis of lymphoma cells, shows promising clinical effect[6]. Metabolites may also function as signalling molecules, and therefore again provide novel therapeutic targets.

Training opportunities

This project provides a broad training in cancer biology and immunology, with comprehensive coverage of standard and advanced techniques including lymphoma models, advanced flow cytometry, confocal imaging, and measurement of epigenetic modification. For study of metabolism, you will develop expertise in stable isotope resolved metabolomics, extracellular flux measurement, using the Seahorse platform, and the bioinformatic analysis of these data. We collaborate with the National Physical Laboratory for mass spectrometry imaging (MSI), which you will have the opportunity of involvement with. You will combine MSI with high dimensional imaging to comprehensively map metabolism within tissues. You will also analyse human clinical samples obtained from patients with lymphoma.

References

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10. Application of multi-modal long-read single-cell sequencing to understand the role of clonality in Multiple Myeloma development[2,3,4] – Dr. Cribbs

Primary Supervisor: Dr. Adam Cribbs
Additional Supervisors: Prof. Udo Oppermann, Dr. Karthik Ramasamy, Dr. Sarah Gooding
Eligibility: Track 2, 3 and 4 students are eligible to apply for this project.

Project Summary
There are a reported 21,352 protein coding genes within the human genome, which speculatively express more than 100,000 different proteins. If we include T cell receptor, B cell receptor and antibody diversity then there are likely to be many millions of unique proteins. The diversity of the human proteome exceeds the genome, in part because of alternative splicing and recombination events, which can create many more combinations of substrates from the same gene or combinations of gene segments, respectively. Typically, droplet based single-cell sequencing methods (Drop-seq, In Drops and 10X chromium), the most widely adopted single-cell sequencing techniques, are only capable of measuring the 3' or 5' end of a transcript. Therefore, translocations and alternative splicing are difficult or nearly impossible to measure. Long-read sequencing methods present the ideal solution for measuring full-length transcripts. However, these methods have limitations, with low basecalling accuracy and sequencing quality, which makes their application to single-cell sequencing challenging.

In collaboration with the Oppermann group, work in the Cribbs lab focuses on developing novel single-cell technology and computational analysis frameworks that empower new modes of treatment for disease. Recently we have developed scCOLOR-seq1, a method to overcome low basecalling accuracy making long-read single-cell transcriptomic sequencing highly accurate. This technology opens the possibility of measuring translocations, variant calling and alternative splicing. We have begun to apply this technology to understand the development of drug resistance in Multiple Myeloma (MM).

We will now extend this work by developing and then applying multiplexed (transcriptomic and epigenomic) long-read single-cell sequencing assays to drug resistant models and primary patient samples. Resulting data will be analysed with the aid of a variety of machine learning techniques to develop a classifier to identify clones associated with disease progression and drug resistance. We aim to develop a robust approach to drug resistance classification for use by clinicians, as well as to investigate biomarkers of drug resistance.

Research objectives
Our ultimate aim is to apply our long-read single-cell technology to primary MM patient samples and then generate computational models that help us to identify the clonal origins of drug resistance in patients with relapsed MM.

Work package 1: *Establish multiplexed scCOLOR-seq in MM proteosome drug resistant cell lines.*
We have further developed scCOLOR-seq into a multi-plexed assay capable for measuring more than one analyte simultaneously. The student will apply this assay to investigate the transcriptomic and epigenomic (i.e. open chromatin) of drug resistant cell line models. Specifically, the aim of this work package will be to identify genomic signatures of drug resistance that can then be used to develop a machine learning model (work package 2).

Work package 2: *Development of a computational analysis strategy and machine learning models to identify MM clones.*
The student will evaluate the accuracy and utility of a variety of unsupervised and supervised classification and machine learning algorithms (e.g. k-means/hierarchical clustering, linear discriminant analysis, support vector machines, Neural Networks and others) to identify the clonal origin of MM drug resistance. Specifically, we would like to develop a classifier model using data from long-read sequencing (structural variation, isoform expression, gene expression) that can identify clonal features of proteasome inhibitor drug resistance. This classification model will become important for understanding the molecular mechanisms that underpin the early stages of drug resistance. We will then use the knowledge from this model and apply it to work package 3, to see if these features can predict drug resistance in MM patient samples.

Work package 3: *Apply multiplexed scCOLOR-seq in primary bone marrow samples from MM patients before and after the development of proteasome inhibitor resistance*.
Having understood the genomic signatures that help to infer clonality in drug resistance cell lines, working with our clinical colleagues, we will next apply this to MM patient bone marrow samples. Our aim of this work is to identify molecular signatures that predict the development of proteasome inhibitor resistant myeloma. We will use the knowledge developed during work package 1 and 2 to inform us of the most important gene and structural variants that may be driving drug resistance.

Translational potential
The stated aim of this project is to study the clonal nature of Multiple Myeloma drug resistance and evaluate drug targets for therapy. By its very definition, this is likely to identify novel therapeutic intervention points within the development of Multiple Myeloma. We have extensive collaborations with several pharmaceutical partners, and we will utilise these interactions to explore the translational potential of targets.

Training opportunities
The student will receive training in the necessary cellular, molecular, and epigenetic biology for this project. This will involve wet-lab workflows for generating short-read and long-read single-cell sequencing data. Extensive training in computational biology will be provided so that the student can analyse their own data. Specifically, this will include software development, data analytics, statistics and computational pipeline development. Outside the lab, the student will be expected to attend regular seminars with high profile external speakers, journal clubs and training in presentation skills, scientific writing, and data management.

References
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11. High resolution mapping of the chromatin landscape in chronic lymphocytic leukaemia to identify the effects of mutations in the non-coding genome [1,2,3,4] – Associate Prof. Davies

Primary Supervisor: Associate Prof. James Davies
Additional Supervisors: Dr. Anna Schuh
Eligibility: Track 1, 2, 3 and 4 students are eligible to apply for this project.

Project Summary
The vast majority of mutations in cancer genomes are in non-protein coding DNA. We have developed a platform for identifying the effects of non-coding mutations, which combines functional genomics including ultra-high resolution chromosome conformation capture (3C) techniques with machine learning approaches. We have set up a collaboration to apply this technology to a large whole genome sequencing dataset from patients with chronic lymphocytic leukaemia (CLL). Using our methods we will predict the effects of mutations in CLL genomes and particularly focus on recurrently mutated regions and mutations near known oncogenes. Our primary aim is to identify genes, whose expression is recurrently altered in CLL through mutations in the non-coding genome. This will potentially lead to new therapeutic targets and improved prognostication for patients.

Research Objectives
The falling price of whole genome sequencing now means that it costs less to sequence a patient's genome than many commonly used radiological investigations. As a consequence, whole genome sequencing is likely to become commonplace in routine cancer diagnostics. At present genetic prognostication in malignancy is largely based around large scale chromosomal rearrangements and detection of mutations in the coding sequence. However, over 95% of mutations in cancers are in the non-coding genome and it is being increasingly recognised that mutations in non-coding DNA can be driver mutations in malignancy. However, methods to systematically characterise the effects of such mutations are not well developed and it is challenging to differentiate mutations that alter prognosis from passenger mutations. Interestingly, many of the genes that are recurrently mutated in malignancies including CLL occur in proteins that play key roles in regulating gene expression such as transcription factors (P53, ZNF292, ZMYM3) or the chromatin state (ARID1A). An improved understanding of gene regulation in CLL is very likely to be useful for improving prognostication and it may identify novel therapeutic targets.
Anna Schuh's laboratory have recently performed paired tumour and germline whole genome sequencing on 540 patients with CLL as part of the 100,000 Genome Project (Genomics England). This has identified large numbers of mutations in the non-coding genome including hotspots, which are recurrently mutated in multiple samples. However, the functional effects of these mutations have not been characterised in detail.
It is challenging to identify how mutations in the non-coding genome lead to altered phenotypes for two main reasons. First, in contrast to the coding genome, there is not a straightforward universally applicable grammar by which the sequence is translated into phenotypic changes. Secondly, many important genes are controlled by distal regulatory elements known as enhancers, which may lie a million base pairs (bp) away from the genes they control. Consequently, it is difficult to identify which genes are being controlled by an enhancer and being affected by a mutation in that regulatory element. We have recently developed approaches to solve these two main challenges.
Jim Hughes' group have developed computational approaches that allow the effects of sequence changes to be predicted in the non-coding genome. This includes a machine learning model, based on 694 DNaseI hypersensitivity and ATAC-seq datasets (Schwessinger et al., Nature Methods 2020). Importantly, this can predict when sequence changes create novel regulatory elements as well as when they damage previously identified enhancers. Our approach has the potential to identify an important set of as yet unidentified genes where mutations in regulatory elements increase expression and lead to increased malignant potential. Since mutations in the coding sequence generally do not lead to a gain of function, these genes will not previously have been identified by conventional analysis of protein coding mutations.
James Davies' laboratory, has recently developed a novel high resolution chromosome conformation capture (3C) method (Micro Capture-C (MCC)), which allows the enhancers controlling a gene to be determined with

around 500 times better resolution than previous methods (Hua et al., Nature 2021). It is known that enhancers come into close proximity with the gene promoter to modulate gene expression and these physical contacts can be used to identify which elements are controlling which genes. Previous 3C methods have limited ability to resolve contacts much below 1kb; by contrast the key proteins controlling gene expression, such as transcription factors, bind short DNA sequences (~7-22 bp). The increased resolution afforded by MCC allows footprinting of transcription factor binding sites, in a similar fashion to DNaseI hypersensitivity.
Aim 1: To use machine learning approaches to identify regulatory elements that are recurrently mutated in CLL. The Schuh laboratory have previously identified hotspots in the non-coding genome, which are recurrently mutated in CLL. As part of a collaboration with Jim Hughes we will apply the machine learning approached we have previously developed to predict the effects of these mutations on enhancer function. This will involve training the previously established model on ATAC-seq data obtained from patients with CLL.
Aim 2: To develop an MCC panel to link the regulatory elements that are identified in Aim 1 to the genes they control. The Davies laboratory has recently developed an ultra-high resolution 3C method, which allows the regulatory elements controlling a gene to be characterised in unprecedented detail. The method is based on targeted oligonucleotide capture and this enables hundreds of genes / regulatory elements to be analysed in parallel. We will generate 3C libraries from 10-20 patients with CLL and simultaneously generate other datasets including ATAC-seq, RNA-seq and ChIP-seq for CTCF to annotate the MCC data.
Aim 3: To validate the regulatory elements identified in Aims 1&2 using genome editing at a small number of loci. The Davies laboratory also has expertise in genome editing, including primary cells from haemopoietic lineages. This will allow the findings from Aims 1&2 to be validated in primary cells or cell models. Outside of the scope of this project the Davies laboratory is developing high throughput genome editing screens, which could be applied to characterising the effects of mutations at scale.

Translational potential

This project has potential to have a very significant impact in two areas. First, it is likely that we will identify novel genes and pathways that are important in the pathogenesis of CLL. This will lead to a better understanding of the disease and in turn we will potentially identify novel therapeutic targets. Secondly, this study will allow us to go on to develop a pipeline for systematically identifying the effects of non-coding mutations in CLL. This would be important because it would potentially allow improved prognostication and improve treatment decisions for patients. Importantly, these methods for identifying the functional effects of mutations in the non-coding genome could be applied broadly to whole genome sequencing from potentially any tumour type.

Training opportunities

All basic approaches in molecular and cellular biology (PCR, Cloning, Cell culture etc.). We also offer training in more specialised functional genomics techniques of chromatin immunoprecipitation, 3C, RNA-seq and single cell techniques. We also have expertise in using cutting edge genome editing technology to modify the genome sequence in both cell lines and primary cells. There will be excellent training in the state-of-the-art FACS facility at the WIMM including isolation and purification of populations of leukaemic cells.
We also have expertise in bioinformatics. We are particularly interested in developing novel bioinformatic approaches to analyse next generation sequencing data. We have recently developed novel methods of analysing 3C datasets, which allow the data to be generated at much higher resolution than was previously possible. There is likely to be the opportunity to undertake the dedicated 3 month Centre for Computational Biology training course in bioinformatics.

References

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12. The association of obesity with care and outcomes for patients undergoing drug treatment for advanced malignancy recorded within the SACT database [1,3,4] – Dr. Dodwell

Primary Supervisor: Dr. David Dodwell
Additional Supervisors: Dr. Simon Lord
Eligibility: Track 1, 3 and 4 students are eligible to apply for this project.

Project Summary
Obesity will overtake smoking as the most significant environmental risk factor for the development of cancer within the next 10 years. Although the impact of obesity on cancer incidence has been much studied, the impact of obesity on treatment and outcomes following cancer diagnosis is unclear.
Some publications have reported the association between obesity and cancer outcomes but there are conflicting results. Most of these studies were of small size and made use of data collected from randomised controlled trials (RCTs). Only a tiny fraction of all patients participate in RCTs and these are highly selected and commonly unrepresentative of patients treated in routine care.

Hypothesis and Objectives: We intend to test the hypothesis that obesity in routine care is linked to poorer survival outcomes in patients undergoing therapy for advanced cancer. The study will initially consider the prevalence of obesity in cancer patients in England receiving systemic therapy and describe how body mass index (BMI) changes over the cancer patient journey. Associations between obesity and patient survival will be assessed according to tumour type, therapy class and multiple patient and treatment characteristics. Additionally, the relationship of obesity to intentionally reduced dosing will be described and related to outcomes.

Setting and Methods: The SACT dataset is unique worldwide in terms of its national coverage, size, mandated data collection within NHS England and detailed information on cancer type, demographics, performance status, co-morbidities, drug information, outcomes, and opportunities for linkage to other databases within National Cancer Registration and Analysis Service (NCRAS) providing additional co-morbidity information, cancer stage, hospital episodes and mortality. The SACT dataset provides an opportunity to examine outcomes in routine care and follow up is of sufficient duration to allow meaningful analyses of survival following the start of drug treatment in large cohorts of patients with common advanced cancers. Data collection was mandated from 2014 and the SACT dataset now contains >730,000 patients. Notably, body mass index (BMI) is included and is collected at multiple timepoints as this is required for dose calculation for most SACT.
For example, between April 2017 and March 2018, 41,165 breast cancer patients receiving SACT were captured by the database of which 86% had height and weight available.1 We already have ethical approval (REC ref: 19/NS/0057) and approval for release of data from the Office for Data Release in Public Health England (PHE).

Trajectories of BMI will be estimated based on repeated measurement through treatment. Non-parametric survival models will be used to assess survival for the full cohort and according to tumour and treatment types. Results will be adjusted for multiple variables including tumour stage, comorbidity, and performance status.

Research objectives
We intend to test the hypothesis that obesity in routine care is linked to poorer outcomes in patients undergoing therapy for advanced cancer.

Objectives
Describe the prevalence of obesity in patients undergoing cancer therapy in England across tumour types.
Describe how body mass index changes during treatment across tumour and treatment types.
Consider the association between survival and BMI.
Compare outcomes for capped and uncapped dosing of cytotoxic chemotherapy in obese cancer patients.

Work package 1:
Describe prevalence of obesity for all cancer patients receiving SACT in England. Describe the prevalence of obesity for patients with advanced cancer receiving SACT in England across different age groups and ethnicities. Describe prevalence of obesity for cancer patients receiving SACT for specific tumour types: melanoma, colon, breast, endometrial, gastric, pancreas, gallbladder, hepatocellular cancers. Describe how BMI changes for patients on SACT for all patients and across different tumour types from first to later lines of therapy.

Work package 2:
Consider adjusted survival outcomes according to BMI, from point of first SACT treatment for all cancer patients in England. Consider adjusted survival outcomes according to BMI for specific tumour types: melanoma, colon, breast, endometrial, gastric, pancreas, gallbladder, hepatocellular cancers. Consider how changes in BMI impact on survival.

Planned initial analyses are for a) first line checkpoint immunotherapy in melanoma, b) first line CDK4/6 inhibitors for hormone receptor positive advanced breast cancer c) first line tyrosine kinase inhibitors in renal cancer, d) first line sorafenib in hepatocellular cancer e) first line chemotherapy in colorectal, pancreatic, and oesophageal cancer.

Work package 3:
The proportion of patients receiving cytotoxic chemotherapy whose dose is capped based on a high BMI will be described. Comparison of adjusted survival outcomes between obese patients with capped and uncapped dosing will be carried out across specific tumour types.

Translational potential
This study will be by far the largest study of its type enabling interrogation of the interaction between obesity and outcomes for specific tumour types, stages, and therapies. It will allow description of how BMI changes throughout the treatment course.
The relationship between reduced SACT dosing and survival, an ongoing controversy in the context of obesity, will be explored. No cancer therapeutics have been developed to treat obese cancer patients or specific biomarkers defined that may select cancer phenotypes for specific therapy. Our intention is that information from this study will help inform angles of investigation into tumour biology to inform research for specific tumour types and therapeutics.

Training opportunities
Develop skills in curation and analysis of large clinical datasets.
Training in health sciences, statistical analysis and use of advanced statistical software packages.
Develop skills in medical writing.

Supervisors
Simon Lord is a medical oncologist and the Director of the Early Phase Clinical Trials Unit at the University of Oxford. He has a research interest in how obesity can alter drug response and the potential to repurpose drugs to target tumour metabolism.[2,3]

David Dodwell is an oncologist and senior clinical research fellow to the Early Breast Cancer Trialists' Collaborative Group at the University of Oxford. He is the UK lead for the SACT database and chairs the clinical group, which provides input and advice to this project to examine the safety, efficacy and cost-effectiveness of systemic cancer therapy used in England.[1,4]

References
1.Bright CJ, Lawton S, Benson S, Bomb M, Dodwell D, Henson KE, McPhail S, Miller L, Rashbass J, Turnbull A, Smittenaar R. Data resource profile: the systemic anti-cancer therapy (SACT) dataset. International journal of epidemiology. 2020 Feb 1;49(1):15-l. **2**. Lord SR, Cheng WC, Liu D, Gaude E, Haider S, Metcalf T, Patel N, Teoh EJ, Gleeson F, Bradley K, Wigfield S. Integrated pharmacodynamic analysis identifies two metabolic adaption pathways to metformin in breast cancer. Cell metabolism. 2018 Nov 6;28(5):679-88. **3**. Lord SR, Collins JM, Cheng WC, Haider S, Wigfield S, Gaude E, Fielding BA, Pinnick KE, Harjes U, Segaran A, Jha P. Transcriptomic analysis of human primary breast cancer identifies fatty acid oxidation as a target for metformin. British journal of cancer. 2020 Jan;122(2):258-65. **4**. Wallington M, Saxon EB, Bomb M, Dodwell D (last author). 30-day mortality after systemic anticancer treatment for breast and lung cancer in England: a population-based, observational study. The Lancet Oncology. 2016 Sep 1;17(9):1203-16.

13. Novel approaches to circulating tumour cell detection and enrichment to combat bone metastasis [1,2,3] – Dr. Edwards

Primary Supervisor: Dr. Claire Edwards
Additional Supervisors: Dr. Jason Davis, Dr. Ian Mills, Dr.Todd Morgan
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Project Summary
Circulating tumour cell (CTC) burden is an established hallmark of poor prognosis prostate cancer and as such is strongly associated with metastasis (*1*). CTCs represent an important source of biological information regarding prostate cancer progression, however exploiting this information has proved complicated, largely reflecting challenges in CTC detection and isolation (*2*). The biological features of CTCs resemble the features of bone metastasis in a number of important ways, not least in their expression of metabolic enzymes associated with the pentose phosphate pathway (PPP) (*3, 4*). Aligned with this, our preliminary studies suggest an association between the pentose phosphate pathway and prostate cancer bone metastasis. In the current proposal, we propose to combine novel approaches to CTC detection and enrichment (*5*) with delineating the role of CTC metabolic reprogramming in prostate cancer metastasis. We bring together a novel and powerful collaboration spanning laboratories, disciplines and institutions to address this challenge, providing a unique multidisciplinary and translational research experience for a DPhil student.

Research objectives
1. **Enhance** the sensitivity of CTC detection and enrichment in blood samples from prostate cancer patients.

2. **Profile** the enriched CTCs to provide more comprehensive data on their metabolic and metastatic status.

3. **Evaluate** the metastatic potential of enriched CTCs through in vitro and in vivo studies using the isolated cells.

CTC detection and enrichment: We will adapt a nanoparticle-based enrichment strategy developed by Prof. Jason Davis (Dept. of Chemistry) and used successfully to isolate exosomes from blood samples. In the first instance we will assess the detection and enrichment sensitivity of this approach using EpCAM as a cell surface target and a combination of electroanalytical and optical (ELISA) detection methodologies. EpCAM is an established CTC marker and the basis for approved CTC testing including through the CellSearch platform. We will benchmark the nanoparticle-based approach using well-annotated samples collected and evaluated head-to-head using established CTC platforms. Having evidenced enhanced enrichment efficiency using a nanoparticle-based approach we will adapt this to incorporate other cell surface markers which may strongly associate with the metastatic potential of CTCs – for example PSMA. PSMA-PET imaging is increasingly used to detect oligometastatic disease and is therefore a natural choice. A strength of nanoparticle-based enrichment is the capacity to multiplex for a range of cell surface markers. We will further expand multiplexing to include cell surface receptors involved in signalling in the bone niche (eg. CXCRs) and/or acting as metabolite transporters (eg. MCT2 and MCT4), determining whether cell-surface markers are present on distinct or overlapping populations of CTCs.

CTC profiling: Benefitting from the expertise of Prof. Todd Morgan (University of Michigan), we will assess metabolic and metastatic gene expression, including G6PD and other PPP genes and genes associated with bone metastasis, within the captured cells. We will determine whether there are differences in metabolic/metastatic profiles in distinct populations of CTCs, and whether metabolic/metastatic profiles in CTCs are associated with clinical features such as progression to advanced disease, metastatic site and extent of the associated bone disease.

Metastatic potential of CTCs: CTCs will be used in short term culture assays, either alone or in combination with bone cells (including bone marrow stromal cells, osteoblasts, bone marrow adipocytes, immune cells), using custom-designed approaches to evaluate bone metastatic potential, including transcriptomic profiling, morphology and migration. Using the expertise of Prof. Edwards in preclinical murine models of cancer and bone,

enriched CTCs will be used in patient CTC-derived models of prostate cancer bone metastasis to study metastatic potential in vivo.

Translational potential

Circulating tumour cells contain a wealth of information regarding disease progression with the potential to impact diagnosis, prognosis, treatment approaches and disease monitoring. As such, the translational potential of our project is extremely high. By developing new methods for better detection and enrichment of CTCs, this will improve the sensitivity of these cells to better inform clinical decision-making. Mechanistically, our studies will uncover features of CTCs that are associated with metastasis, provding novel insights into the metastatic process and identifying novel metabolic biomarkers that predict prognosis and/or therapy response.

Training opportunities

This is an exciting opportunity to gain expertise in a range of cutting edge techniques that span chemistry, cell and molecular biology, in vivo models and clinical analysis. These include nanoparticle theranostics, transcriptomic profiling using single cell or bulk RNA-Seq, in vivo PDX models of prostate cancer bone metastasis, and working with clinical samples and analysis of associated data.

References:

1. L. C. Hofbauer et al., Novel approaches to target the microenvironment of bone metastasis. Nature reviews. Clinical oncology, (2021). **2.** J. S. Chung et al., Circulating Tumor Cell-Based Molecular Classifier for Predicting Resistance to Abiraterone and Enzalutamide in Metastatic Castration-Resistant Prostate Cancer. Neoplasia 21, 802-809 (2019). **3.** J. Chen et al., Metabolic classification of circulating tumor cells as a biomarker for metastasis and prognosis in breast cancer. Journal of translational medicine 18, 59 (2020). **4.** J. Chen et al., Metabolic reprogramming-based characterization of circulating tumor cells in prostate cancer. Journal of experimental & clinical cancer research : CR 37, 127 (2018). **5.** C. Jiang et al., Serum neuronal exosomes predict and differentiate Parkinson's disease from atypical parkinsonism. J Neurol Neurosurg Psychiatry 91, 720-729 (2020).

14. A Theoretical Examination of Cancer Vaccine Peptide Presentation[4] – Prof. Elliot

Primary Supervisor: Prof. Tim Elliott
Additional Supervisors: Prof. Eamonn Gaffney
Eligibility: Track 4 students are eligible to apply for this project.

Project Summary

Understanding the discriminants of therapeutic success and response variation is a fundamental problem in immuno-oncology. This project proposes to extend existing mechanism- based models for the intra-cellular handling of potential therapeutic peptides and proteins that can induce a $CD8^+$ T cell response in the context of tumour vaccination. The study will firstly develop and refine the current modelling framework to accommodate further intracellular dynamics and to recapitulate recent experimental observations of features that modulate antigen presentation to $CD8^+$ T cells and its variation with HLA allotypes and inflammation for example. This will provide a framework for hypothesis generation, with a view to suggesting novel directions for developing a rational, evidence-based framework in vaccine design for tumour intervention, accommodating features that are likely to modulate govern variability in patient response.

Background: Immunotherapies are increasingly being used to treat a wide variety of cancers and present both enormous potential and challenges [1]. Therapeutic vaccines aim to stimulate the immune system by inducing a $CD8^+$ T cell response against the tumour, via the MHC-I pathway (Fig 1), for instance by introducing therapeutic peptides that can subsequently be expressed on MHC-I. Hence, fundamental questions concern which peptides should be presented to the immune system. While HLA profiling via mass spectroscopy offers promising results for identifying mutant tumour peptides. While previous studies have suggested identifying such peptides [2], actually inducing immune responses against identified peptides remains a challenge [3], *with ~90% false positives reported* [4], while characterising the variability in response presents further difficulties. However, the need for developing an understanding of peptide presentation and mechanisms driving its variability. In light of such challenges, TJE has designed, implemented and validated initial computational models for levels of antigen presentation by MHC-I in terms of cytosolic peptide production rates, intracellular competition between peptides and peptide editing in the processing pathway [5].

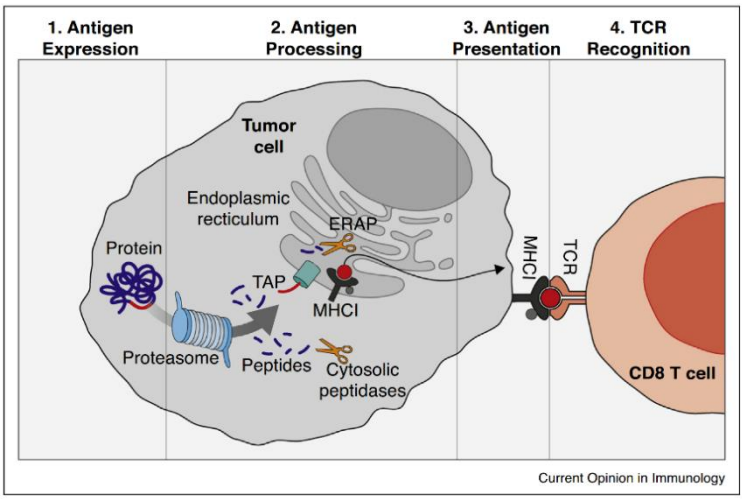

Fig.1. A summary of the MHC (major histocompatibility complex)-I pathway within an antigen presenting cell (APC) from cytosolic protein to CD8+ T cell activation. Tumour vaccines can induce, inter alia, exogenous proteins and long peptides for APC internalisation or present short peptide directly to surface APC MHC-I, depending on design.

Research objectives

Objective I. Enzyme Kinetics. The first objective of this DPhil project is to extend the computational model developed by TJE to refine its treatment of peptide loading onto MHC-I and to test the hypothesis that incorporating the non-linearities of enzyme dynamics, such as Michaelis Menten kinetics, will improve model agreement with existing datasets. Specifically, given newly available data together with extensive legacy observations.

Objective II. Tapasin (TPN) Dependent and Independent Pathways. Previous studies have assumed the tapasin (TPN) pathway, as schematically illustrated in Fig. 1, is dominant which is commonly the case. However, further TPN independent pathways can be more relevant for some HLA alleles, while downregulation of the TPN pathway can occur in cancer, with important clinical relevance, as it has been linked to a poorer prognosis in many types of cancer, for example non-small cell lung cancer [6].

II.i Global down regulation of the TPN pathway. Thus, the next objective will be to examine the impact of competition between TPN dependent and TPN independent pathways, firstly when there is a global down regulation of the TPN pathway.

II.ii Competition between pathways. A more refined study will then examine different peptides competing for MHC-I presentation and which differ in their propensity to be presented via either pathway.

Thus, the study will leverage recent datasets to generalise the models to accommodate the interplay between TPN dependent and TPN independent pathways, for example examining and making predictions for the features of the peptide repertoires that can be presented on MHC-I via TPN-dependent and TPN-independent routes and how this may change as TPN is downregulated.

III. Inflammatory Cytokines. Inflammatory cytokines such as IFNγ upregulate MHC-I expression and levels of TPN and often feature in the tumour microenvronment. Examining recent datasets investigating how inflammatory cytokines impact on peptide presentation will enable a model parameterisation for inflammatory environments enabling further understanding of how features of the dysregulated tumour microenvionment impact of peptide selection in MHC-I presentation.

All these investigations will constitute a study in parameter estimation and model selection, as well as rational model simplification, using mechanism-based modelling techniques, that will integrate the study with the expertise and supervision of MCC and EAG, who run a Mathematical Immunology Research Group in Oxford.

Proposed outcomes: The first outcome will be a refined computational model for the levels of cell surface presentation of MHC-I-peptide complexes, which will generate a publication. A second outcome will be a publication based on competing TPN-dependent and TPN-independent pathways for a global down regulation of TPN, with a further publication examining competition between peptides presented predominantly either by the TPN pathway or a TPN-independent pathway. A final publication will be for a reparameterisation of the models for inflammatory environments. It is also anticipated the student will also acquire non-primary publications in providing modelling support for further experimental studies within THE's laboratory.

The final outcome will be **writing a DPhil thesis,** with a concomitant literature review of the field modelling antigen processing.

Translational potential

The potential of this proposed research concerns an improved fundamental understanding of peptide selection for cancer therapeutic vaccinations. This will impact peptide screening and choice for potential treatments, as well as patient stratification in understanding which features of peptide propensities for presentation via TPN dependent and independent pathways will be associated with variation in patient responses for particular types of tumour micro- and immune- environments.

Training opportunities

The project is suitable for a student with an engineering/physics/mathematics background and a keen interest in applying these skills within a biomedical setting. Research level training in quantitative mechanism-based modelling for cellular and immunological sciences will be provided, especially techniques related to model development and simplification, as well as scientific computing and Bayesian parameter estimation and model selection. Importantly training in the cellular, cancer and immunological sciences will also provided to ensure expert subject knowledge underpins the student's research. More general research, communication, teaching, innovation and career development skills will be provided by MSD-wide training and also by the group meetings of the Wolfson Centre of Mathematical Biology within the Mathematical Institute, which recognises the particular difficulties of inter-disciplinary research.

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15. Discovery of potent agonist peptides for tumour-reactive T cells[1,2,3] – Dr. Fernandes

Primary Supervisor: Dr. Ricardo Fernandes
Additional Supervisors: Prof. Tao Dong
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Project Summary

T cells probe the surrounding environment using the T-cell receptor (TCR) to scan peptides presented by the major histocompatibility complex. The nature and potency of the T cell response towards pathogens or tumour cells are determined by the signalling output from two distinct classes of immune receptors: the TCR and co-receptors, which includes activating and inhibitory checkpoint receptors such as CD28 or PD-1 and CTLA-4, respectively. The latest advances in single-cell sequencing have facilitated the identification of TCRs from clonally expanded, tumor-infiltrating T cells. However, the identification of agonist peptides is still notoriously challenging. This project aims to establish a framework to identify potent agonist peptides recognised by effector and regulatory T cells of interest, with a strong focus on identifying peptides recognised by TCRs from expanded tumour-infiltrating lymphocytes (TILs).

Identifying antigens recognised by the TCR is challenging given the extreme diversity of the three individual components involved: peptide antigens, TCR and MHC. We aim to identify peptides, neoantigens and mimotopes, recognised by the TCR of clonally expanded CD8+ effector T cells in tumour settings (Fig. 1). To this end, we will engineer large (> 109) peptide-MHC libraries to be displayed at the surface of yeast cells, after which we will use an affinity-based screen to identify peptides recognised by TCRs of interest. This affinity-based approach will be complemented by a functional screen using an engineered system in mammalian cells. In this recently developed approach, the peptide-MHC library is fused to a CAR-like signalling module and displayed in T cells. This functional-based selection hijacks the unique sensitivity and specificity of the CD28/CD3 signalling modules to report on a productive TCR/pMHC interaction. Sorting of cells based on the upregulation of activation markers such as CD69 and CD25 will be used to isolate agonist peptides of different potency. The combination of affinity- and activity-based selections will guide the identification of potent agonist mimotopes, self-peptides or neoantigens using custom-built algorithms to rank closely related wild-type peptides. The identification of peptides recognised by tumour-reactive T cells will facilitate their expansion and detection using peptide-MHC molecules. Moreover, following isolation or activation with agonist peptides, tumour-reactive T cells will be characterised using single-cell transcriptomics and proteomics, for example. Agonist peptide identification combined with single-cell sequencing and quantitative proteomic analysis of relevant T cells will expand our current understanding of the role of diverse T cell subsets during an anti-tumour immune response. Furthermore, the discovery of disease-related agonist peptides opens the possibility to modulate T cell responses by peptide immunisation, an essential first step towards achieving in vivo expansion and activation of tumour-specific T cells. This research plan thus aims to contribute towards the development of relevant immunotherapies in cancer settings and a better understanding of T cell function.

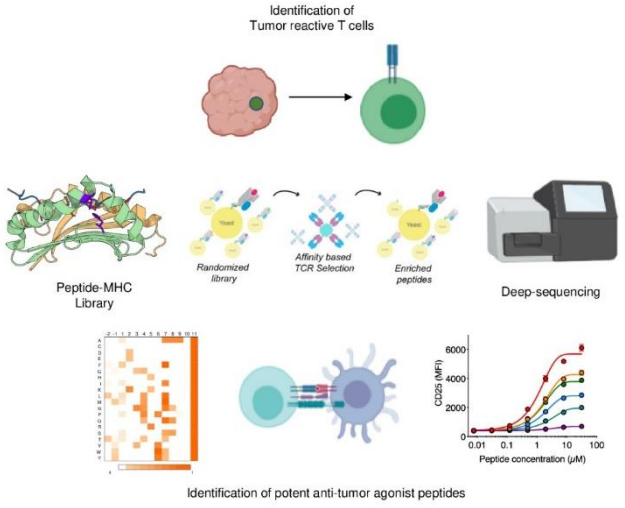

Figure 1. General overview of the experimental approach to discover peptide antigens to elicit robust anti-tumor T cell responses.

Research objectives

This research plan aims to develop a framework for identifying peptides recognised by T cells of interest with a particular focus on tumour-reactive T cells. We will focus on finding antigens for orphan TCRs from CD8+ TILs isolated from melanoma and glioblastoma patient samples. Candidate peptide antigens will be extensively characterized *in* vitro with functional and biophysical assays. We anticipate two primary outcomes. First, the

engineered peptide-MHC library displayed by yeast and mammalian cells will be made readily available to the scientific community. Second, we expect to identify potent peptide agonists which will facilitate the identification, isolation and activation of tumour-reactive T cells. These reagents will also be made available to the scientific community working in this field.

Translational potential

The discovery of agonist peptides is notoriously challenging and has limited the possibility of expanding (in vivo) tumour-reactive T cells *in vivo*. We expect that the described approach will establish a rapid and facile method to discover peptide antigens for tumour-reactive T cells. Checkpoint inhibition blockade using antibodies against PD-1 and CTLA-4 to enhance T cell activity has shown great promise in the clinic, but in most patients, this approach fails to produce durable responses. We anticipate the next stage of immunotherapy development to involve a combination of checkpoint blockade - eliciting broad but unspecific potentiation of T cell responses - with antigen-specific stimulation of tumour-reactive T cells. The identification of peptide antigens for T cells involved in anti-tumour responses is expected to guide the selection of TCRs for adoptive cell transfer and the development of high-affinity TCRs and peptide vaccines for immunotherapy.

Training opportunities

The student will receive training in molecular biology, protein design, expression, purification and biophysical characterisation and various cellular assays. Moreover, the student will be trained in protein engineering, library design and selection using yeast- and mammalian-display. T cell signalling assays will be used to validate candidate antigens, which will provide an opportunity for training in flow cytometry and RNA-seq. This training will allow the candidate to drive fundamental and applied research in academia and industry. At the end of this project, the candidate will be in a great position to lead the development of new protein drugs from conceptual design to implementation and thorough validation in an area of great interest in T cell biology and immunotherapy. The student will have full access to the facilities and resources available within the Department and across the broader community at the University of Oxford.

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16. A spatially resolved 3D multi-omic Atlas for cancer analytics in the human brain[2,3] – Associate Prof. Fischer

Primary Supervisor: Associate Prof. Roman Fischer
Additional Supervisors: Associate Prof. Olaf Ansorge
Eligibility: Track 2 and 3 students are eligible to apply for this project.

Project Summary

'Oncometabolomics' links metabolic cancer signatures to genetic subtypes of primary and metastatic brain cancers. For example, glioblastomas (GBMs) may be associated with D-2-hydroxyglutarate (2HG) (1, 2). Although the mutations leading to cancer associated phenotypes are often known, there are no data on the spatially resolved proteomic context that is driving oncogenesis at the molecular level. To resolve this missing link, we have developed a spatial proteomics workflow to contribute the first integrated three-dimensional 'oncomap' of GBM. Specifically, we will use laser-capture microscopy (LCM)-derived samples for ultra-deep LC-MS/MS analysis (3) in the 3-dimensional context of human brain tissue. This technology is to be complemented by/integrated with mass spectrometric tissue imaging (MSI) using MALDI (4). The project will use resection specimens of human GBM, serially sectioned for three-dimensional reconstruction and integration of digital microscopy, metabolomics, proteomics and potentially transcriptomics (Figure 1). Our project focusses on the transition from already established 2-dimensional workflows into 3-dimensional space and the generation of the first 3-dimensional proteomic map of human glioblastoma derived tissue (5).

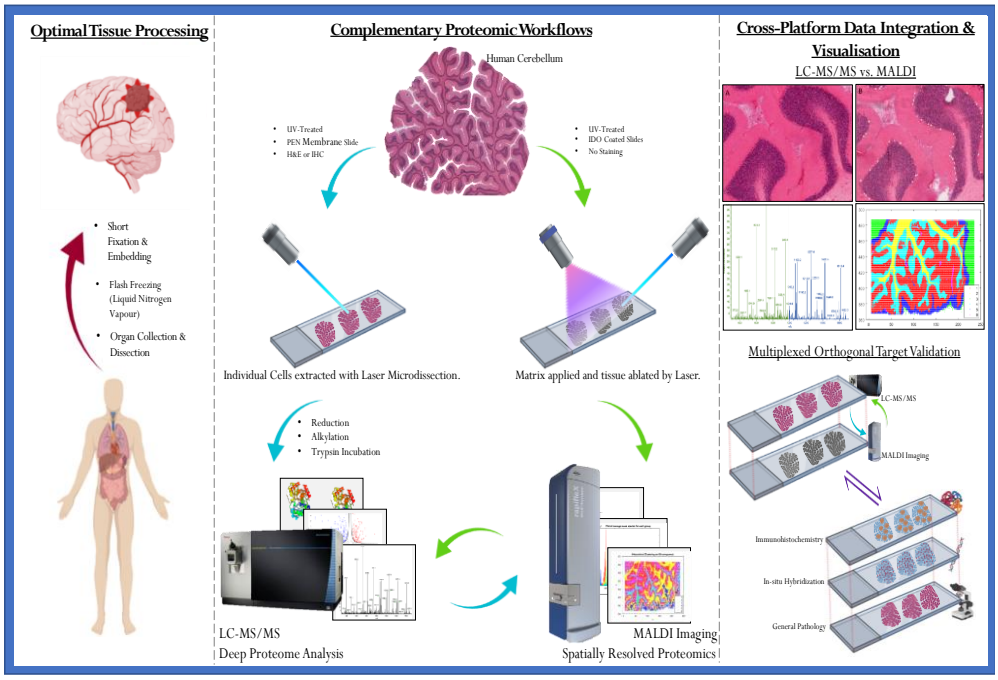

Figure 1 We have developed a novel liquid nitrogen vapour (LNV) freezing method and short-fixation protocol (**left**). Laser micro dissection combined with state-of-the-art mass spectrometry and MALDI imaging provide complementary data for mapping tumour biology at molecular level (**centre**). Integration of LC-MS/MS and MALDI MSI datasets of serial sections to generate 3-dimensional proteomic maps, followed by target validation at (sub-)cellular resolution (using existing Perkin Elmer / Codex platforms at the University of Oxford (**right**).

Research objectives

This project will lead to the generation of the first 3-dimensional proteomic map of a biological macro structure. Recently established methodology for 2-dimensional proteome mapping within a tumour (Figure 2) will be refined in order to increase spatial resolution (towards single cell), followed by expanding the approach into 3 dimensions. The resulting data will be integrated in a 3-dimensional atlas, spanning individual molecular resolution up to spatially integrated multi-omic data at pathway level. This Atlas will serve as an interactive online resource and exemplar in collaboration with the Big Data Institute (Alberto Santos Delgado/Philip Charles) using and advancing the established "Clinical Knowledge Graph" multi-omic integration tool. This first-of-its kind project will lay the groundwork for such analysis of other biological structures such as organs and other tumours and will help understanding of molecular processes in the spatial context of the tissue.

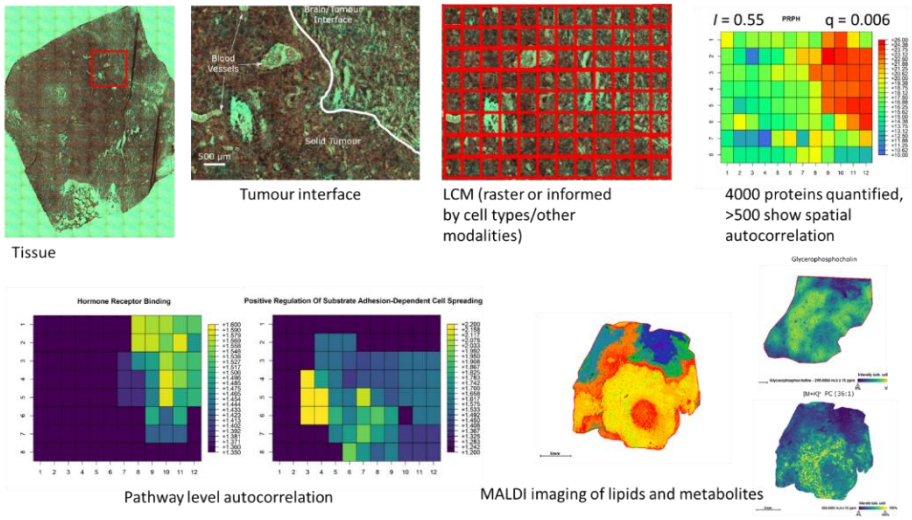

Figure 2 Established workflows for state-of-the-art proteomics and MALDI imaging reveal the spatial resolution of the cancer proteome in an AT/RT brain tumour and discover localized phenotypes at molecular and pathway level. This project aims to further advance this methodology for 3-dimensional visualisation of the deep cancer proteome within the spatial context of the tumour (i.e., glioblastoma) in order to discover druggable pathways in dependency of individual disease type and progression (real data shown).

Translational potential

While the spatial aspect of tumour biology is currently largely ignored, its understanding is relevant for drug development, target discovery and drug delivery. Our preliminary data shows that the spatial proteome together with the activity of relevant pathways in tumour tissue is highly variable and depends on factors such as nutrient supply/proximity to blood vessels and other localized factors. Efficiency of treatment therefore often depends on the ability of drugs to reach the desired location. This project aims to inform about the spatial distribution and activity of cancer and immunology related molecular signatures and pathways in order to better exploit the efficiency of drugs in the 3-dimensional space of tumours such as glioblastomas.

Training opportunities

The candidate will acquire highly transferable skills in mass spectrometry, proteomics, metabolomics, data analytics/integration and oncometabolomics:

- Multi-disciplinary training in the 'final frontier' technologies of tissue 'omics', which is thought to disrupt diagnostic pathology in the next decade.
- Training on key global health priority areas: "new technologies and infrastructure", "precision medicine", "discovery science" with a focus on an area of unmet need: neuro-oncology and neurodegeneration (CRUK and MRC Neurosciences and Mental Health Board priorities).
- Direct access and training on state-of-the-art key technologies/equipment such as laser capture microdissection, high throughput LC-MS/MS, MALDI imaging
- Multi-omic data integration and visualisation (Collaboration with Big Data Institute)

References:

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17. Mechanistic analysis of the cancer-associated Aurora A-PP6 axis in chromosome mis-segregation and micronucleation, and cGAS/STING signalling [1,2,3,4] Dr. Gruneberg

Primary Supervisor: Dr. Ulrike Gruneberg
Additional Supervisors: Prof. Francis Barr
Eligibility: Track 1, 2, 3 and 4 students are eligible to apply for this project.

Project Summary

Mounting evidence shows that in many cancers, chromosome segregation errors linked to micronucleation underpin solid tumour evolution by promoting cellular heterogeneity within tumours, ultimately leading to metastasis. Micronuclei arise when a small number of chromosomes are not incorporated into the main nucleus of the progeny cell, which is different to classical chromosome instability where all chromosomes are captured in one intact nucleus. Due to their size and mechanism of formation, micronuclei are structurally and functionally defective and do not efficiently compartmentalise DNA away from the cytoplasm or ensure its error-free replication. These are crucial differences explaining why micronuclei are the major site of chromothripsis, a chromosome shattering process that acts as a catalyst for cancer evolution. To defend against this danger, cells possess an innate immune surveillance mechanism for cytosolic DNA which initiates an interferon and NF-B signalling response upon detection of micronuclei. This process involves the cyclic GMP-AMP synthase (cGAS) and the interferon response adaptor stimulator of interferon genes (STING) [1].
Despite this knowledge, there are few cases where the mechanism of cancer-associated micronucleation is understood, thus hampering progress in the area of cancer biology. One tractable example is the Aurora A-PP6 oncogene-tumour suppressor axis, discovered by our labs, which is crucial for efficient bipolar spindle formation and chromosome segregation [2, 3]. Aurora A is a mitotic kinase and classical oncogene frequently amplified in cancers whose activity is regulated through auto-phosphorylation counteracted by protein phosphatase 6 (PP6) [2, 3]. Cancer-associated loss of function "driver" mutations in PPP6C, the catalytic subunit of PP6, trigger chromosome mis-segregation with micronucleation. This is most prominent in melanoma, where PPP6C loss-of-function "driver" mutations are found in >12% of cases [4, 5]. However, whether the cGAS/STING response is triggered by these micronuclei or other changes to genome integrity is currently not known. In this new collaborative project, we will investigate how the different classes of chromosome segregation errors and micronucleation events seen following dysregulation of the Aurora A-PP6 axis link to cGAS/STING signalling. Additionally, we will explore the potential for evasion of the cGAS/STING response in PP6-mutant and Aurora A amplified tumour cells.

Research objectives

Primary tumour cells with loss-of-function mutations in PPP6C, cultured cell lines depleted for specific PP6 subunits or carrying tumour-associated loss-of-function mutations in PPP6C, have increased rates of chromosome mis-segregation and high levels of micronucleation (Figure 1). These micronuclei are specific sites for DNA damage, in agreement with the idea they are undergoing chromothripsis [2, 3]. **This gives us a unique cancer-relevant platform to investigate cGAS/STING signalling during the micronucleation process.** Our immediate research aims are to follow the formation of individual micronuclei in PP6-deficient cells, and determine whether they are immediately recognised by the cGAS/STING immune surveillance system and trigger immune signalling, or whether this occurs only after multiple aberrant cell divisions. The participation of all three groups is essential to deliver the in-depth analysis of cell cycle and cell cycle checkpoint biology (Gruneberg), cell cycle kinase-phosphatase biochemistry (Barr) and innate immune signalling pathways (Greaves) needed for the project.

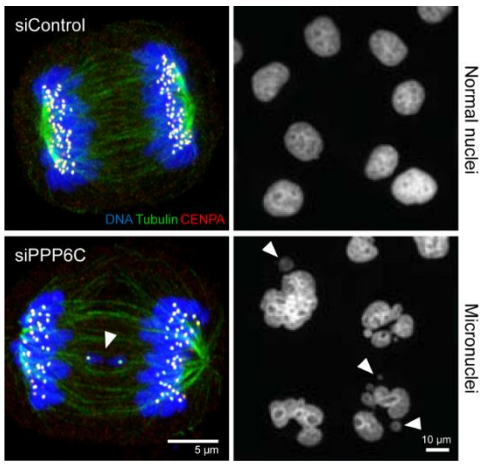

Figure 1. Cells lacking PPP6C have lagging chromosomes in mitotic exit and display high levels of micronucleation (arrowheads).

To investigate the origin and the potential longer-term consequences of cGAS/STING signalling, we will use both PPP6C depleted or PPP6C knock out tissue culture cells of different origins (transformed diploid HCT116, highly aneuploid HeLa, and non-transformed RPE1 cells) and LB373-MEL human melanoma cells carrying homozygous loss-of-function point mutations in PPP6C. The commonly used A375 melanoma cell line has a splice site mutation in PPP6C and expresses a truncated unstable protein from one allele, providing a further "*hypomorphic*" model. These cell lines are already available in our labs, and we use STR profiling from ATCC for lineage validation.

2.1. Origin of micronuclei in PP6-mutant cells. Depletion or mutation of PPP6C results in widespread micronucleation in the affected cells. However, it is not clear yet whether the micronuclei are a consequence of defective DNA damage repair or telomere function in S-phase or aberrant chromosome segregation during mitosis. To clarify the etiology of the micronuclei in PPP6C-deficient cells, we will use high resolution fixed and live cell microscopy of PPP6C-depleted or knock-out cells expressing fluorescent nuclear envelope and centromeric markers available in our groups. Detection of micronuclei by the innate immune system is only possible upon rupture of the micronuclear membrane allowing access of the cGAS DNA sensor to micronuclear chromatin. We will therefore monitor the integrity of the micronuclear membranes using cell lines expressing fluorescently tagged nuclear membrane components and investigate whether micronuclei of different origins, *e.g.* DNA fragment versus whole chromosome, are differentially susceptible to nuclear envelope disruption.

2.2. Analysis of the cGAS/STING pathway in PPP6C-deficient cells. To investigate whether the micronuclei present in PPP6C-deficient cells are detected by the cGAS/STING pathway, we will use a combination of biochemistry and fixed and live cell imaging. We will also exploit our established assays for innate immunity and NF-κB signalling and address whether these micronuclei trigger pro-inflammatory signalling. Our findings will be compared with characterised situations in which cGAS/STING signalling is known to be initiated through RnaseH2b knock-out [1]. Our hypothesis is that cells that have been acutely depleted of PPP6C or that have been selected in the laboratory for the knock-out of PPP6C, will display cGAS/STING signalling upon micronucleation but that bona fide PPP6C-deficient tumour cells will have evaded the signalling pathway to avoid detection by the innate immune system.

Translational potential

A key goal in cancer research is to find changes or dependencies that will allow selective killing of the tumor cells without harming untransformed bystander cells, and provide better early-stage diagnostic tools. Our proposed work has potential benefits in both these areas:

- PPP6C mutations act as early "drivers" for tumorigenesis by promoting genome instability. However, these cells possess an immediate weakness in the form of significantly increased micronucleation, detected by the innate immune system. The cGAS/STING mediated innate immune response to PPP6C micronuclei, and whether this is evaded by tumours in some cases, provides a model for the development of therapies that exploit micronucleation.
- Nuclear morphology has long been used as a diagnostic hallmark for cancer, e.g. the PAP smear test, however underpinning molecular correlates for many cancers have remained elusive. Mutations in PPP6C destabilise the protein and absence of PPP6C in conjunction with nuclear morphology abnormalities could thus be developed as an early-stage biomarker.

Training Opportunities

The project combines molecular biology, cell cycle analysis, genomics, classical biochemistry and advanced microscopy approaches with immunological assays to measure innate immune signalling. There will therefore be extensive training opportunities in all these areas by experts in the field.

References

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18. Investigating CBP/p300 bromodomain inhibition to exploit synthetic lethality in CBP-deficient lung cancers[1,2,3] – Prof. Hammond

Primary Supervisor: Prof. Ester Hammond
Additional Supervisors: Prof. Stuart Conway
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Project Summary

Genetic screens to identify synthetic lethality relationships are now common and have identified numerous potentially exploitable relationships. However, few of these have yet to be realised in the clinic due in part to a lack of effective inhibitors. Recently, the paralogues CBP and p300 were found to be synthetic lethal and this, combined with the knowledge that many cancers are CBP-deficient, has highlighted the potential efficacy of targeting of p300. We have developed inhibitors of the CBP and p300 bromodomain which prevent the functions of these proteins. We aim to investigate the effect of these compounds in CBP-deficient lung cancers. This approach has the potential to offer a novel therapeutic approach for the treatment of a subset of lung cancers.

Research objectives

The paralogues, CREBBP/CBP and EP300/p300 are master transcriptional coregulators that modulate the expression of hundreds of genes. CBP and p300 are bromodomain-containing histone acetyl transferases (HATs), which place acetyl marks at lysine K18 and K27 of histone H3 and act as transcriptional coactivators for various DNA-binding transcription factors. Unsurprisingly, their dysfunction and dysregulation are implicated in diseases including cancers and neurodegeneration.[1] Genome-wide profiling has shown that 10-15% of non–small cell and small cell lung cancers have loss-of-function mutations in CBP. Mutations are also prevalent in other types of other human cancers, including lymphomas (29–33%), leukaemias (18%), and bladder cancers (15–27%).[2] Functional screening for synthetic lethal genes in CBP-deficient cancers identified p300 as the most prominent synthetic lethal gene in these cancers.[2] This raises the possibility that compounds that inhibit p300 and/or CBP function, would allow exploitation of this synthetic lethal interaction. However, to date, no such compounds have been reported, preventing this approach being tested.

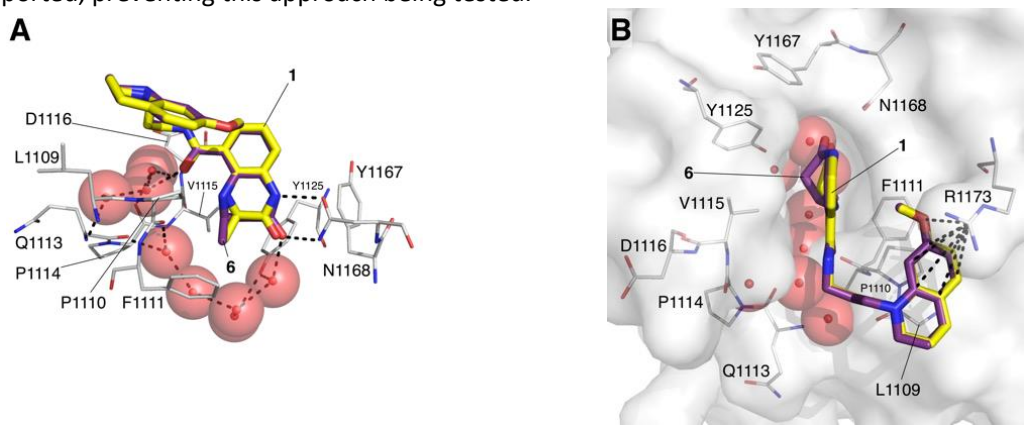

Figure 1. X-Ray crystal structure of **6** bound to the CREBBP bromodomain (PDB code 6YIM, carbon = purple, protein surface from this structure shown) overlaid with the X-ray crystal structure of **1** bound to the CREBBP bromodomain (PDB code 4NYX, carbon = yellow).[3] **A**. The side orientation shows that the headgroups of each compound form the same hydrogen-bonding interactions with the bromodomain, and that the KAc-mimicking methyl and carbonyl groups of both molecules overlay very closely. **B**. The top orientation shows that the interaction with R1173 is present for both molecules.

The bromodomain of CBP/p300 works in synergy with the HAT domain, and acts to guide the sites of histone lysine acetylation. We developed the first high affinity inhibitors of the CBP bromodomain,[3,4] and have recently reported more selective bromodomain ligands that are effective tool for probing CBP/p300 bromodomain function in cells.[5] These compounds modulate cellular lysine acetylation and show antiproliferative effects in prostate cancer cell lines. Here we aim to investigate the effects of these compounds, and further analogues that are being developed, in CBP-deficient cancers. To date, there have been no studies on the effect of CBP/p300

bromodomain inhibition in cancers that lack a functional form of one of these proteins. This approach might allow us to identify compounds that are more effective in these cancers, compared to normal cells. The objective of this project is to use bromodomain inhibitors to target CBP mutant lung cancers through synthetic lethality.

Translational potential

Despite the elegance of synthetic lethality, there is only one example of synthetic lethality being exploited in clinically. PARP inhibitors are used in patients who carry germline mutations in either the BRCA1 or BRCA2 genes, which result in defects in DNA damage repair.[6] One of the main challenges in this area has been the identification and mechanistic characterisation of further proteins that display robust synthetic lethal interactions. The potential of this project is to validate an entirely novel therapeutic approach to the treatment of lung cancer which would also have inevitable impact on the treatment of other cancer types.

Training opportunities

Profs Hammond and Conway have worked together collaboratively for over 10-years. This includes 12 papers (published or in press), >6 million in joint funding, graduation of 4 co-supervised PhD students and 3 current co-supervised students. This project offers the opportunity to join a well-funded, collaborative and interdisciplinary team. The student will be based in the Hammond lab in the Oncology department. The techniques used will include, but are not limited to, tissue culture, western blotting, qRT-PCR, RNA-seq, microscopy, FACs, use of radiation sources, hypoxia chambers, spheroid growth and in vivo work.

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19. Evaluating the molecular basis for tumour cell sensitivity to OXPHOS inhibition[1,2,3] – Prof. Higgins

Primary Supervisor: Prof. Geoff Higgins
Additional Supervisors: Dr. Karl Morten, Dr Jon Lees
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Project Summary

Reprogramming tumour metabolism by inhibiting oxidative phosphorylation (OXPHOS) has gained significant traction as a therapeutic strategy. Several different OXPHOS inhibitors have entered clinical trials either as single-agent treatments or in combination with established therapies such as radiotherapy and chemotherapy. OXPHOS inhibitors currently in development include compounds targeting specific components of the electron transport chain as well as novel mitochondrial metabolism disruptors targeting all components of the ETC. This proposal seeks to identify which of these strategies and clinical compounds are likely to be the most effective treatment in different therapeutic settings and identify the molecular features of tumour cells which determine their sensitivity to OXPHOS inhibition.

Research objectives

There is growing interest in the development of OXPHOS inhibitors as anti-cancer therapies 1 alone or in combination with other treatments. OXPHOS upregulation can be driven by genetic mutations such as in components of the SWI/SNF complex, rendering cells particularly sensitive to single-agent OXPHOS inhibition 2. Separately, reducing the oxygen consumption rate of tumour cells by inhibiting OXPHOS has been shown to reduce tumour hypoxia in mouse models 3, and has been successfully translated in a clinical trial of patients with non-small cell lung cancer 4. Reversing tumour hypoxia in this way has been shown to increase tumour sensitivity to radiation 3, and is also expected to enhance the effects of immune checkpoint inhibitor therapy 5. It has also been shown that OXPHOS inhibition can enhance the effects of chemotherapy treatment such as platinum via an increase in reactive oxygen species mediated damage 6. Oxidative phosphorylation can be disrupted either by targeting individual or multiple components of the electron transport chain (Figure 1). Several drugs have been developed or repurposed as OXPHOS inhibitors and are currently being investigated in clinical studies.

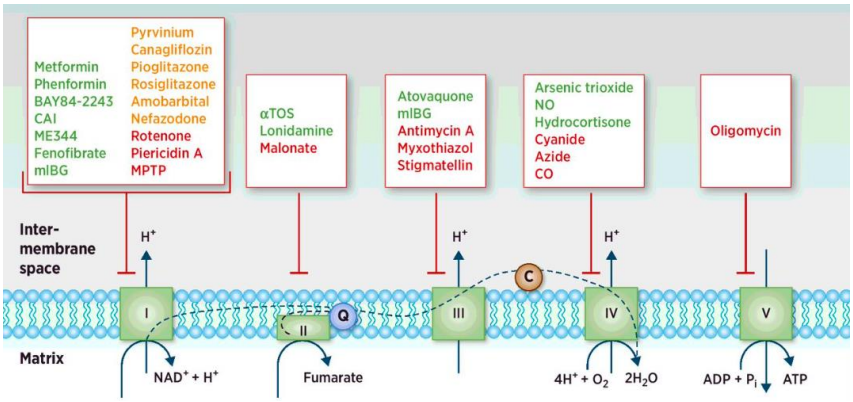

Figure 1) Inhibitors of OXPHOS. The OXPHOS metabolic pathway generates ATP by transport of electrons to a series of transmembrane protein complexes in the mitochondrial inner membrane, known as the electron transport chain. The dotted line indicates the flow of electrons through complex I, complex II, Coenzyme Q10 (Q), complex III, cytochrome c (C), and complex IV, with O2 acting as the terminal electron acceptor. Examples of compounds of therapeutic potential being studied as OXPHOS inhibitors in vivo or in the clinic are shown in green, those being studied in vitro are shown in orange, and classical mitochondrial poisons are shown in red. αTOS, α-tocopheryl succinate; CAI, carboxyamidotriazole; CO, carbon monoxide; mIBG, meta-iodobenzylguanidine; MPTP, 1-methyl 4-phenyl 1,2,3,6 tetrahydropyridine; NO, nitric oxide (adapted from 1).

The objectives of this proposal are:

Objective 1. Identify the molecular basis for alterations in cellular sensitivity to OXPHOS inhibition.

It is currently unclear which tumour abnormalities drive cellular response to OXPHOS inhibition, and therefore which patients are likely to benefit from treatment. Based on published CRISPR screen data, our bioinformatics collaborator (Dr Jon Lees) used in silico modelling to cluster a large panel of cell lines into two groups – those 'sensitive' or 'resistant' to OXPHOS inhibition. We have been able to experimentally validate the reliability of these groupings with high reproducibility using two different OXPHOS inhibitors. In depth genetic analysis of the OXPHOS sensitive cell line cluster will help us to identify commonly occurring mutations linked to the OXPHOS sensitive phenotype. The mutant genes of interest will be knocked down or transfected into 'sensitive' or 'resistant' cell lines. Having identified the molecular basis for differences in sensitivity, we will subsequently explore the mechanistic basis for these differences. We will also aim to validate these preclinical findings using the tumour material from patients recruited into the 'ATOM' and 'ARCADIAN' clinical trials led by the Higgins group, where lung cancer patients have been treated with the OXPHOS inhibitor atovaquone 4.

Objective 2. Identify the optimal clinical compounds for ongoing clinical studies
Differences in potency of OXPHOS inhibition, generation of reactive oxygen species, pharmacokinetics, and side-effect profiles means that it is currently unclear which of the inhibitors currently in clinical trials are likely to be the most therapeutically beneficial. Using both in vitro, and relevant in vivo models we will establish which of the prominent OXPHOS inhibitors currently being tested clinically is most effective as a) single agent treatments, b) as hypoxia based radiosensitisers c) at enhancing the efficacy of immunotherapies.

This work will be done in collaboration between the Higgins and the Karl Morten groups who have overlapping interests in OXPHOS. The proposal combines the clinical and radiation expertise of the Higgins group and mitochondrial biology expertise of the Morten group. The two groups have very recently started working together, and their collaborations which will be strengthened by this proposal.

Translational potential
Identifying the best OXPHOS inhibitors for specific clinical indications will enable us to prioritise the clinical translation of these compounds. Likewise, identifying molecular features which predispose tumours to OXPHOS inhibitors will ensure that future clinical trials are able to enrol only those patients likely to respond to intervention.

Training opportunities
The student will be taught all of the necessary laboratory techniques including 2D and 3D cell culture, generation of stably transfected cell lines, flow cytometry, fluorescence microscopy as well as specialised techniques such as metabolic analyses using the Seahorse flux analyzer and in vitro T-cell assays. There will also be the opportunity for the student to undertake training for the in vivo xenograft studies of OXPHOS inhibitors in combination with radiation and immune checkpoint inhibitors. Training may also be given in relevant patient sample analysis including RNASeq and immunohistochemistry.

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20. Studying the role of a chromatin remodelling factor (ATRX) in malignancy [1,2,3] – Dr. Higgs

Primary Supervisor: Dr. Douglas Higgs
Additional Supervisors: Prof. Richard Gibbons
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Project Summary

The X-encoded, SWI/SNF-like chromatin remodeling factor ATRX interacts with the histone chaperone DAXX, to deposit the histone variant H3.3 at sites of nucleosome turnover. ATRX was originally identified as the cause of a syndromal form of intellectual disability associated with variable degrees of alpha-thalassaemia; so-called ATR-X syndrome. Over the past 10 years ATRX has emerged as an important tumour suppressor gene that is mutated in a wide range of cancers. Mutations in ATRX cause widespread changes in a variety of nuclear processes including transcription, replication and DNA repair, and is consistently found mutated in cancers which maintain their telomeres via the alternative (ALT) pathway. In this project we will examine the mechanisms by which ATRX normally controls such nuclear processes at its most thoroughly studied target, the alpha globin gene cluster which lies at the telomeric region of human chromosome 16 (16p13.3). Specifically, we will determine how ATRX alters cell behaviour in patients with a condition called myelodysplasia which often precedes the development of acute myeloid leukaemia.

Research objectives

In collaboration with UK and international colleagues we have identified and stored material on 130 patients who have a rare form of ⍰-thalassaemia which occurs in the context of a pre-malignant condition called the myelodysplastic syndrome (MDS). Many of these patients go on to develop acute myeloid leukaemia. These patients have no pre-existing forms of ⍰⍰ thalassaemia (AT) and so this condition is acquired specifically in the pre-malignant clones of cells in MDS: hence the condition is referred to as the ATMDS syndrome. When we analyse the bone marrow cells of patients with ATMDS we find a distinct constellation of mutations in epigenetic readers, writers and erasers which are also found in other patients with MDS but, importantly, in addition, most patients with ATMDS have mutations in ATRX. The gene encoding this protein was originally discovered in our laboratory in 1995 as a cause of X-linked ⍰⍰thalassaemia associated with developmental abnormalities (ATR-X syndrome) and ATRX has more recently been recognised as a tumour suppressor gene in a wide variety of malignant tumours, in adults and infants, including glioblastoma, melanoma, pancreatic neuroendocrine tumours and a wide range of sarcomas

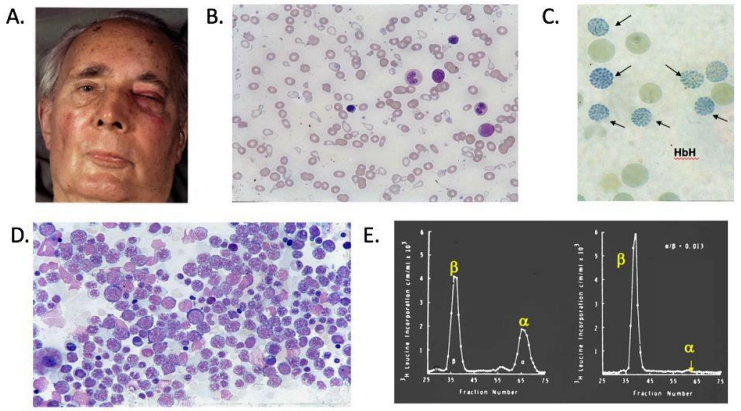

Figure 1. A.. A patient with MDS B. the blood film showing hypochromic microcytic red blood cells. C. Cells containing HbH inclusions which are tetramers of excess β-globin chains (β_4) seen in patients with α-thalassaemia. D. a bone marrow smear showing the features of MDS. E. Globin chain synthesis in a normal individual (left) and a patient with ATMDS (right). There is an almost complete absence of α-globin synthesis in this individual.

By understanding the role of ATRX in vivo, we hope to understand its normal role in gene expression, replication and DNA repair and how this is perturbed in malignancy. Analysis of naturally occurring mutations often provide important clues to the mechanism of disease. The key scientific question in this project is how do mutations in ATRX alter gene expression. We have been studying both primary cells from patients with ATMDS and ATR-X syndrome and developing much needed erythroid cell models of these diseases. This has been challenging for a variety of reasons but recently we have shown that using single cell analysis we are now able to identify a sub-population of erythroid cells that appear to be more affected by ATRX mutations than others and we are currently investigating

why this should be so. Some clues to this will come from analysing the impact of other genes that are mutated in ATMDS syndrome: appropriate cell lines in which such genes have been mutated individually and in combination are now edited and available for further studies. The aims of this project will therefore be to further characterise primary cells and the recently established erythroid cell models of ATMDS syndrome using transcriptional, epigenetic and chromosome conformation studies to analyse how □□globin expression is perturbed in this pre-malignant condition. In turn, this model will provide entirely new information on how ATRX mutations give rise to other forms of cancer

Translational potential

Since we originally identified the ATRX gene we have also shown that the ATRX protein is part of a complex together with a histone chaperone DAXX and the histone variant H3.3. Mutations in all three components of this complex have now been associated with a wide variety of malignant tumours. Almost all of such tumours maintain their telomeres via the so-called alternative (ALT) pathway of telomere maintenance. This aspect of ATRX is also being studied independently by a member of Oncology (Dr David Clynes) in collaboration with Professor Richard Gibbons (Co-supervisor). The current proposal aims to study the role of ATRX in gene expression. There seems little doubt that understanding the normal biological role of this complex will be of importance in understanding its role in the development of cancer.

Training opportunities

The laboratory includes senior and junior post- docs plus students and research assistants with a wide range of experience in genomics, cell biology and the analysis of gene regulation. The facilities at the MRC Weatherall Institute of Molecular Medicine include genomics, sequencing, flow cytometry, genome engineering, advanced imaging, single cell biology and computational biology. In the course of this project we anticipate that the student will acquire skills in a wide range of genomics including ATAC-seq, ChIP-seq, RNA-seq and a wide range of chromosome conformation capture techniques. In addition, the laboratory has established expertise in genome engineering including CRISPR and base-editing. Importantly, we ensure that all students obtain a thorough training in computational biology to analyse such data

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21. Vesicle transport of cancer invasion-promoting proteinase to the leading edge: a crucial mechanism of cancer invasion [2,3,4] – Associate Prof. Itoh

Primary Supervisor: Associate Prof. Yoshifumi Itoh
Additional Supervisors: Associate Prof. Marco Fritzsche
Eligibility: Track 2, 3 and 4 students are eligible to apply for this project.

Project Summary

Cancer invasion and metastasis are the life-threatening features of malignant cancer. It is well accepted that the invasion process relies on the degradation of extracellular matrix (ECM) by a type I transmembrane proteinase, membrane-type 1 matrix metalloproteinase (MT1-MMP)1. One of the crucial regulatory mechanisms of MT1-MMP to promote cancer invasion is localisation at the leading edge, and disturbing this process would inhibit cancer invasion. The leading-edge localisation is achieved by a targeted intracellular transport of MT1-MMP-containing vesicles. We recently identified three kinesin motor proteins (KIFs) responsible for the MT1-MMP vesicle transport in invasive HT1080 fibrosarcoma cells. We found that KIF3A and KIF13A coordinate the vesicle transport of MT1-MMP to the leading edge, while KIF9 inhibits the process by transporting the vesicle to other membrane domains2. To further understand the mechanism of MT1-MMP localisation at the leading edge, it is crucial to identify adaptor molecules that allow KIFs to recognise MT1-MMP-containing vesicles and investigate their dynamic interaction during an invasion. This DPhil project will identify the adaptor molecules necessary for MT1-MMP vesicle transport using the BioID2 proximity labelling system and proteomics. Upon verifying the adaptor molecules, the dynamic interaction of MT1-MMP, adaptor molecules, and KIFs are extensively analysed by live-cell imaging under 2D and 3D culture conditions using state-of-the-art Super-resolution cell imaging equipment. Achieving this project will reveal the mechanism of cancer invasion, which may lead to identifying novel therapeutic targets.

Research Objectives

Background: Invasion is the dreadful feature of malignant cancer, causing tissue destruction and metastasis to the distal organs. The invasion process can be divided into the following steps. (1) attachment to ECM through ECM receptors, (2) degradation of the ECM at the direction of migration using proteinase, and (3) moving the cell body into the degraded area by re-organising cytoskeletons, which is called “three step theories” of cancer invasion. In the ECM degradation step, a membrane-bound metalloproteinase, MT1-MMP, is considered a crucial enzyme. MT1-MMP is a type I transmembrane proteinase that promotes invasion of different cancers, including breast, stomach, liver, colorectal, bladder, and pancreatic cancers, melanoma, fibrosarcoma, and others. Therefore, understanding the regulatory mechanism of MT1-MMP is essential to understand the invasion process of different cancer. One of the critical regulatory steps of MT1-MMP is its cell surface localisation at the leading edge. MT1-MMP is known to localise at different forms of the leading-edge cell membrane structures, including lamellipodia, filopodia, invadopodia, focal adhesion, and localisation to these specialised membrane domains is achieved by a targeted intracellular transport of MT1-MMP-containing vesicles1. Vesicle transport is carried out by kinesin motor protein (KIF) along the microtubule cytoskeleton. We have recently identified three KIFs responsible for MT1-MMP vesicle trafficking. KIF3A and KIF13A coordinate the vesicle transport of MT1-MMP to the leading edge (Fig 1), while KIF9 inhibits the process by transporting the vesicles to other membrane domains2. The next step is to investigate how these three KIFs recognise the MT1-MMP vesicles and analyse their dynamic interaction to traffic the vesicles to different membrane domains, allowing us to understand the precise mechanism of MT1-MMP localisation at the leading edge, a crucial process for cancer cell invasion. Therefore, the objectives of this DPhil project are following:

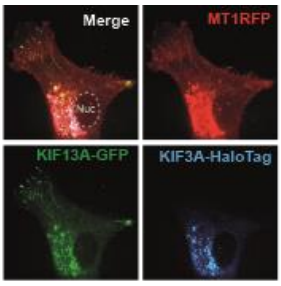

Fig. 1. Both KIF3A-HaloTag and KIF13A-GFP colocalise with MT1-RFP vesicles at perinuclear area, while only KIF13A-GFP-positive MT1-RFP were found at cell periphery

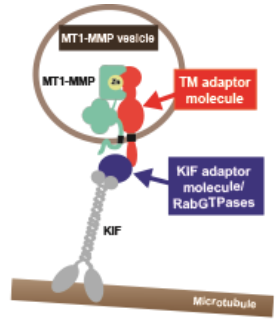

Fig. 2. Possible involvement of a trans-membrane (TM)-adaptor molecule and a KIF adaptor molecule(s) for MT1-MMP-specific vesicle transport

Objective 1. Identify adaptor molecules for each KIF to recognise MT1-MMP containing vesicles by BioID2 proximity labelling and proteomics

For KIFs to recognise MT1-MMP-containing vesicles, it needs at least one transmembrane adaptor protein that interacts with MT1-MMP through the ectodomain and one cytoplasmic adaptor protein or Rab small GTPases that connects the vesicle with KIFs (Fig 2). We will identify these molecules by using the BioID2 system followed by proteomics. BioID2 is a proximity promiscuous biotin ligase derived from the A. aeolicus biotin ligase BirA, and it biotinylates molecules within a 10 nm radius3. By expressing BioID2-fused KIFs and MT1-MMP, all proximity molecules to KIFs and MT1-MMP will be biotinylated. Biotin-labelled molecules are then isolated and identified by LC-MS/MS. Common molecules identified between MT1-MMP and each KIF are the candidate for adaptor proteins for each KIF. These molecules will be verified by gene silencing followed by analysing MT1-MMP activity on the cell surface and co-localisation with KIFs and MT1-MMP.

Objective 2. Investigate the dynamic mechanism of coordination of KIF3A and KIF13A for MT1-MMP vesicle transport to the leading edge and antagonising effect of KIF9 in 2D and 3D culture

Knockdown of KIF3A or KIF13A significantly reduced ECM degradation and invasion of HT1080 cells. We found that both KIF3A and KIF13A co-ordinately transport MT1-MMP vesicles to the leading edge. First, KIF3A and KIF13A transport the vesicle from the Trans Golgi to the early endosome, and KIF13A alone takes over the vesicles from the early endosome to the plasma membrane. To analyse how the KIF3A and KIF13A interact with and dissociate from the vesicles, GFP-KIFs, Halo-APKIF3A (Adaptor Protein for KIF3A), SNAP-APKIF13A (Adaptor Protein for KIF13A), and MT1-RFP will be expressed in cells and analysed by super-resolution live-cell imaging using TIRF-SIM. Also, these cells will be analysed in 3D culture using Lattice light-sheet microscopy.

KIF9 knockdown increased ECM degradation by HT1080 cells. This increased degradation was due to enhanced MT1-MMP vesicle transport by KIF3A and KIF13A, suggesting that KIF9 antagonises these KIFs by trafficking the vesicles to other membrane domains2. We will reveal how KIF9 acts on MT1-MMP vesicles together with APs identified for KIF9 (APKIF9). Does it traffic the vesicles before KIF3A and KIF13A bind, or do they compete with each other? Where does KIF9 traffic the vesicles? We will ask these questions by carrying out live-cell imaging of cells expressing MT1-RFP, KIF9-GFP, SNAP-APkif9, and Halo-KIF3A and Halo-KIF13A using Lattice light-sheet microscopy and Confocal microscopy with Airyscan 2.

Translational potential

The mechanism of cancer invasion is not fully understood, and there is no drug available targeting invasion process. Ability of cancer cell invasion rely on its ability to degrade ECM, which not only allows cancer cells to migrate, but modify their microenvironment to their like. MT1-MMP is a crucial proteinase that promotes the invasion, but it is still not clear how MT1-MMP localises to the leading edge for invasion. Outcome of this project would not only help understanding the invasion process of soft tissue sarcoma that we use as a model in this project, but also other cancer types that use MT1-MMP for their invasiveness, including squamous cell carcinoma, melanoma, lung cancer, colorectal cancer, prostate cancer etc. Understanding the mechanism would allow us to identify a novel target molecule that disturbs leading edge localisation of MT1-MMP, which effectively inhibit cancer cell invasion. Knowledge gained from the project is expected to open up an opportunity to develop novel therapeutic intervention.

Training opportunities

We are based at the Kennedy Institute, a world-renowned research centre and is housed in a state-of-the-art research facility. Full training will be provided in a range of cell and molecular biology techniques. A core curriculum of 20 lectures will be taken in the first term of year 1 to provide a solid foundation in musculoskeletal sciences, immunology and data analysis. Students will attend weekly departmental meetings and will be expected to attend seminars within the department and those relevant in the wider University. Subject-specific training will be received through our group's weekly supervision meetings. Students will also attend external scientific conferences where they will be expected to present the research findings. This project will provide depth of experience and knowledge of cell biology, proteomics, molecular biology, super-resolution and high-resolution live cell imaging microscopies and analyses.

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22. Common pathways for regulating viral and episomal DNA transcription provide new approaches to treat cancer[1,2,3] – Associate Prof. Kriaucionis

Primary Supervisor: Associate Prof. Skirmantas Kriaucionis
Additional Supervisors: Prof. Jane McKeating
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Project Summary
Gene amplifications are common genetic alterations found in different cancers. Amplified oncogenes enable higher expression of respective genes contributing to tumorigenesis. Interestingly, recent work demonstrated that amplified copies of genes could reside not only within chromosomes, but outside of chromosomes in the form of circular extrachromosomal DNA (ecDNA). How cells perpetuate and transcribe ecDNA is an active area of investigation. Like ecDNA, some viruses replicate via circular DNA, which resides in nucleus. The aim of the project is to determine whether viruses and cancer-amplified ecDNA share similar mechanisms of transcription and replication. Using hepatitis B virus (HBV) as a model, the project aims to reveal shared mechanisms of transcription and determine the potential of interfering with it for the treatment of cancer. The project opens collaboration opportunity between groups working in cancer epigenetics and virology.

Research objectives
Tumours originate when a cell acquires mutations resulting in the appearance of new traits including proliferation and spreading, that are detrimental to the whole organism. Some mutations are known to inactivate genes restricting tumour phenotypes (tumour suppressors), others result in the gain of new functions. It has been well established that a gain of function can be acquired by amplification of oncogenes, however how cells maintain multiple copies of genes has been only recently elucidated. Interestingly, a substantial number of cancers maintain amplified genes as circular extra chromosomal DNA (ecDNA). A recent study observed that ecDNA is common in many cancers and on average 14% of tumours carry ecDNA, whereas in normal tissues ecDNA is undetectable[1]. Moreover, the presence of ecDNA is linked with poor prognosis. As ecDNA does not have centromeres it is not faithfully inherited during cell division. Consequently, cells with different quantities of ecDNA are produced leading to tumour heterogeneity and fuelling tumour evolution[2,3]. Both features are likely to contribute to the poor outcomes of treatment, manifesting as resistance and relapse. Current efforts are focussed on identifying the molecular mechanisms that maintain ecDNA, that could provide new therapeutic targets.

Circular DNA in cells is common in virus infection for example, both Epstein-Barr virus (EBV) and hepatitis B virus (HBV) replicate via circular DNA which localize to the nucleus. HBV infection leads to the formation of covalently closed circular DNA (cccDNA)[4] that serves as a template for viral transcription and persists in cells[5], resulting in a persistent infection and increased risk of hepatocellular carcinoma. Current HBV therapies are thought to fail mainly due to their inability to eliminate cccDNA, which acts as a viral reservoir. Viruses have evolved to evade the host machinery that inactivates episomal DNA and recently discovered pathways illustrate how HBV encoded proteins regulate episomal DNA transcription[6]. We recently reported that the low oxygen environment of the liver, which is often found in tumors, enhances the transcriptional activity of HBV cccDNA[6], suggesting that hypoxic pathways may modulate ecDNA. Our central hypothesis is that viruses co-opt mechanisms to maintain their circular DNA analogous to the ones employed by tumours for their maintenance of ecDNA. This project will determine how proteins, which are known to regulate transcription and replication of HBV cccDNA, can regulate the expression and replication of tumour resident ecDNA.

We have validated tumor derived cell lines that carry ecDNA and these will be manipulated using state of the art gene overexpression and deletion techniques to explore the mechanisms underlying expression and replication of ecDNA. Our goal is to discover new targets for cancer therapies that will focus on tumours with frequent presence of ecDNA (glioblastoma, oesophageal adenocarcinoma and ovarian cancers).
The project is a collaboration between Skirmantas Kriaucionis and Jane McKeating research groups. Skirmantas Kriaucionis studies how chromatin and epigenetic mechanisms affect gene regulation[7], and, in particular, how DNA modifications are being interpreted and turned over in cancer cells. Jane McKeating is an internationally recognised

molecular virologist with an interest in hepatitis viruses and their interplay with hypoxia signalling pathways[8].The studentship will build on the complementary expertise of gene regulation and virology and will explore this vibrant area of tumour biology.

Translational potential

Whilst there has been substantial progress in treatments of some cancers (e.g. breast, melanoma) resulting in around two fold increase in patients surviving 10 years, treatment progress of brain, oesophageal, lung, pancreatic and liver cancers has been much lower. Less than 20% of patients after diagnosis of these cancers survive 10 years (Cancer Research UK). Interestingly, a recent study identified that around 60% of glioblastomas, 40% oesophageal adenocarcinomas and 25% of lung cancers have evidence of ecDNA. Identification of important components, which act in instructing transcription and replication of ecDNA will enable subsequent efforts in drug discovery, which will aim to disrupt mechanism maintaining functional ecDNA.

Training opportunities

The student will learn cell culture and gene manipulation of cell lines. Molecular biology techniques will be used to produce DNA constructs, which will be delivered to cells using transient transfection methods. CRISPR/Cas9 will be used to engineer cell lines with deletions of genes, which will be identified as likely players in ecDNA maintenance. Gene expression will be quantified using quantitative PCR, flow cytometry and massively parallel sequencing techniques. Chromatin states will be examined using chromatin immunoprecipitation. The student will gain broad expertise in viral biology and epigenetics.

References:

1. Kim, H. et al. Extrachromosomal DNA is associated with oncogene amplification and poor outcome across multiple cancers. Nat Genet 52, 891–897 (2020). **2.** Turner, K. M. et al. Extrachromosomal oncogene amplification drives tumour evolution and genetic heterogeneity. Nature 543, 122–125 (2017). **3**.deCarvalho, A. C. et al. Discordant inheritance of chromosomal and extrachromosomal DNA elements contributes to dynamic disease evolution in glioblastoma. Nat Genet 50, 708–717 (2018). **4.** Wei, L. & Ploss, A. Hepatitis B virus cccDNA is formed through distinct repair processes of each strand. Nat Commun 12, 1591 (2021). **5.** Lythgoe, K. A., Lumley, S. F., Pellis, L., McKeating, J. A. & Matthews, P. C. Estimating hepatitis B virus cccDNA persistence in chronic infection. Virus Evol 7, veaa063 (2021). **6.** Turton, K. L., Meier-Stephenson, V., Badmalia, M. D., Coffin, C. S. & Patel, T. R. Host Transcription Factors in Hepatitis B Virus RNA Synthesis. Viruses 12, 160 (2020). **7.** Cusack, M. et al. Distinct contributions of DNA methylation and histone acetylation to the genomic occupancy of transcription factors. Genome Res 30, 1393–1406 (2020). **8.** Liu, P. J., Balfe, P., McKeating, J. A. & Schilling, M. Oxygen Sensing and Viral Replication: Implications for Tropism and Pathogenesis. Viruses 12, 1213 (2020).

23. Defining DNA repair mechanisms to target in precision cancer therapies [1,2,3] – Prof. Lakin

Primary Supervisor: Prof. Nick Lakin
Additional Supervisors: Prof. Peter McHugh
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Project Summary

Inhibitors of DNA repair have emerged as powerful agents in cancer therapy, either as monotherapies that exploit synthetic lethal interactions, or by increasing the efficacy of chemo- and radiotherapies[1]. Principal in this strategy is inhibition of Poly(ADP-ribose)-polymerases (PARPs), enzymes that regulate DNA strand break repair, and PARP inhibitors (PARPi) are being used to treat tumours with defects in homologous recombination (HR)[1]. However, despite the success of PARPi in the clinic, our understanding of the pathways that PARPs regulate and how these integrate into models of PARPi toxicity in different tumour backgrounds is limited. Defining these relationships will identify novel pathways and targets that are synthetic lethal with HR, offering alternative avenues to treat tumours that are resistant to PARPi. By combining our expertise in PARP biology and DNA repair[2-6] with cutting edge genome editing, cell biology, proteomics and biochemistry, this PhD project will address this fundamentally important question by characterising novel PARP-dependent mechanisms that repair stalled/damaged replication forks. By exploiting degron technology to conditionally deplete the HR factor BRCA1, we developed a model to assess synthetic lethality with HR in different genetic backgrounds. This identified that recovery of stalled replication forks by Rad52-dependent break-induced replication (BIR) is synthetic lethal with HR and importantly, that this is regulated by PARP1. Building on these findings, the aims of this multidisciplinary hypothesis-driven research will be to: a) define how PARP1 regulates replication fork recovery by BIR; b) identify and characterise targets that are ADP-ribosylated (ADPr) by PARP1 in response to replication stress; c) develop biochemical assays to assess the activities of DNA repair enzymes regulated by PARPs. Our long-term vision is to exploit these findings to develop novel strategies and inhibitors that exploit synthetic lethal interactions with HR in PARPi-resistant tumours.

Research outcomes

Background: Given PARPs regulate single strand break (SSB) repair, the current paradigm for synthetic lethality between PARPi and HR is that inhibitors trap PARPs at SSBs, resulting in protein-DNA adducts that create DNA replication blocks that require HR for repair[7]. Our previous work challenged this model by identifying that gene disruption of PARP1, as opposed to trapping at DNA breaks, is a major determinant of synthetic lethality with HR[6]. Building on this work, we exploited genome editing to knock-in a degron onto the *BRCA1* gene, allowing conditional depletion of BRCA1 in defined genetic backgrounds to assess novel synthetic lethal interactions with HR. As expected, disruption of PARP1 in combination with BRCA1 depletion is toxic to cells. Strikingly, this toxicity is greater than disrupting a canonical SSB repair gene (XRCC1), indicating PARP1 regulates a pathway other than SSB repair that is synthetic lethal with HR. Our unpublished data indicate this pathway is Rad52-dependent break-induced replication (BIR), a process that repairs stalled/damaged replication forks in the absence of HR. The overall goal of this PhD project is to define the role of PARP1 in regulating BIR by pursuing the following aims:

- Define how PARP1 regulates BIR
- Identify targets that are modified by PARP1 to regulate BIR
- Test how PARP1 regulates BIR activities *in vitro* to screen for inhibitors of these pathways.

Regulation of BIR by PARP1

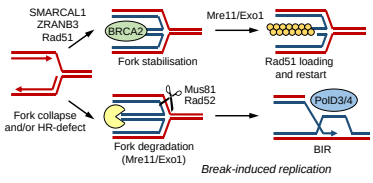

Fig 1: Replication fork recovery by Rad51-dependent (upper) and –independent (break-induced replication [BIR]; lower) mechanisms.

We will more clearly define where in the BIR pathway PARP1 functions. Initiation of BIR requires processing of replication forks by a variety of nucleases, including Mre11, Exo1, DNA2 and Mus81 (Fig 1). Given the requirement for DNA breaks in PARP1 activation, we will assess whether these factors are required to activate PARP1 in response to replication stress. Using high-resolution microscopy and biochemical assays (e.g. iPOND) we will assess which BIR factor(s) require PARP1 to assemble at stalled/damaged replication forks. These assays will be complemented by

DNA fibre analysis and plasmid reporter assays to assess the requirement for PARPs and BIR factors in replication fork restart and BIR. Together, these data will identify how PARP1 is activated in response to replication stress and the factors that it regulates to promote BIR.

Identify targets that are modified by PARP1 to regulate BIR

Having identified where in the BIR pathway PARP1 functions, we will define the mechanistic basis of this regulation. In collaboration with M. Nielsen (University of Copenhagen), we used mass spectrometry (MS) to detect ADPr of 113 proteins on 170 sites in response to replication stress induced by hydroxyurea (HU). This includes 8 HR factors (17 sites in total) and 2 BIR proteins (Mre11 [2 sites]; PolD3 [1 site]). We will validate HU-induced Mre11 and PolD3 ADPr by IP of endogenous or epitope-tagged proteins from cells and assessing ADPr with ADPr-specific antibodies. Generation of mutants at these sites and testing their ADPr status will confirm site-specific ADPr. Following validation, site specific mutations will be knocked in to endogenous genes using genome editing and the impact on BIR assessed using a variety of assays including the ability of cells to survive replication stress, assembly of BIR factors at stalled/damaged replication forks, cell based assays for BIR activities etc.

Biochemical characterisation of PARP-dependent BIR activities

We will assess the impact of ADPr on the biochemical activities of the factors identified above, with specific reference to nuclease and DNA polymerases. Initially, this will focus on Mre11 and PolD3, but the strategy will be extended to other BIR factors if required (e.g. Mus81). Purified Mre11 and PolD3 will be assayed for nuclease and DNA polymerase activities respectively, either before or after ADPr with PARP1. Different aspects of activities will be analysed, including nuclease/polymerase speed and polarity, in addition to substrate specificity of nucleases towards replication fork and other DNA structures. Proteins mutated at ADPr sites (see above) will be incorporated into this analysis to confirm whether any differences in activities induced by PARP1 is dependent on the ADPr sites. This analysis will complement genetic/cell biology approaches to assess the role of PARPs in BIR described above. Importantly, in the longer term they will also serve as a platform to screen for inhibitors that impact on the ADPr-specific activities identified.

Translational potential

This work will define novel synthetic lethal interactions with HR. Findings will be progressed to the clinic by exploiting the biochemical assays described to develop inhibitors against these pathways that can then be used to treat breast and ovarian tumours that have developed resistance to PARP inhibitors.

Training opportunities

Training will be provided in a variety of cutting-edge techniques including genome editing, high resolution/content microscopy, cell-based assays to assess DNA repair, protein expression/purification and biochemical assay development.

References

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24. Chemical functionalisation of nanobodies targeting the Hedgehog pathway [3] – Dr. Lanyon-Hogg

Primary Supervisor: Dr. Thomas Lanyon-Hogg
Additional Supervisors: Prof. Christian Siebold
Eligibility: Track 1 students are eligible to apply for this project.

Project Summary

Hedgehog (HH) signalling plays fundamental roles in cell proliferation and survival. Aberrant expression of the Sonic HH (SHH) ligand has been linked to the initiation and progression of numerous cancers, including pancreas, breast, lung, prostate, and stomach. We have developed single chain camelid antibody ("nanobody") binders of several key proteins in the HH pathway, that exhibit high affinity for their target proteins and have been validated by structural studies. This DPhil will develop these nanobody tools using synthetic chemistry modification guided by structural biology to produce reagents for a variety of purposes, including therapeutic blocking of HH signalling and biomarkers for early-stage cancer detection.

Research objectives

HH signaling is essential for embryonic development and plays fundamental roles in tissue homeostasis, cell proliferation and survival in adults.[1,2] HH signal transduction is activated by binding of the secreted morphogen SHH to the cell-surface receptor Patched (PTCH), which releases the inhibitory function of PTCH on the G protein-coupled receptor Smoothened (SMO).[3,4] SMO subsequently relocates to the primary cilium leading to activation of the transcriptional effector GLI, which ultimately regulates expression of SHH target genes, including PTCH and GLI.[5] Ectopic expression SHH ligand has been linked to the initiation and progression of numerous cancers, such as pancreas, breast, lung, prostate, and stomach cancers.[6]

We have developed nanobodies against several key proteins in the HH pathway, including SHH, PTCH and SMO. These nanobodies have low- or sub-nanomolar affinity for their targets, and we have used them in cellular assays and to aid structural solution of target protein complexes (**Fig 1**).[7,8] Nanobodies have also been used to activate HH signalling *in vivo.*[9] The **research objectives** of this proposal are to modify our high-affinity nanobodies, using synthetic chemistry guided by structural biology, to build in new functionality for analysis and inhibition of the HH pathway. This collaboration will support progression of nanobody binders towards tools for clinical applications, and we can already produce >50 mg of monodisperse nanodies for studies. The research objective will be achieved through the following aims:

Aim 1. Production of nanobodies and synthetic chemical modification. Different functional groups will be produced using synthetic chemistry and attached to individual nanobodies, including: fluorophores attachment (e.g. sortase tagging,[10] cysteine modification) to allow fluorescence imaging of the target; formation of reactive warheads to allow covalent bonding to targets (e.g. dehydroalanine[11]); fusion to moieties triggering interalisation and degradation, or immune stimulating small-molecules (e.g. SZU-101[12]); nanobody-drug conjugates to deliver selective chemotherapy payloads (e.g. monomethyl auristatin E[13]).

Aim2. Characterisation of functionalised nanobodies in established biophysical assays for maintenance of target-nanobody protein-protein interactions, and cellular assays to probe for HH pathway signalling activity, including measurement of GLI mRNA. We will further use fluorescent nanobodies as detection reagents to measure localisation/internalisation of target proteins in the primary cilium, the HH signalling antenna.[2]

Proposed outcomes. This project will generate a suite of highly selective nanobody tools that can modulate the HH pathway function for therapeutic effect. Nanobodies can also be used as imaging reagents to detect the presence/absence of HH pathway components, providing potential biomarkers for early stage HH pathway activation in cancer. Development of these tools will also have substantial academic impact in the potential of synthetic chemical modification of nanobodies to introduce new and unnatural functionality into these already powerful molecules.

This project will involve collaboration between the Lanyon-Hogg laboratory, performing synthetic chemistry and modification of nanobodies, and the Siebold laboratory, performing nanobody production and biophysical protein-protein interaction studies and cellular assays. The Siebold group have solved structures of nanobodies in complex

with SHH/PTCH/SMO, which will guide selection of residues for mutation/modification. This award will be instrumental in facilitating this highly-interdisciplinary research programme, requiring a skillful and dedicated DPhil candidate to drive progress across the different disciplines. Funding will support design, production and analysis of functionalised nanobodies, priming transition to preclinical models through subsequent grant applications and collabroations within the CRUK Oxford Centre and more broadly with the CRUK community. Nanobodies hold substantial therapeutic promise, and this DPhil will provide a roadmap for future studies exploring use as multifunctional reagents.

Translational potential

Upregulation of HH signalling is linked to formation of numerous cancers, which can be targeted by drugs that inhibit HH signaling. SMO inhibitors have received FDA approval for basal cell carcinoma; however, these are compromised by the rapid emergence of resistance mutations in SMO blocking inhibitor binding.[14] The monoclonal antibody 5E1 binds to SHH and inhibits medulloblastoma growth in mouse models;[15] however, nanobodies have several advantages over antibodies due to their smaller size, higher solubility and stability, making nanobodies particularly well suited for specific and efficient antigen targeting in tumours. With lower production costs, nanobodies represent a very attractive therapeutic. In this project, we will build new functionality into high-affinity nanobody binders to allow covalent inhibition of the target protein, or triggering internalisation and degradation. We will also investigate the use of nanobodies to deliver chemotherapies to increase potency in cells.

HH signalling is a driver of growth of pancreatic cancer, one of CRUK's cancers of unmet need. PDAC is the fourth leading cause of cancer related deaths in the UK, due to late diagnosis and ineffective treatments. Inhibition of HH signaling reduces PDAC growth, specifically limiting stromal proliferation allowing deeper penetration of chemotherapeutics [16,17] and in a phase II clinical trial the combination of HH inhibitors with standard of care chemotherapeutics showed improved survival [18]. Our functionalised nanobodies could therefore have significant therapeutic benefit to PDAC patients as combination therapies.

In addition to acting as a potential anti-cancer therapeutic, the fluorophore-labelled nanobodies could be used as tools to detect overexpression of HH pathway components. This is particularly relevant for PDAC as there are currently no means to reliably detect early stage disease, leading to decreased patient prognosis. Imaging using functionalised nanobodies could be a powerful biomarker method for PDAC diagnosis at an early stage when treatment options are still available.

Training opportunities

The student will receive training in protein expression and purificaftion from bacterial cells using established protocols for nanobody production. They will also learn synthetic organic chemistry including reaction planning, COSHH, synthetic methods, and analysis techniques (NMR, MS). Further training will be given in structure-guided design software (PyMol, Flare), techniques for protein modification (sortase tagging, cysteine labelling, dehydroalanine formation) and characterisation of proteins (MS, in-gel fluorescence, Western blot). For analysis of functionalised nanobodies, the student will learn cell culture and methods for determination of HH pathway activity (GLI mRNA qPCR, immunofluorescence microscopy). In addition to practical skills, training will be provided in soft skills including project planning, writing and presentation, which will be delivered as part of a team working environment that prioritises diversity and inclusivity.

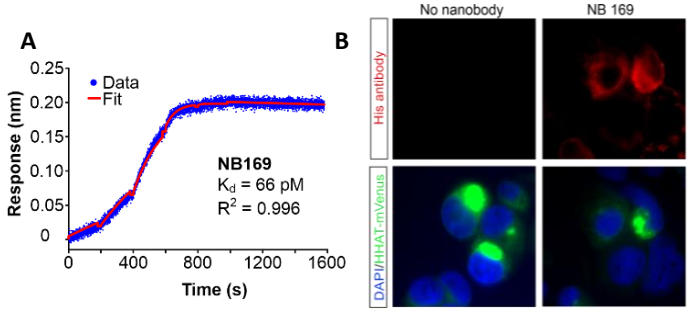


Fig1. A) Exceptionally high-potency nanobody-target binding;[8] **B)** His_6-nanobody-target binding in cells;[8] **C)** structure of nanobody in complex with PTCH extracellular domain.[7]

References: **1**. Lanyon-Hogg *Trends Biochem. Sci.* 2017, 566; **2**. Kong *Development* 2019; **3**. Radhakrishnan *Nat. Chem. Biol.* 2020, 1303; **4**. Kowatsch *Curr. Opin. Struct. Biol.* 2019, 204; **5**. Rodgers *ACS Chem. Biol.* 2016, 3256; **6**. Rubin *Nat. Rev. Drug Discov.* 2006, 1026; **7**. Rudolf *Nat. Chem. Biol.* 2019, 975; **8**. Coupland *bioRxiv* 2021, 2021.07.08.451580; **9**. Zhang *P Natl Acad Sci USA* 2020, 28838; **10**. Williamson *Nat Protoc* 2014, 253; **11**. Jones *Rsc Chem Biol* 2020, 298; **12**. Gao *Sci Rep* 2016, 39598; **13**. Akaiwa *Chem. Pharm. Bull.* 2020, 201; **14**. Byrne *Nature* 2016, 517; **15**. Coon *Mol. Cancer Ther.* 2010, 2627; **16**. Olive *Science* 2009, 1457; **17**. Bailey *Clin. Cancer. Res.* 2008, 5995; **18**. De Jesus-Acosta *J Clin Oncol* 2014, 32;

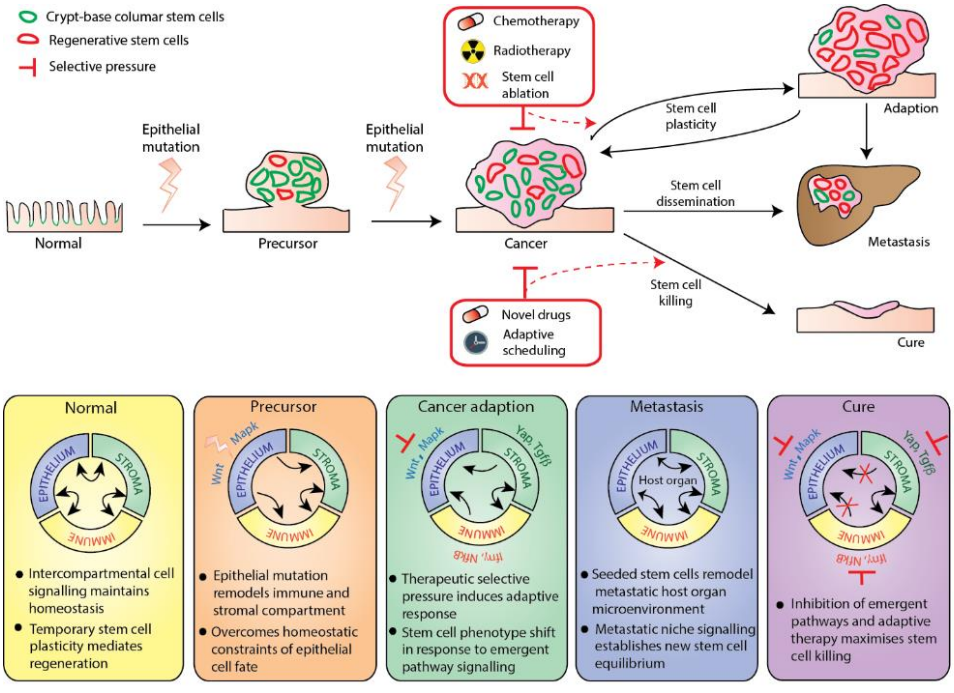
25. Stem cells and adaptive molecular phenotype in colorectal cancer (STAMP-CRC)[1,2,3,4] – Prof. Leedham

Primary Supervisor: Prof. Simon Leedham
Additional Supervisors: Dr. Alex Gordon Weeks
Eligibility: Track 1, 2, 3 and 4 students are eligible to apply for this project.

Project Summary

Tumour heterogeneity plays a key role in cancer adaption and resistance to therapies, but understanding genetic heterogeneity alone cannot paint a complete picture. The forces of natural (and therapeutic) selection act upon phenotypic characteristics, and phenotype is a function of both the genotype and the microenvironment. The capacity to measure and understand relevant cancer cell phenotypic variation is key to monitoring neoplasia evolutionary trajectory. We believe that cancer stem cell molecular phenotype is an informative readout of dynamic evolutionary change within a tumour and is an important, and currently unmeasured metric that can improve prediction of tumour response to treatments, biologically inform existing therapy scheduling and drive the development of cancer cell adaption drug targets. Here we will assess demonstrable cross-species stem cell phenotypic heterogeneity in intestinal tumours, investigate the driving co-evolutionary interaction between the mutant epithelium and surrounding stromal/immune cell compartments, and assess the spatio-temporal impact of therapeutic selective pressures.

Background: Adult tissues are complex ecosystems, dictated by the inter-dependence of developmentally distinct, but interacting cellular compartments – the epithelium, stroma and immune system, all embedded within a secreted extracellular matrix. These compartments co-evolve: through organ development, maintenance of homeostasis, tissue regeneration and the initiation, invasion and dissemination of cancer cells. This complex intercompartmental crosstalk, mediated by conserved cell signalling pathways, establishes a dynamic equilibrium with powerful cell-extrinsic influence that shapes and constrains epithelial cell fate determination, both in homeostasis and disease. Disruption of this equilibrium, as occurs in inflammation, can temporarily alter epithelial cell fate inducing dedifferentiation and return to stemness for a wide range of cells1. This cell fate plasticity underpins rapid intestinal regenerative capacity but is co-opted and corrupted in carcinogenesis. In this setting, punctuated epithelial mutation initiates permanent disruption of signalling crosstalk, eventually overcoming tissue homeostatic constraints and driving pathological intercompartmental co-evolutionary trajectories.



Here we propose that dynamic epithelial cell plasticity in established lesions is regulated by the combination of key cell-intrinsic (epi)mutation(s) and disrupted microenvironmental signalling pathways. We believe that malleable cell fate is responsible for tumour adaptive responses following therapeutic selective pressures, and that this intercompartmental interaction can be assessed using our newly developed techniques to measure cancer stem cell molecular phenotype (Gilvasquez, Nasreddin submitted).

Research Objectives

We propose an intercompartmental co-evolution model of colorectal carcinogenesis where epithelial accumulation of somatic mutation results in remodelling of the stromal and immune microenvironment which, in turn, signals back, regulating cancer stem cell plasticity and promoting tumour adaption to therapeutic selective pressures. We believe that this intercompartmental interaction can be 'read out' through cancer stem cell molecular phenotype. Mapping the signalling landscape that promotes stem cell plasticity is key to biologically informing current intervention scheduling, in order to exploit the trade-offs required for tumour adaptive change (evolutionary steering), as well as developing the next generation of treatments that target emergent adaptive signalling pathways.

Objective 1. Assess the impact of therapeutic selective pressures on adaptive stem cell behaviour

Our prelim data shows that application of a therapeutic selective pressure can result in profound adaptive shifts in the stem cell molecular phenotype without notable impact on conventional clinical measurements of tumour response (e.g tumour size, cell proliferation etc). We hypothesise that stem cell molecular phenotype may be an informative, but currently unmeasured metric that could drive adaptive scheduling and evolutionary steering of current therapies, whilst developing and testing novel treatments to target emergent tumour adaptive signaling pathways. Mouse models are necessary for testing temporal progression of tumours, and preclinical drug and radiation therapies can be used to apply therapeutic selective pressures. We will use state-of-the art, disease-positioned mouse models of aggressive and treatment refractory tumours (e.g *Braf* driven right sided lesions, and CMS4), and test the effect of adaptive drug scheduling and novel treatments using stem cell phenotype to guide therapy type and timing. We will use genomic sequencing, including single cell transcriptomics where appropriate, established multiplex immunohistochemical staining and tissue ecology measures to assess outcome.

Objective 2. Understanding and predicting stem cell dynamics in primary and metastatic tumours

Metastasis represents an evolutionary bottleneck and induces a stem cell clonal reset in a new host organ. Understanding the relationship between the stem cell dynamic key of the primary tumour and any distant organ metastasis is key to determining whether key phenotypic characteristics of the primary tumour can be used to predict dissemination or metastatic response to therapy. We will undertake combined molecular and morphological stem cell phenotyping of paired primary tumours and metastases in mouse and human tissue. This will allow us to map the metastatic stem cell landscape in different secondary organs, assess for a correlative relationship between the primary tumour stem cell phenotype and secondary deposits in stage IV cancers, and assess for organ specific cell-extrinsic pathways that influence the stem cell equilibrium in different metastatic niches. Results will be used to predict optimal regimens for preclinical testing of evolutionary steered metastatic therapies.

Translational potential

Understanding the interaction of the immune and stromal components of colorectal cancer is central to developing the next generation of drugs that are able to target and abrogate the emergent pathways that mediate tumour adaption and drug resistance. This project will combine use of state-of-art, disease-positioned mouse models together with human tissue to iteratively assess tumour microenvironmental interactions and then preclinically test new agents, with the aim of de-risking clinical trial progression.

Training opportunities

This would suit a student from any of the eligible pathways with an interest in understanding cancer heterogeneity and tracking tumour evolution and adaption. The project will involve mouse modelling and preclinical drug testing but requires no previous experience. Clinical training can be arranged for medically qualified candidates.

References:

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26. Understanding the role of infection in responses to cancer immunotherapy, using single cell approaches [1,2,3] – Prof. Lu

Primary Supervisor: Prof. Xin Lu
Additional Supervisors: Prof. Sir. Andrew McMichael
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

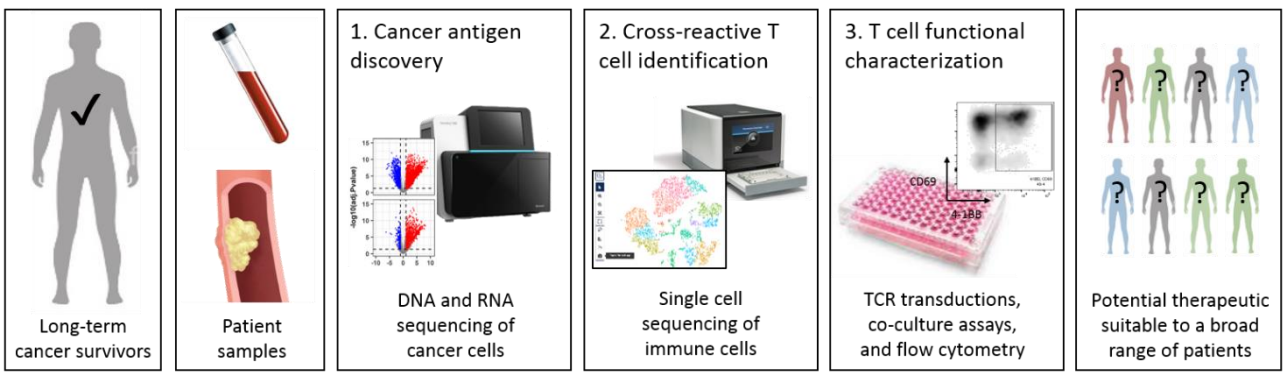
Project Summary

Immunotherapy has greatly improved the outcome of previously untreatable cancers, but only a limited proportion of patients respond to this treatment. Understanding the molecular mechanisms behind patients' durable responses to immunotherapy is the biggest challenge in the field. This project will investigate an exciting hypothesis that latent immune responses against infectious diseases can be reactivated by immunotherapy and contribute to cancer elimination. The study will use a unique collection of blood, tumour and normal tissue samples from long-term oesophageal cancer survivors, collected before and after immunotherapy in an experimental medicine clinical trial. These samples are being analysed using cutting-edge methodologies and this project will dissect cancer/viral cross-reactive responses, using single cell transcriptomic and proteomic technologies as well as cloning, gene transduction, co-culture assays, and flow cytometry analysis. The student will be supervised by world-leading experts in immunology and cancer biology, joining a team effort to improve the implementation of immunotherapy in oesophageal cancer.

Research objectives

Background: Oesophageal cancer is the 6th most common cancer and its incidence is rising rapidly. Despite recent advances in treatment options, oesophageal cancer continues to have a poor prognosis and it is identified as a cancer of unmet clinical need [1]. Immunotherapies that block the PD1-PDL1 axis strongly reinvigorate immune responses and eliminate tumour cells. The use of anti-PD1 to treat oesophageal cancers was recently approved after clinical trials demonstrating its clinical benefit [2]. However, as not all patients respond to these therapies, the biggest challenge in the field is to identify the molecular requirements for successful and durable responses. We have preliminary data indicating that a significant proportion of tumour infiltrating lymphocytes harbour T cell receptors (TCR) known to recognize antigens from Epstein-Barr Virus, Cytomegalovirus and Influenza virus, so we hypothesize that latent immune responses against infectious agents that cross-react against cancer cells can be reactivated by immunotherapy and contribute to the immune control of cancer. Studying patients that responded to immunotherapy and survived more than 5 years after treatment is a great opportunity to find such immune responses.

Objectives and outcomes: The overall aim of this new line of research is to identify the mechanisms behind long-lasting control of cancer after immune checkpoint therapy. The specific objective of this project is to characterize immune responses against cancer and viral antigens present in the tumours and peripheral blood of long-term cancer survivors. The final outcome is to identify new cancer-specific TCR, which will have the potential for development into novel immunotherapeutics to treat checkpoint therapy refractory patients.



Approaches (see figure): The student will have access to samples from a cohort of oesophageal cancer patients treated with anti-PDL1 only or in combination with anti-CTLA-4. A unique collection of blood, tumour and normal tissue samples obtained at baseline and after immunotherapy will be available. The student will dissect patients' immune responses at single cell level, using genomic, transcriptomic, and cellular assays. Cancer antigens will be discovered using transcriptomic and genomic data already obtained from the tumour samples. Antigen-specific T cells will be identified using 10x genomics single cell sequencing and barcoded multimers, as well as data mining in cancer and microbial TCR databases (VDJdb and GLIPH). Cancer-specific TCR will be identified using co-culture of transduced immune cells and cancer cells or peptide pools. Candidate virus-specific TCR will be screened against peptide pools and yeast libraries (with collaborators, if required).

Translational potential

Through addressing a novel hypothesis, this project has the potential to identify new biomarkers for predicting response to immunotherapy and to generate new immunotherapeutic molecules to treat patients with oesophageal cancer. If our hypothesis proves correct, the presence of certain infectious agents could be used as correlates of response to immunotherapy. Irrespective of the outcome, the TCR molecules identified by the student could be used in future adoptive cell transfer therapies to control cancer in a broad range of patients.

Training opportunities

The student will be trained in state of the art molecular and cellular biology techniques including single cell transcriptomics and proteomics, TCR cloning and transduction, immune cellular assays, and multi-colour flow cytometry. This project will also involve learning how to work with clinical trial samples. The student will have opportunities to integrate with the wider scientific and clinical communities in Oxford through established collaborative networks – both in immunology and cancer - and with national and international communities at conferences. The student will benefit from the training and career development programme at the Ludwig and Nuffield Department of Medicine, which includes: regular oral presentations, journal clubs, and skills development in writing, data management and public engagement.

References:

1. Smyth, E., Lagergren, J., Fitzgerald, R. et al. Oesophageal cancer. Nat Rev Dis Primers 3, 17048 (2017). 2. Ilson, D. H. Adjuvant Nivolumab in Esophageal Cancer — A New Standard of Care. N Engl J Med 2021; 384:1269-1271

27. Modelling cyclic hypoxia and its effects on cancer cells and therapy [4] – Prof. Maini

Primary Supervisor: Prof. Philip Maini
Additional Supervisors: Prof. Ester Hammond
Eligibility: Track 4 students are eligible to apply for this project.

Project Summary
Regions of low oxygen, hypoxia, occur to some degree in most solid tumours as a result of the poorly formed tumour vasculature and the high metabolic demand of cancer cells. Less understood is the phenomenon of cycling hypoxia, which describes the fluctuations in oxygen level which also occur in tumours. Cycling hypoxia has been described as leading to aggressive disease, therapy resistance and increased metastasis. Cycling hypoxia affects DNA replication and cell cycle progression leading to an accumulation of mutations and DNA damage. We have developed a new mathematical model that can account for recently published experimental data that show how the periodic exposure of cells to limiting oxygen levels impacts their progress through the cell-cycle. The aim of this project is to take this model, developed under the simplifying assumption that the system is spatially well-mixed, and extend it to the more biologically realistic case of an environment in which oxygen levels vary spatially, as well as temporally. This will then allow us to investigate in depth how cycling hypoxia can lead to tumour heterogeneity in response to treatment (such as radiotherapy) and to explore how best to regulate oxygen levels in order to enhance the efficacy of treatment.

Research Objectives
Background: In normal cells, the DNA damage response (DDR) maintains genetic stability by promoting cell cycle arrest to allow time for DNA repair or cell death (apoptosis). The DDR is activated early during tumourigenesis as an anti-cancer barrier in response to oncogene activity and physiological stresses. However, continuous activation of the DDR results in a selective pressure for the outgrowth of mutated cancer cells, with aberrant cell-cycle progression and apoptotic control. Exposure to insufficient oxygen levels, i.e., hypoxia, is a key driver of loss of cell cycle control and apoptotic resistance. Hypoxic regions are commonly found in solid tumours as a result of the uncontrolled proliferation of tumour cells and abnormal structure of tumour vasculature. Exposure to severe levels of hypoxia (< 0.1% O2), which are only observed in pathophysiological conditions, leads to replication stress and consequent activation of DDR and the pro-apoptotic p53 tumour suppressor. Such levels of hypoxia are commonly denoted as radio-biological hypoxia (RH) due to their association with resistance to radiotherapy. This latter observation shows how important it is to understand the effects of cycling hypoxia on the cell cycle. We have developed a new mathematical model for the cell cycle that takes into account recent experimental data from the Hammond lab (having confirmed that previous mathematical models in the literature cannot account for these data).

Objectives: The model we have developed has been analysed and used to make predictions on the effects of cycling hypoxia on the cell cycle in a scenario in which there are no spatial effects, the so-called “well-mixed” system. While this is useful to compare and validate our model with experimental data, it is not clear how applicable it is to the clinical setting in which the tumour cell micro-environment is highly spatially heterogeneous. Our aim is to now explore this model in the context where oxygen levels fluctuate not only in time, but also vary in space, and to see what predictions it makes on the resulting heterogeneity of cell response to radiation treatment. The model will then provide us with an in silico setting that will allow us to explore different treatment protocols, for example enhancing hypoxia may kill more cells but may lead to more radiation resistance in the surviving cells, while reducing hypoxia would limit resistance but allow more cells to survive. In such cases, where there are contrasting factors at play, mathematical models allow us to explore, very efficiently, the effects of different treatment protocols. In this way, we can determine which treatment strategies would be optimal for maximising tumour kill.

Approaches: Our present model consists of a coupled system of differential equations which describe the evolution of a cell population through different stages of the cell cycle. To extend this to a spatial context will require us to develop an agent-based model, in which each agent would be a cell, equipped with its own cell cycle model. By imposing spatial heterogeneity in oxygen levels across the tumour, and by including oxygen uptake, we will simulate tissue responses to varying levels of cycling hypoxia and then model how such tissue would respond to radiotherapy.

We envisage that for large tumours, this model will become computationally intractable so we will also use a coarse-graining approach to determine a partial differential equation model for cell density allowing the model to be simulated more efficiently.

Proposed Outcome: The outcome will be a new spatial model for tumour cell dynamic response to cycling hypoxia which will be used to suggest how combination therapies can be used to enhance treatment.

Translational potential

This work fits into the theme of Cancer Big Data (Maini and Byrne are two of the Cancer Centre Theme Leaders). It will provide an example of how multidisciplinary research can combine to provide advances that cannot be attained by pursuing a single disciplinary approach. In this case, it will allow us to suggest new strategies for how to adapt radiation treatment in light of the tumour micro-environment and, in turn, how to modify the micro-environment to enhance the effect of treatment.

Training opportunities

The student will be trained in mathematical modelling of multiscale systems using ordinary, partial, and delay differential equations, hybrid agent-based models, computational techniques. The student will be able to attend relevant Masters-level lectures if required and will be part of the large mathematical oncology group at the Wolfson Centre for Mathematical Biology (WCMB), which has weekly group meetings during term. The WCMB, as a whole, also meets weekly for research skills training sessions, as well as presenting research, and there are formal weekly seminars given by external expert speakers. The student will also join the Hammond lab for meetings. Hence, the student will emerge with a broad background in mathematical modelling in biology, with detailed expertise in the multiscale modelling of cancer and its clinical implications.

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28. Exploiting synthetic defects in metabolism and DNA repair to improve the treatment of glioma and AML [2,3] – Prof. McHugh

Primary Supervisor: Prof. Peter McHugh
Additional Supervisors: Dr.Daniel Ebner
Eligibility: Track 2 and 3 students are eligible to apply for this project.

Project Summary
Current treatments for gliomas, an aggressive form of brain tumour, are non-specific and do not significantly increase clinical survival rates. Likewise, treatments for acute myeloid leukaemia (AML), especially relapsed disease, remain challenging. We have discovered a potential synthetic lethal response in tumours which exhibit a mutation in the isocitrate dehydrogenase (IDH) gene: IDH mutations are present in ~80% of gliomas and ~20% of AMLs. Specifically, the loss of several related DNA repair factors and IDH mutation leads to loss of cancer cell viability. Combining mechanistic cellular studies with our ongoing studies on small molecule DNA repair inhibitors could provide a route to treat these aggressive diseases.

Research objectives
Following the sequencing of thousands of glioblastoma samples, IDH was found to be mutated in in around 80% of tumours and in a significant subset of AML. A specific IDH1 (R132H) mutation occurs in about 70% of glioma tumours. IDH1 and IDH2 play key roles in redox metabolism, catalysing the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG) and CO2 producing NADPH (Cohen et al., 2013). The cancer-associated mutant forms of IDH enzymes produce 2-hydroxyglutarate (2-HG), termed an 'oncometabolite', a metabolic intermediate that helps tumour cells survive and proliferate. 2-HG acts as a competitive inhibitor of enzymes using α-ketoglutarate as a co-substrate, including a family of metal dependent dioxygenases, the ALKB family (Fig. 1) (Rohle et al., 2013).
ALKBH2 and ALKBH3 are non-heme, α-KG-dependent dioxygenases which repair alkylation DNA damage. ALKBH2 is predominantly involved in repairing 1-methyladenine (1-meA) lesions on double-stranded DNA whilst a substrate for ALKBH3 is 3-methylcytosine (3-meC) lesions on single-stranded DNA (Dango et al., 2011, Fedeles et al., 2015).
Following a genetic screen we observed a synthetic lethal response to ALKBH2 and ALKBH3 loss that is induced by loss of several DNA repair factors. This, in turn, implied that cancer cells harbouring IDH mutations could be sensitive to simultaneous inhibition of DNA repair genes by virtue of their reduced ALKB activity. Follow-up studies suggest that this hypothesis is correct.

Experimental plan
We will explore the detailed biology of the synthetic relationship of DNA repair defects with the most important clinically relevant IDH1 R132H patient mutation, as well as ALKBH2/3 disruption. By combining repair pathway loss (looking across all the major DNA repair pathways) with IDH mutation and ALKBH2/3 disruption, we will establish the full extent of this relationship. These findings will be validated in isogenic, matched glioblastoma and AML cell lines with and without IDH mutations, allowing us to explore this potential mechanism of synthetic lethality in a relevant cancer setting. These cell lines will be carefully validated to ensure an accurate understanding of the alterations made during the genome engineering process.
We will next characterise the nature of the DNA repair defects observed in repair defective IDH mutant (and ALKB deficient) cells using a wide range of well-established cellular, genetic and biochemical assays available to us. We will also define the pathway to cell death in cancer cells lacking ALKBH2/3 or mutated in IDH1 upon DNA repair pathway loss. Furthermore, inhibitors of IDH and ALKBH2/3 are available, via our collaborator Prof. Chris Schofield (Chemistry Oxford) (Woon et al., 2012). Molecules that target both wild-type IDH1 but also selectively inhibit the R132H form (as well as other clinically observed variants) have been developed and will be used to test our hypothesis that in IDH mutated cells can be killed through DNA repair pathway inhibition.
We will employ IDH wt/Mutant isogenic cell line pairs to execute two cell-based high throughput screens. The first is designed to identify novel therapeutically translational anti-cancer small molecules for the treatment of IDH mutant tumours. The second will employ pooled CRISPR/Cas9 pooled screening to identify novel synthetic lethal targets in the DNA Damage Repair pathway with IDH mutant cell lines. Both small compounds and novel CRISPR targets will be validated in high-grade IDH mutant astrocytoma cell lines derived from aggressive patient tumours that have progressed from low-grade IDH mutant astrocytomas. The application of these clinically relevant cell lines to validate

the novel targets identified in the primary screens is a critical step in the potential development of therapeutic drugs. In summary, we aim to test the potential synthetically lethal interaction between DNA repair genes and IDH mutation with a view to it becoming a targetable clinical pathway in IDH mutant gliomas and AML, providing a specific therapeutic approach for cancers which currently have a poor clinical prognosis.

Translational potential

This proposal addresses a key priority of the Cancer Research UK and the Oxford Centre as it uses basic science to validate novel approaches to two difficult to treat cancers, AML and glioma.

Training opportunities

Cell culture, genomic engineering (CRISPR-Cas9 and base/prime editing), large-scale screens, general molecular biology methods, DNA damage and repair assays, advanced microscopy, cell sorting methods, protein purification chemical biology, protein science/enzyme inhibition, and biochemical assays.

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1.Cohen AL, et al. (2013) IDH1 and IDH2 mutations in gliomas. Curr Neurol Neurosci Rep, 13: 345. **2.** Dango S, et al. (2011) DNA unwinding by ASCC3 helicase is coupled to ALKBH3-dependent DNA alkylation repair and cancer cell proliferation. Mol Cell, 44: 373-84. **3. F**edeles BI, et al. (2015) The AlkB Family of Fe(II)/α-Ketoglutarate-dependent Dioxygenases: Repairing Nucleic Acid Alkylation Damage and Beyond. J Biol Chem, 290(34), 20734-42. **4.** Jalbert LE, et al. (2017) Metabolic Profiling of IDH Mutation and Malignant Progression in Infiltrating Glioma. Sci Rep, 7: 44792. **5.** O'Connor MJ (2015) Targeting the DNA Damage response in cancer. Mol Cell, 60(4), 547-560. **6.** Rohle D, et al. (2013) An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells. Science, 340: 626-30. **7.** Woon EC, et al. (2012) Dynamic combinatorial mass spectrometry leads to inhibitors of a 2-oxoglutarate-dependent nucleic acid demethylase. J Med Chem, 8;55(5), 2173-84

29. Underlying molecular and epigenetic mechanisms of Ikaros function in Acute Lymphoblastic Leukaemia and Multiple Myeloma[1,2,3] - Associate Prof. Milne

Primary Supervisor: Associate Prof. Thomas Milne
Additional Supervisors: Associate Prof. Anindita Roy
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Project Summary

Relapsed and refractory Acute Lymphoblastic Leukaemia (ALL) is the second major cause of cancer death among children. ALL is generally a disease of lymphocyte progenitors that become transformed through various mechanisms, but the transcription factor Ikaros plays a key role in ALL progression and resistance to treatments. Multiple myeloma (MM) is a disease of mature lymphoid cells called plasma cells, which puts it at the opposite end of the lymphoid development spectrum from ALL. Interestingly however, MM is united with ALL in that the transcription factor Ikaros has an important, although possibly contrasting, role in both diseases. In particular, Ikaros is essential in MM, and is a target of degradation by immunomodulatory imide drugs (IMiDs) such as lenalidomide. To better understand how MM develops resistance to drugs such as lenalidomide, we are proposing to better understand the molecular mechanisms of Ikaros function in both ALL and MM, with a particular focus on how Ikaros controls gene expression.

Research objectives

Although ~90% of children with Acute Lymphoblastic Leukaemia (ALL) now survive beyond 5 years, current treatment regimens have failed to improve outcomes in a distinct subset of high-risk paediatric ALLs, making relapsed and refractory ALL the second major cause of cancer death among children [1]. Since paediatric ALL generally has a relatively low burden of somatic mutations [1,2], it is possible to track mutations associated with treatment failure. One mutation associated with both high-risk ALL as well as the development of resistant clones after treatment is alterations (often deletions) in the *IKZF1* gene, which codes for the transcription factor (TF) Ikaros [1]. *IKZF1* is a widely expressed gene that is generally important for normal haematopoietic development and is also more generally expressed in leukaemia [3,4]. As well being involved in ALL, Ikaros is a target of lenalidomide and pomalidomide, drugs that are part of the class of immunomodulatory imide drugs (IMiDs) used to treat multiple myeloma [5]. Multiple myeloma (MM) is in the same developmental pathway as ALL, but rather than being a disease of progenitor B cells, MM arises from a terminally differentiated transformed plasma cell. Despite being at the opposite end of the developmental spectrum from ALL, the importance of Ikaros is something that links together these two otherwise quite different diseases. This provides an opportunity to better understand the molecular details of Ikaros function in two different contexts, where it has important, but potentially opposite molecular roles. The molecular function of Ikaros is not completely understood, but it can interact with both activating and repressing complexes, as well as mediate interactions between enhancers and promoters through epigenetic mechanisms [3]. Enhancers are key regulatory elements in the genome that control gene expression; and differential enhancer usage may contribute to patient outcomes. What is not understood is exactly how Ikaros controls enhancer activity, and how resistance to lenalidomide impacts this activity. This project has three major goals:

AIM 1) To determine if Ikaros can activate enhancers *de novo* through epigenetic mechanisms
AIM 2) To determine if Ikaros regulates enhancers of important gene targets in MM and ALL
AIM 3) To determine if iMiD resistance in MM is due to acquisition of alternate enhancer activation pathways and thus loss of Ikaros essentiality.

AIM 1) Most work on enhancers (including our own [6,7]) has concentrated on methods of removing specific factors to determine their effect on endogenous enhancer function. This is essentially a loss of function approach, and provides useful information on what factors are necessary for enhancer function. However, to really understand what each factor contributes to enhancer behaviour, gain of function approaches are required to test for sufficiency. To accomplish this, we used a TetO array inserted into a gene desert region in mouse ES cells (Figure 1). By fusing a protein of interest to the TetR DNA binding domain, it is possible to anchor a protein or domain of interest at this gene desert region and determine whether it can recruit specific activities *de novo*. Our preliminary findings demonstrate that anchoring a specific TF transactivation (TA) domain is sufficient to cause histone acetylation (H3K27ac) and initiate transcription from regions more than 50kb distal to the TetO locus by creating new enhancer-like interactions (Figure 1). However, not all TFs we have studied have this ability to create enhancer-like elements *de novo*, and some instead recruit repressive complexes

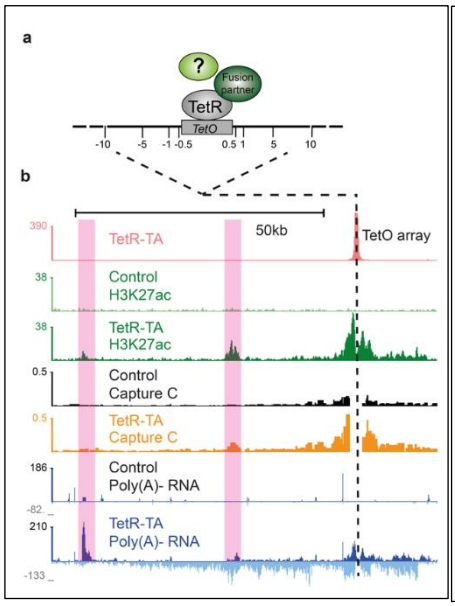

Figure 1: A transcription factor transactivation domain (TA) that is sufficient to create a* de novo *enhancer in a gene desert. (a) *An array of 14 TetR binding sites (TetO) in a gene desert region inserted into chromosome 8 in mouse ES cells. Expression of the TetR-TA in these cells anchors the TA domain to the* TetO *array.* ***(b)*** *TetR only (control) and TetR-TA cell lines showing TetR-TA ChIP-seq (top), H3K27ac ChIP-seq (green), NG Capture C (black and orange), a high resolution 3C technique (see publications 6 and 7) showing interactions with the* TetO *array. Poly(A)- RNA-seq (blue) across the region containing* TetO *is also shown. Pink highlighted regions indicate two novel enhancer-like elements that appear in the TetR-TA line. Dashed line indicates position of* TetO *array.*

and set up a repression domain (not shown). Since Ikaros is known to recruit both repressive and activating complexes, we want to fuse different domains of Ikaros to TetR and use this system to determine which activity predominates. We have a particular interest in using NG Capture C to determine if Ikaros can create enhancer-promoter interactions *de novo* (Figure 1). Our expected outputs are that we will have a clear understanding of what factors Ikaros alone can recruit, and to determine if it has a role in initiating enhancer contacts as proposed in the literature [3]. This will be the first step in understanding its function in leukaemia.

AIM 2) Once we've established the functional capabilities of Ikaros in our gene desert system, we will perform knockdowns of Ikaros, and iMiD treatments to degrade Ikaros, in leukaemia and myeloma cell lines and primary samples. We will then perform RNA-seq, ChIP-seq and use 3C approaches such as NG Capture C to directly identify gene targets regulated by Ikaros to determine if it is necessary for enhancer-promoter interactions at endogenous loci. The expected outcome is that we will be able to directly determine if Ikaros is responsible for maintaining enhancer function in leukaemia. Prof Anindita Roy will be essential in helping with the ALL work while both Dr Sarah Gooding and Prof Udo Oppermann will be essential for working with MM samples.

AIM 3) We will then create iMiD-resistant cell lines and also acquire primary lenalidomide and pomalidomide resistant samples from the WIMM-based MyelomaBio biobank, to discover how the regulatory profile at the enhancers of key genes has been altered by iMiD resistance. This will be done through the use of genome-wide ChIP-seq approaches as well as NG Capture C at key gene targets to detect altered enhancer activity and altered enhancer-promoter interactions.

Translational potential

The current practice in myeloma treatment is to use different IMiD and CELMOD IKZF1-degrading drugs in sequence. The efficacy of using these drugs sequentially can be limited, but predicting which patients will continue to respond is not currently possible. This project will provide an opportunity to better understand how drug resistance develops, how we can track it, and will aid in developing new approaches for drug-resistant patients. Also, lenalidomide is not commonly used to treat ALL, and better understanding Ikaros function in ALL may identify whether certain ALL patients may benefit from the use of iMiDs in combination with other treatments in high-risk ALL.

Training opportunities

Interdisciplinary by design, this project will involve interactions with several labs across Oxford and will use a broad range of cutting-edge technologies. This includes state of the art techniques for the analysis of gene regulation at a genome-wide level (ATAC-seq, ChIP-seq, RNA-seq), technologies for analysing the 3D genome in high resolution (3C techniques such as Capture C, see publication 6 and 7 for examples), advanced molecular biology, genome editing (e.g. CRISPR/CAS9), as well as computational biology. Training will be specifically provided in the use of basic bioinformatics pipelines, as well as more substantial opportunities for focused training in bioinformatics. At the end of the DPhil, the expectation will be that the candidate will be able to generate as well as analyse their own genome-wide datasets.

References:

1) Waanders, E. *et al. Blood Can Discov* **1**, 96-111, doi:10.1158/0008-5472.BCD-19-0041 (2020). **2)** Andersson, A. K. *et al. Nat Genet* **47**, 330-337, doi:10.1038/ng.3230 (2015). **3)** Read, K. A. *Immunol Rev* **300**, 82-99, doi:10.1111/imr.12936 (2021). **4)** Harman, J. R. *et al.* **Milne T.A.** *Gen Res*, doi:10.1101/gr.268490.120 (2021). **5)** Martinez-Hoyer, *Exp Hematol* **91**, 22-31, doi:10.1016/j.exphem.2020.09.196 (2020). **6)** Crump, N. T. *et al.* **Milne T.A.** *Nat commun* **12**, 223, doi:10.1038/s41467-020-20400-z (2021). **7)** Godfrey, L. *et al.* **Milne T.A.** *Nat commun* **10**, 2803, doi:10.1038/s41467-019-10844-3 (2019).

30. Understanding intracellular complement regulation in colorectal cancer [1,2,3] – Dr. Olcina

Primary Supervisor: Dr. Monica Olcina del Molino
Additional Supervisors: Prof. Benedikt Kessler
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Project Summary

C3 is a central innate immune player[1]. Until recently, we though that C3 was mostly present in the blood and that its function was limited to fighting infections[1]. However, we have recently found that C3 is also present inside cancer cells, and that tumours having high levels of C3 are the most difficult to treat. C3 proteolytic cleavage is central to mounting inflammatory responses in the extracellular space[1]. More recently, C3 cleavage products, have been found to function intracellularly in non-canonical roles[2–4]. Despite rising interest in complement's role in cancer, comprehensive intracellular characterisation of C3 cleavage products has not been carried out to date. Understanding what activation fragments are present in cancer cells can help us recognise how complement components get cleaved, what their functions are and how best to target their removal/inhibition. Our data indicate that colorectal cancer cells express intracellular C3 and that C3 appears to be proteolytically cleaved. Surprisingly, C3 cleavage may restrict immune activation in colorectal cancer. We would like to characterise the full spectrum of C3 cleavage products by mass spectrometry which will also allow the identification of C3 proteases. Mapping intracellular C3 cleavage in cancer will allow us to identify C3 functions in cancer and how to target these for therapeutic intent.

Research Objectives

Our preliminary data indicate that intracellular C3 is cleaved, and such cleavage may be key to understanding currently undefined C3 intracellular functions (Fig. 1). While specific antibodies can detect C3 and certain C3 cleavage products, these are unable to reliably detect the whole range of C3-derived fragments. Using colorectal models available in the Olcina laboratory and state-of-the art mass spectrometry and proteomics expertise from the Kessler laboratory we propose characterising C3 proteolytic regulation in colorectal cancer by mass spectrometry. The collaboration between these two groups will be greatly facilitated by this project and the co-supervisor opportunities it will afford. Once a map of C3 cleavage has been attained (aim 1), we will use this to identify C3 processing proteases (aim 2). These studies will be important for identifying which C3 cleavage fragments/domains are involved in regulating cell intrinsic innate immunity signalling and the consequences of such signalling for anti-tumour immune responses (aim 3).

Aim 1: Characterisation of intracellular C3 fragments by Mass Spectrometry: With the Kessler group and the TDI Mass Spectrometry Laboratory, the student will use Liquid Chromatography Tandem Mass spectrometry (LC-MS/MS) to characterise the full range of intracellular C3 fragments in colorectal cancer cells. These studies will be critical for determining substrate preferences and the identification of intracellular proteases.

Outcome*:* Overall, this aim will provide the first characterisation of intracellular C3 cleavage products. Characterisation of C3 proteolytic cleavage will help identify the mechanisms by which C3 regulates immune signalling in cancer.

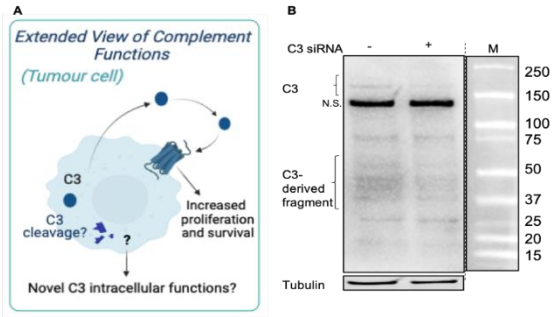

Figure 1. C3 is expressed and cleaved in tumour cells A. *Schematic representation of non-canonical functions of tumour-derived C3. C3 cleavage in tumours is poorly characterised and the functions of C3-derived fragments are largely undefined.* ***B****. Western blot showing expression of C3 and C3-derived fragments in colorectal cancer cells following treatment with scramble (-) or C3 siRNA (+), M = marker, n.s = non specific.*

Aim 2: Protease identification for C3 targeting in cancer*:* The main proteases and regulators involved in controlling intracellular C3 cleavage in cancer are unknown. C3 targeting strategies will be improved with a better understanding of how C3 cleavage is regulated. We propose identifying proteases involved in C3 cleavage by taking two complementary approaches:

i) Cleavage site data from MS experiments will be used to bioinformatically predict candidate proteases which will then be tested experimentally. Specific classes of predicted proteases identified bioinformatically will be initially narrowed down experimentally using protease inhibitors (serine protease inhibitors vs cysteine protease inhibitors vs metalloprotease inhibitors). Once a class of proteases has been identified using broad-spectrum inhibitors, more specific inhibitors will be used where applicable. Ultimately, individual newly identified proteases will be tested as bona fide C3 intracellular regulators *in vitro* using knockdown and overexpression experiments.

ii) CRISPR-Cas9 Screen: We will undertake a targeted CRISPR-Cas9 screen. Genes selected for the targeted screen will include both proteases known to regulate C3 in the extracellular space and those that may not have been previously associated with C3 cleavage. We will also include soluble regulators of C3 convertase decay (e.g., Factor H). This approach will allow us to identify novel intracellular C3 regulators. Protein expression of any hits will be assessed in whole cell lysates nuclear and cytoplasmic fractions to verify that the observed expression is indeed intracellular. Hits will be validated by using knockdown (siRNA and CRISPR-Cas9) and overexpression experiments to assess the contribution of these factors to C3 cleavage.
Outcome. This aim will yield the Identification of C3 proteases in colorectal cancer and will therefore provide important insights into intracellular C3 regulation and future therapeutic targets.
Aim 3. Biological consequences of C3/C3 cleavage for restriction of anti-tumour inflammation. Our preliminary data indicate that C3 cleavage may reduce effective cell intrinsic innate immunity signalling. Reduced intrinsic innate immune signalling could dampen effective anti-tumour responses mediated by immune cells in the tumour microenvironment (such as T-cells). We therefore hypothesise that targeting C3 or its cleavage would be a good strategy to stimulate anti-tumour immunity. Together with other members of the Olcina laboratory the student will assess whether targeting C3 or its cleavage can "reprogram" the immunosuppressive tumour microenvironment by enhancing pro-inflammatory responses that enhance T-cell cytotoxicity. These assays will complement future mechanistic and *in vivo* studies aimed at identifying the specific functions of C3 cleavage products in cell intrinsic innate immune signalling.
Outcome: This aim will provide functional data addressing the effects of tumour derived autocrine C3/C3 cleavage for T-cell mediated responses in colorectal cancer.
Overall, we anticipate this project will result in at least one high impact publication in which the student will be first author. Importantly, this DPhil project will greatly accelerate future mechanistic studies to be carried out by additional laboratory members. The DPhil candidate will also be a co-author in these publications

Translational potential
These studies will help us understand C3 intracellular regulation and functions and how to target C3 for enhancing anti-tumour immune responses. We will undertake this programme using colorectal models since high C3 expression is a prominent feature of the colorectal cancer subtype (CMS4) with the worst prognosis[5]. Although CMS4 tumours are immune infiltrated, immune activation in these patients appears insufficient to mount an appropriate anti-tumour response. These data suggest that stimulation of immune populations already present in the tumour microenvironment would benefit these patients. Since intracellular C3 may restrict immune activation and is high in CMS4, we hypothesise that it would be a good target to stimulate anti-tumour immunity in CMS4. However, to effectively understand how intracellular C3 restricts immune activation we must first understand how it is activated intracellularly and which activation fragments are important for its functions. Comprehensive mapping of intracellular C3 cleavage products and identification of C3 proteases in colorectal cancer cells will be the essential first step to a better understanding of C3 function in cancer.

Training opportunities
Through this DPhil project, the student will acquire knowledge on the role of the complement system, an innate immunity pathway with emerging functions for tumour progression. The student will acquire experience in using colorectal cancer models with clinically relevant molecular phenotypes while working both independently as well as in a team. The student will also benefit from the proteomics processing and analysis pipeline established by the TDI Mass Spectrometry Laboratory headed by co-supervisor Prof. Kessler. The student will also acquire experience in performing CRISPR-Cas9 screens.

References:
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31. The immune landscape of the pancreas during neoplastic transformation [1,2,3,4] – Prof. O'Neill

Primary Supervisor: Prof. Eric O'Neill
Additional Supervisors: Prof. Tim Elliott
Eligibility: Track 1, 2, 3 and 4 students are eligible to apply for this project.

Project Summary
Pancreatic ductal adenocarcinoma (PDAC) is one of the leading causes of cancer death with a 5-year survival below 5%. Poor survival statistics arise from the lack of early symptoms resulting in advanced stage at diagnosis together with mostly ineffective chemotherapeutic regimes. A major difficulty to identifying individuals at-risk of PDAC earlier is that symptoms are vague, non-specific and indicate a very low progression rate even in conditions known to pre-dispose to the disease. Therefore, identifying key biological events associated with progression form pre-neoplastic or benign lesions is key to providing biomarkers that can properly diagnose early. Pancreatic intraepithelial neoplasia (PanIN) lesions are the most common precursor and proposed to originate from both smaller pancreatic ducts and via dedifferentiation of the acinar cells. Inflammation of the pancreas is well described to promote PanIN formation and progression to PDAC, but as not all PanINs progress to invasive carcinoma in situ, diversity of the immune landscape in early lesions is likely to be important in the establishment of disease. Upon progression to PDAC the microenvironment shows extensive fibrotic stroma, an abundance of T-regulatory (Tregs) and M2-polarised tumour associated macrophages (TAMs), blocking immune effector functions. Although there is low immunogenicity, there is variability across in patients with low CD4/CD8 T-cell infiltrates associated with poorer survival.

The aim of this project is to understand the fine balance between the immune system and the emerging cancer.

This project will characterise the establishing tumour immune-microenvironment (TIME) in pre neoplastic tissue to identify key events that result in tumour initiation, but also highlight how evasion mechanisms get embedded and therefore potential intervention strategies for PDAC.
The KPC model (LSL-Kras$^{G12D/+}$;LSL-Trp53$^{R172H/+}$;Pdx-1-Cre) is a well-defined mouse model of human pancreatic cancer and this rapidly develops spontaneous tumours. The KC model (Kras$^{G12D/+}$;Pdx-1-Cre) allow identification of early PanIN lesions, with a subset going onto form PDAC with a long latency that more closely mirrors the human condition. We aim to utilise syngeneic orthotopic injection of KPC tumour cells or KC pancreatic ductal organoids together and KC mice to interrogate the immune landscape of benign vs pre-cancerous lesions. We also aim to validate findings in ex-vivo tissue slices of resected PDAC vs comparative healthy tissue, where live tissue responses to interventions can be monitored in real-time.

Research objectives
Our general goals are:
1. To define the Tumour immunological microenvironment of developing neoplasms in the pancreas
2. Identify how the immune suppressive environment becomes established and where intervention strategies are likely to provide benefit.
3. Comparative analysis of human tissue.

The specific aim for Goal 1 - Estabalish the immune landscape of developing lesions in the pancreas:
We have developed an Aurora Cytek panel covering a broad panel of immune cell markers to give an overview of immune cell populations in blood and tissue. Here we aim to use to determine the tissue resident and emerging immune populations present as a tumour initiates and evolves in the pancreas of KC mice. A secondary aim is to determine whether immune profiling give biomarker information for early detection, monitoring and intervention startegies in per-neoplasm to early disease.

The specific aims for Goal 2 - To establish the chronology of how a developing tumour escapes immunoediting and an immune suppressive environment becomes established. A secondary aim is to ascertain key immune signalling events crucial to maintainence of the repressive state and where intervention may induce greater tumlour control.

We also employ an othrotopic model of pancreatic cancer by injection of KPC tumours cells directly into the pancreas, allowing greater tractability around the onset and monitoring immune landscape.

The specific aims for Goal 3 – To explore the relevance of our findings in human pancreatic tissue. We have developed techniques to sustain viability resected pancreatic cancer ex vivo and characterised the immune landscape over time (>12days). We aim to further develop the potential of this technology to monitor and explore surplus pancreatic tissue from the Oxford transplant clinic and non-cancerous resected material to assess immune components and their response to intervention in real time ex vivo.

It is anticipated that the information obtained here will lead to a better understand of early lesion biology and will advance detection as well as potential treatments with immune targeting agents.

Translational potential

The research has very high translational potential: Through this project, we hope to establish in-depth understanding of the relationship between immunity and pancreatic cancer. This project will be a collaboration between Prof. Tim Elliott (Cancer Immunology, NDM), Prof. Eric O'Neill (Pancreatic Cancer, Oncology) as well as research medical oncologists (Mark Middleton and Shivan Shivakumar) and HPB surgeons (Keaton Jones, Mike Silva and Zahir Soonawalla) at the NHS Churchill hospital. As a centre for early diagnosis, pancreatic cancer and immune-oncology any findings here can be rapidly brought forward through our clinical partners to interventional trials.

The findings from this project will be progressed towards clinical application through validation studies in human tissue and to build preliminary evidence for clinical trials. The supervisory team will include one of our collaborating clinicians (as above) as appropriate for clinical specialisation (medical, clinical, surgical) to provide support for medical track students or a more experienced clinician scientist (Mukherjee, Shivakumar, Jones) for academic track students. We have had experience in working with our clinical colleagues to supervise both clinical and academic students and have considerable experience to successfully guide towards D.Phil.

Our interactions with national and international pancreatic cancer and cancer immunology networks will also expose the candidate to world class/ state-of-the-art technology and science.

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32. Mechanisms of therapeutic response and resistance to BCMA-directed therapy in multiple myeloma – a systems biology approach [1,2,3,4] – Prof. Oppermann

Primary Supervisor: Prof. Udo Oppermann
Additional Supervisors: Dr. Karthik Ramasamy, Dr. Adam Cribs, Dr. Sarah Gooding
Eligibility: Track 1, 2, 3 and 4 students are eligible to apply for this project.

Project Summary

Multiple myeloma (MM) is a cancer of immunoglobulin-secreting plasma cells residing in the bone marrow. Myeloma recurrently relapses during its clinical course, with inevitable evolution to a fatal multidrug-resistant phenotype. Although MM survival rates have improved due to the availability of new drug classes including immunomodulatory drugs and proteasome inhibitors, patients eventually become resistant to these therapies. Although several novel drug classes are currently investigated, it is incompletely understood why effective drug molecules only work in subsets of patients. A next wave of therapeutics are targeted against the BCMA (B cell Maturation antigen) cell surface molecule. CAR-T cells, bispecific antibodies and antibody drug conjugates targeting BCMA are all in clinical trials.

One such novel therapeutic agent is Belantamab Mafodotin, an antibody-drug conjugate that targets BCMA, an essential myeloma surface protein, approved by FDA recently. Clinical data is encouraging, however a high proportion of patients do not respond, and drug resistance eventually develops in many others. The proposed DPhil project will investigate in depth the mechanisms underlying Belantamab Mafodotin drug resistance using a systems-based approach to correlate genomic, epigenomic and proteomic data in ex vivo systems and patient-derived myeloma samples (including banked sequential samples from Belantamab treated patients). This will inform on patient populations that are likely to benefit from a targeted therapy, and on markers of emerging resistance.

Research objectives

Background:

Multiple myeloma (MM) is a clonally heterogeneous cancer of antibody-producing plasma cells residing in the bone marrow. Therapeutic outcomes depend upon underlying genomic and mutational alterations besides evolution in the clonal landscape following exposure to sequential therapy combinations. MM is currently incurable, and it is suggested that the combination of therapy-driven clonal evolution together with the ability of malignant plasma cells to adopt a reversible, microenvironment-dependent dormancy are fundamental reasons why even deep remissions inevitably relapse. It is known that lower minimal residual disease (MRD) levels following induction and consolidation therapy correlate with longer progression free and overall survival. In order to advance towards deeper remissions and potentially towards a cure, suitable approaches to identify optimal patient populations for a given treatment and strategies for targeting persistent and therapy resistant tumour cells are required.

B-cell maturation antigen (BCMA, CD269, TNFRSF17) is a member of the TNF-receptor superfamily and is preferentially expressed in mature B-lymphocytes and malignant plasma cells. The receptor binds to its ligands BAFF and APRIL, leading to NF-kappaB and MAPK8/JNK activation and thus may transduce signals for cell survival and proliferation. Belantamab Mafodotin (GSK2857916) is a novel antibody-drug conjugate (ADC) that targets BCMA, and upon receptor binding and internalisation displays strong cancer cell responses mediated in part by its chemotherapeutic drug conjugate auristatin, a microtubule-targeting agent. The responses to single agent Belantamab Mafodotin therapy in relapsed myeloma are very exciting, showing an exceptional overall response rate of 60% in a Phase 1 study in an extensively pre-treated myeloma patient population . This monotherapy is a step change in available therapeutics for MM. However, responses are durable only for a proportion of patients: some patients are primary refractory to this agent whilst others respond but eventually relapse. Accordingly, the mechanisms driving both response and relapse require systematic scientific evaluation.

Objectives and proposed work: in order to investigate the mechanisms underlying therapeutic response and resistance to BCMA-ADC therapy, a two-tiered approach will be taken. Our laboratory has established a defined set of human myeloma cell lines which represent distinct genomic aberrations and reflect the main determinants of risk status found in myeloma patients. Isogenic wild-type and drug-resistant cell lines are available, including various proteasome inhibitor (PI; bortezomib, carfilzomib) and immunomodulatory drugs (IMiD; lenalidomide,

pomalidomide), currently the main myeloma therapeutics. These cell lines have been extensively characterised using a systems approach including transcriptomic (RNAseq), proteomic (including phosphoproteomic and ubiquitomic, as well as mass cytometry) and epigenomic (ATACseq, Hi-C, CHIPseq) techniques. Single-cell data are currently generated to understand the clonal trajectories underlying PI and IMiD resistance. In this DPhil project, the student will first use these well-characterised tools to investigate the responses to Belantamab Mafodotin using the suite of techniques described above. Selected cell lines will then be used to generate BCMA-ADC resistance and will also include experiments with the chemotherapeutic payload auristatin as single agent. Collectively this will deliver essential ex vivo information on candidate pathways that define therapeutic responses to BCMA-directed drugs. By investigating bone marrow samples from patients treated with Belantamab Mafodotin using the systems approach detailed above, the student will be in a position to correlate ex vivo data with patient data. This will be complemented by patient samples obtained from the MAGNETISMM trial investigating BCMA-CD3 bispecific antibody treatment (Elranatamab). Single-cell readouts (single cell seq and mass cytometry) used for the patient bone marrow assays will provide important datasets and information on the interactions between myeloma cells and the bone marrow immune environment and allow to compare different therapeutic approaches against BCMA in MM.

Outcome: Data generated in this project will inform on mechanisms of response and resistance and mode of action of a novel therapeutic in myeloma. The project will provide the student with state-of-the art systems approaches to investigate drug resistance. It is anticipated that the successful student will be trained to advance a future career as clinician scientist or as biomedical researcher in academia or industry

Translational potential

This systems approach will deliver new information on how primary and acquired drug resistance against a novel therapeutic develops, by defining the dynamic changes in the integrated myeloma cancer and bone marrow microenvironment. The generated large data sets will be instrumental to identify mechanisms underlying the pathways that determine both primary resistance and which are altered during the acquisition of drug resistance. This information is essential to (i) select the appropriate patient population that will best respond to therapy and furthermore (ii) will provide target driven hypotheses to possibly overcome acquired drug resistance to BCMA targeted therapeutics.

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33. Prediction of tumour recurrence after oesophageal cancer surgery using multi-modal machine learning [4] – Dr. Papiez

Primary Supervisor: Dr Bartlomiej (Bartek) Papiez
Additional Supervisors: Dr. Sheraz Markar
Eligibility: Track 4 students are eligible to apply for this project.

Project Summary

Recent developments to standard oesophageal or gastric cancer treatments, such as surgery, chemotherapy, and radiation therapy, show that the overall survival rate for oesophageal cancer has doubled over the last 20 years. The patients after undergoing the specified treatment predominantly surgery, can be monitored by various protocols to detect recurrence, for example by standard symptom based follow-up which may trigger further investigation, or by the intensive surveillance protocol, which uses a wide spectrum of clinical measures including clinical assessment, imaging, and endoscopy. However, currently established protocols for monitoring treated oesophageal or gastric cancer to detect cancer recurrence are heterogenous, and thus at present remain inconclusive to establish whether an intensive surveillance protocol impacts on the overall survival and health-related quality of life.

Recently, an international multicentre retrospective observational study (ENSURE) of consecutive patients undergoing surgery with curative intent for oesophageal and gastro-oesophageal junction (GOJ) cancers (2009 – 2015) across 20 European and North American cancer centres was undertaken. ENSURE includes two surveillance options namely the standard surveillance based on symptomatic follow-up (SS), and the intensive radiological surveillance (IS) which extends clinical assessment by routinely collected Computed Tomography (CT) and Positron Emission Tomography (PET). In the absence of robust scientific evidence and no consensus on surveillance strategy after treatment oesophageal, ENSURE offers the possibility to investigate whether advantages exist for the IS protocol to improve oncological outcome and if so to develop guidelines for randomised controlled trials (RCT).

Recent developments in Machine Learning (ML), in particular Deep Learning (DL), have been shown to yield results of comparable accuracy to the human experts in various clinical applications including grading of diabetic retinopathy from fundus retinal imaging (Ting2017), detection of pneumonia from chest X-rays (Rajpurkar2017), or classification of skin cancer using dermatology imaging (Esteva2017). While those ground-breaking solutions create opportunities to automate some medical procedures, they are inherently limited as these approaches utilise clinical imaging acquired at one time point during patient care to detect or diagnose disease for example retinopathy progression (Arcadu2019). Given the longitudinal nature of ENSURE study including CT/PET scans taken at 6, 12, 18, 24, 30, and 36 months, there is an unmet clinical need to develop deep learning models that can extract relevant features from such imaging to detect tumour recurrence after oesophageal cancer surgery. Furthermore, the project could also investigate the use of other clinical variables available in ENSURE study and develop multi-modal deep learning models that could combine both imaging and non-imaging data to model patient survival rates, or predict cancer recurrence.

Research objectives

A. **Development of a machine learning model to detect tumour recurrence in patients after oesophageal cancer surgery using longitudinal CT/PET imaging available in ENSURE study.**

The ENSURE study included 2147 patients within the intensive surveillance group, who received PET-CT or CT scans at least annually for at least 3 years as part of the post oesophageal cancer surgery follow-up. This has generated a repository of at least 8588 PET-CT or CT scans from the study, which be used within this study. The majority of intensive surveillance patients (74%) used CT as part of their follow-up and thus the majority of available images will be from CT surveillance. Within this cohort from the intensive surveillance group, 62% of patients developed recurrence within 3 years of surgery, ensuring the event rate is adequate for the proposed study. We have applied and gained approval from the ENSURE study group, along-with all centres to transfer radiological images to a central repository within the Big Data Institute (BDI) in Oxford.
The raw PET/CT data set (or a part of it) will be annotated by the expert radiologist(s) to identify the location and the relevant anatomy for the reccurent tumor. We envisage that the project will start investigation on supervised (or weakly-supervised) detection of the reccurent tumor based on the information contained in PET/CT and available annotations. Several deep learning approaches have been proposed in computer vision aiming to detect objects of interest in natural images (fast R-CNN (Ren2016), YOLO (Bochkovsky2020), or SSD(Liu2016) to name a few), however

few methods exist for 3D object detection in medical context (Chen2021).Furthermore, we also envisage development of the method, which learns the strategy to automate selection of the cases that are important to annotate to further improve the tumor recurrence detection.

B. Development of machine learning model to predict patient's after treatment progression (cancer recurrence) using multi-modal i.e. imaging (PET/CT) and non-imaging (clinical assessments) data

In this part of the project, we envisage the investigation of the recent machine learning methods for survival analysis such as DeepSurv (Katzman2018) or DeepHit (Lee2018), and the development of the method, which can be applied to the detection of tumor reccurence using multimodal imaging and non-imaging dat available in the ENSURE data set. The important dimensions of research development in this objective is consideration of longitudinal nature of the data included in the ENSURE study (Lee2019).

Up to date, there has been little work done to explore machine learning methods to predict disease progression (Jin2021) at a patient level using longitudinal (sequential) large data sets combining both imaging and non-imaging data. In particular, the most recent approaches (Rahman2020) for prediction of early recurrence after oesophageal cancer surgery utilised only routinely collected clinical and pathological data without considering clinical imaging. Therefore, the project offers a unique opportunity for novel methodology developments, which may be also applicable in other types of tumors where intensive surveillance is also available (e.g. colorectal cancer).

Translational potential

There are over 5,200 new cases of oesophageal or gastric cancer diagnosed per year in the UK, with over 4100 deaths per year attributed to these cancers. Furthermore, approximately 60% patients with locally advanced and localised disease treated with curative intent will develop tumour recurrence and die within 3 years of completing the treatment. The major challenge to an intensive surveillance strategy is the presumed cost associated with regular CT or PET imaging. The burden of this cost largely lies on a radiologist carefully reviewing the images to detect recurrence, which is something that will be addressed in this delivered research. Thus it is expected that prediction of recurrence after oesophageal cancer surgery is of significance for both clinicians and patients and to the wider healthcare system (Niu2020). Such machine learning models could assist clinicians in decision-making processes and improve the overall quality of patient management.

Training opportunities

The Big Data Institute (BDI) is a world-renowned research centre and is housed in a brand new state-of-the-art research facility. Full training will be provided in a range of Health Data Science topics (Currently BDI is hosting CDT in Health Data Science for DPhil students). The student will have also opportunity to attend the research seminar offered at the BDI and the Institute of Biomedical Engineering (IBME) as the primary supervisor is a member of Imaging Hub at the IBME (https://eng.ox.ac.uk/biomedical-image-analysis/). The student will be expected to attend relevant seminars within the department and those relevant in the wider University. Subject-specific training will be received through our group's weekly supervision meetings. Students will also attend external scientific conferences where they will be expected to present the research findings.

References:

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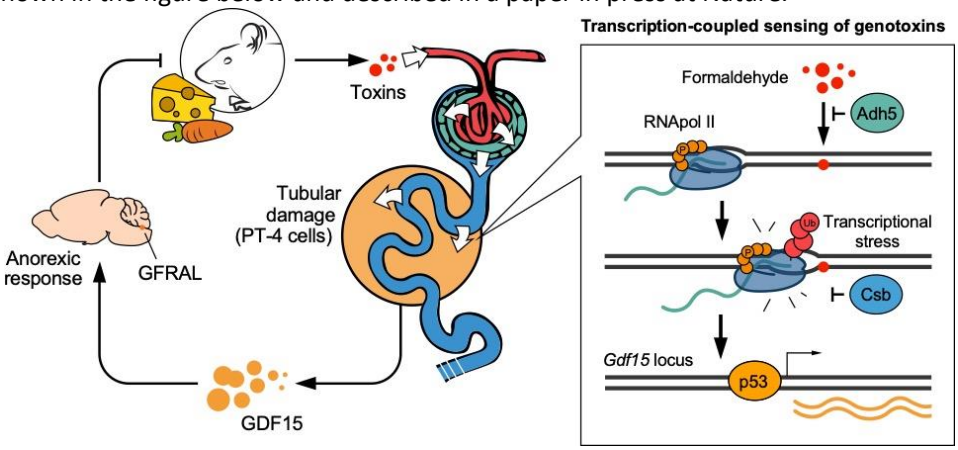
34. DNA damage induced cachexia in cancer and other pathological states [1,3] – Prof. Patel

Primary Supervisor: Prof. KJ Patel
Additional Supervisors: Dr.Ross Chapman
Eligibility: Track 1 and 3 students are eligible to apply for this project.

Project Summary
We have recently uncovered that metabolism produces the genotoxic aldehyde – formaldehyde. The DNA damage endogenous formaldehyde causes needs to be repaired by two key repair pathways – the Fanconi anaemia DNA crosslink repair pathway and Transcription coupled nucleotide excision repair pathway (TC-NER). When these repair pathways fail then this leads to catastrophic physiological consequences, in the case of TC-NER this causes the human illness Cockayne syndrome. This project concerns our mouse model for Cocakayne syndrome which results is premature ageing and a severe progressive wasting condition (cachexia). Our research identified a key role for the kidney in sensing this DNA damage, and if this is activated then it releases a hormone GDF-15 that instructs anorexia and remodels metabolism to cause cachexia. This same mechanism is also activated when we are subjected to cancer chemotherapy and may underpin the loss of appetite and weight loss that occurs when patients are subjected to such treatment. This PhD project will set out to better understand this new kidney anorexia response with an emphasis to understand the factors that activate it and how it induces cachexia. Using a combination of unique mouse models and the creation of reporter mice you will develop new models for this response. Using somatic cell genetics and CRISPR CaS9 directed genomic screens you will identify new components of this response and determine how they function. Finally using state of the art metabolic approaches, you will identify factors like formaldehyde that induce this response specifically in murine models where cachexia is a feature.

Research objective
The main objective of this research is to better understand how this new DNA damage induced endocrine anorexia pathway is activated (drivers) and how it functions. This will require a multidisciplinary approach involving the creation of new mouse strains, somatic genetic screens, and advanced metabolomic methods and platforms. The outline of this pathway is shown in the figure below and described in a paper in press at Nature.



Translational Potential
Cancer patients are often subjected to chemotherapy that causes anorexia and weight loss, understanding why this happens will help improve patient care and survival through treatment. Cachexia is a common feature in early and more so in advanced cancers, it is complete mystery of why this occurs. Our recent discovery may shed light into this complex and common affliction of cancer. https://cancergrandchallenges.org/challenges/cachexia
This research project will to some extent explain a simple mechanism that induces weight loss and food aversion in response to cancer chemotherapy. It may be possible to interfere with this response so that patients undergoing chemotherapy might not suffer these common aversive effects. At a more broader level cachexia contributes to poor

quality of life and also cancer mortality, if we better understand the molecular physiology of what drives cachexia then we might be able to treat it or prevent it from occurring.

Training opportunities

You will work in an integrated small team addressing this complex field. Training in genetic physiology in murine models, genetic screens using state of the art gene editing technology and sate of the art metabolomics.

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35. Development of a cost-effective and rapid NMR-based blood test for the early detection of advanced polyps and colorectal cancer in patients with overlapping clinical symptoms[1,2,3,4] – Dr. Probert

Primary Supervisor: Dr. Fay Probert
Additional Supervisors: Dr.James East, Prof. Daniel Anthony
Eligibility: Track 1, 2, 3 and 4 students are eligible to apply for this project.

Project Summary
Early diagnosis of advanced polyps/CRC greatly increases a patient's chance of survival. However, polyps are often asymptomatic or present with non-specific symptoms that mimic other gastrointestinal (GI) disorders including inflammatory bowel diseases (IBD) and irritable bowel syndrome (IBS). Endoscopy is the 'gold-standard' for discriminating between these conditions and so screening is currently the best method for early detection. However, endoscopy is invasive and can have poor patient compliance. A less invasive alternative is urgently required. The two week wait (2WW) pathway aims to detect CRC early by allowing general practitioners (GP) to 'fast-track' patients with concerning symptoms. However, only 5% of patients referred via 2WW have advanced polyps or CRC. This is due to the overlapping symptoms of CRC and other GI diseases along with referral of non-priority patients. Indeed, 27% of patients referred do not fulfil the 2WW criteria resulting in many patients undergoing endoscopy unnecessarily and a huge strain on the service. While the need for a non-invasive biomarker is widely recognised, there remains no reliable biofluid test able to detect advanced polyps and early CRC or better triage 2WW patients. Indeed, promising circulating tumour DNA and protein markers, while useful in the later stages of CRC, are undetectable in patients with advanced polyps and early stage disease[1,2,3].

We have demonstrated that nuclear magnetic resonance (NMR) metabolite profiles correlate with endoscopic and histological severity in UC and reveals a blood metabolite signature which represents inflammatory activity in IBD[4]. More recently, we identified a metabolite signature of early cancers in a cohort of patients presenting with non-specific symptoms. Within this cohort, we identified a unique metabolite signature of CRC which was distinct from the signature of IBD providing strong evidence that altered homeostasis as a result of malignancy, even in the early stages of disease, sufficiently alters metabolism in a manner that is detectable in blood. Furthermore, the metabolite changes that result from early stage cancer are distinct from those induced by inflammatory diseases suggesting that the blood metabolite profile could be used to distinguish CRC from other GI diseases with overlapping clinical features.

This project will combine metabolomics analysis of blood with multivariate machine learning methods to (1) detect advanced polyps and early stage CRC, (2) discriminate between CRC, IBD, and IBS in a group-wise analysis of patients referred via 2WW, and (3) perform an integrative pathway analysis to uncover the diverse chemical changes in the body associated with these conditions.

The proposed project will develop a new collaboration between the metabolomics, colorectal cancer, and gastroenterology research groups at Oxford bringing together expertise in oncology, chemistry, and mathematics.

Research objectives
The purpose of the proposed investigation is to extend and expand our NMR-based methods[5,6] coupled with machine-learning statistical tools to the identification of intrinsic differences between the blood metabolite profiles of patients with advanced polyps or CRC and other, less urgent, conditions such as IBD, and IBS. This will provide diagnostic blood-based biomarkers of CRC and a method of screening patients using a simple blood test. **The development of such a test would allow (1) less invasive, earlier diagnosis and more frequent screening of CRC, and (2) pre-screening in primary care, reducing the number of unnecessary urgent endoscopy referrals.**

In a prospective cohort of patients referred to the John Radcliffe Hospital, Oxford via the 2WW pathway we will:

- Establish a biobank of blood samples for metabolomics analysis and other CRC research projects in the future;

- Identify blood metabolite biomarkers that detect advanced polyps;
- Develop a mathematical algorithm able to discriminate between advanced polyps/CRC, inflammatory diseases, and other less urgent GI disorders.
- Further our understanding if the biochemical changes identified by integrative pathway analysis along with targeted metabolomics analysis of animal models and cell culture using stable isotope labelling strategies.

The proposed project will establish a collaboration between the metabolomics and CRC research groups which will result in a new 2WW biobank of at least 1500 blood samples to be used for metabolomics, and other research projects in the future. Biobankers are currently employed to aid in sample collection and many samples are already available for analysis via the TGU biobank ensuring a swift start to the project. The student will aid in processing prospective samples, in order to validate their results and models developed during the project. At the rate patients are seen in the 2WW clinic we predict such collection will take ~3 months of part time work. Successful translation of any clinical test is reliant on robustly collected and catalogued prospective samples and so this is a vital step if our research is to be brought to the clinic. We anticipate the data generated will result in at least two high impact publications and provide proof-of-principle that metabolomics analysis of blood can be used to detect advanced polyps/CRC and triage patients. Furthermore, the project will provide novel insight into the distinct chemical pathway changes which occur in these diseases.

While the metabolomics (FP, DA, TC) and gastroenterology teams (ST, AW) have previously published together in the field of IBD, this is the first collaborative project to extend the use of metabolomics to detection of CRC and will be the first project to build a link between the metabolomics and CRC teams (JE, SL). The requested funding will foster collaboration between the chemistry, pharmacology, and mathematics departments along with gastroenterology, and cancer research groups bringing together a multidisciplinary team of clinicians, biologists, chemists, experimental pathologists, mathematicians, and statisticians. Combined, this team has extensive expertise in cancer research, gastroenterology, inflammatory diseases, metabolomics analysis, and the development of diagnostic blood tests. While others have investigated metabolite changes as a result of cancer compared to healthy controls, this is the first time that such a range of expertise has been combined to tackle the important challenge of early detection of advanced polyps/CRC in patients referred to 2WW

Translational potential

The long-term goal of the proposed project is to deliver a cost effective, non-invasive and rapid test to benefit patients. The currently available methods for detecting advanced polyps/CRC are expensive, invasive, and can only be carried out in specialist clinics. Furthermore, referral criteria are non-specific and result in a high proportion of patients referred to 2WW who do not need to be seen urgently as part of the cancer pathway. The ability to screen patients in a primary care setting using a simple blood test would allow more frequent monitoring and thus earlier detection ensuring patients receive appropriate treatment as early as possible. Furthermore, more accurate triage for 2WW would result if fewer invasive endoscopy referrals of low-risk patients.
Any identified diagnostic model and identified biomarkers will generate IP that will be developed towards the clinic using commercially available NMR metabolomics platforms in collaboration with our industrial partner, Numares AG. Numares are an NMR-diagnostics company with vast experience in bringing NMR biofluid tests to market who we have a strong collaboration with. This will ensure rapid translation of our results to the clinc.

Training opportunities

This DPhil project will provide experience in patient recruitment and clinical data collection, the preparation of biological samples for metabolomics analysis, the acquisition of high-field ^{1}H NMR data, and assigning small molecules in complex mixtures. Training will be provided in the use of R statistical software and machine-learning analysis of 'big' data. There is also the opportunity to gain experience in animal models and cell culture to investigate the metabolite changes identified in greater detail.

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36. Mechanisms of Trisomy 21-induced B lymphoblastic leukaemia initiation in Down Syndrome [1,2,3] – Associate Prof. Roy

Primary Supervisor: Associate Prof. Anindita Roy
Additional Supervisors: Associate Prof. Bethan Psaila
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Project Summary

Children with Down syndrome (DS) have an increased risk of acute myeloid leukaemia and acute lymphoblastic leukaemia (DS-ALL)[1] pointing to specific effects of trisomy 21 (T21) on haematopoietic stem and progenitor cell (HSPC) biology[2]. While DS myeloid leukaemia is well understood and is known to originate in fetal cells[3], leukaemia initiation in DS-ALL is poorly understood[4]. Evidence in established DS-ALL show that the most frequent molecular abnormalities affect JAK2 signalling and CRLF2[5]. The peak increase in DS-ALL (27-fold) occurs in the youngest children (1-4y)[1], suggesting most T21-associated leukaemogenic effects occur in early childhood and, as in non-DS-ALL[6], may originate in fetal life. Consistent with this, research in the Roy/Roberts lab has shown severely impaired B-cell development in T21 fetal liver (FL)[7] and, more recently, in fetal bone marrow (FBM) with a distinct lack of fetal specific B progenitors[8, 9]. We hypothesise that perturbation of B-lymphopoiesis in fetal life represent the leukaemia initiating event in DS-ALL and this project aims to investigate the mechanisms driving this process (Figure 1).

Our preliminary investigation to determine the molecular basis for severely impaired fetal B lymphopoiesis in DS using RNA sequencing of normal and T21 FBM HSPC has shown downregulation of chromatin silencing genes, suggesting that epigenetic mechanisms are likely to be responsible. In addition, our recent single cell transcriptomic studies[10], as well as pilot functional data from a previous DPhil student in the lab[9] have pointed towards an important role for the T21 haematopoietic microenvironment in abnormal fetal haematopoiesis in DS. In particular, gene set enrichment analysis has shown upregulation of key interferon (IFN) signalling pathways[9]. This is particularly interesting as four of the key IFN genes are encoded on Hsa21, although whether this pathway is active in a cell intrinsic manner or driven by a T21 FBM microenvironment is not clear. Furthermore, we also have preliminary data from DS ALL patient samples showing that these changes are maintained in the ALL blast cells suggesting that they may play a role in the development of leukaemia. Reduced B-lymphopoiesis and an inflammatory signature are also hallmarks of adult/ageing haematopoiesis.[11, 12]

Research objectives

Aim 1. To determine how T21 shapes the epigenetic landscape of T21 fetal HSPC and associated transcriptomic changes in fetal and DS-ALL cells. **(Collaboration, T Milne)**.

Several Hsa21 genes with known roles in B-cell biology and leukaemia, including *RUNX1, ERG, ETS2, BACH1, HMGN1* are expressed at higher levels in T21 FBM HSPC than in their disomic counterparts. Our previous and preliminary data support accumulating evidence of global transcriptomic changes in fetal DS tissues[2, 7]. In agreement with DS models[13], increased expression of the chromatin regulator gene *HMGN1* on Hsa21 in fetal HSPC, supports the case for systematically characterising the epigenetic landscape in primary fetal HSPC. (Samples: Normal and T21 FL and FBM HSPC (n>20 each) and DS-ALL (n=8) and non-DS ALL (n=8) samples are available in the lab with ethical approval. Bulk RNA-seq, ATAC-seq and methylation data for these samples have already been or are being generated in the lab.)

Experiments: First, ChIP-sequencing of normal and T21 fetal HSPC and ALL blast cells will be used to define the epigenetic landscape in each of the major fetal HSPC populations, and in the DS-ALL blasts themselves. Specifically, we will compare activating/repressing histone marks of T21 fetal HSPC with normal fetal HSPC and with DS-ALL blasts. These techniques have been optimised for small cell numbers (2000-10,000 cells in the Milne lab). Second, to assess the functional importance of any differences, these data will be compared with the matching transcriptomic and chromatin accessibility data that has already been generated from these samples. The analysis will focus on candidate pathways important in i) B-cell development; ii) B-ALL; and iii) inflammation associated genes. DS-ALL blasts will also be compared with cytogenetically-matched ALL blasts from children without DS in order to identify T21-specific and ALL-specific changes. Third, the top ranked genes (or pathways) identified from integrating the epigenetic and transcriptomic data, will be selected for mechanistic experiments (Aim 3).

Aim 2. The role of the FBM microenvironment in perturbing T21 fetal B lymphopoiesis.

In Down Syndrome, the FBM niche consists of cells that also harbour T21. The extent to which the T21 microenvironment drives the inflammatory signals in fetal HSPC and DS-ALL and the mechanisms involved are not clear. Preliminary data from our bulk and single cell transcriptomic studies allowed us to infer the involvement of both IFN and TNFa pathways but no functional studies to validate these pathways have yet been performed. (Samples: Normal and T21 fetal mesenchymal stromal cells (MSC), aw well as FL and FBM tissue sections, are already available and stored in the lab with ethical approval.)

Experiments: *Functional assays*: We have established fetal MSC co-culture systems in our lab which support multilineage differentiation[8]. (i) Crossover experiments will be performed to establish whether T21 MSC cause normal fetal HSPC to lose B-cell potential, and whether normal MSC restore B-cell output of T21 fetal HSPC. (ii) In addition we will test the survival of DS- ALL blasts in T21/normal MSC co-culture system to determine whether a T21 microenvironment maintains DS-ALL blasts better. (iii) Lastly, we will edit T21 fetal HSPC with DS-ALL 1st hit (such as CRLF2 rearrangement) to create a DS-ALL or preleukaemia model, and use in vitro co-cultures on T21 vs normal MSC to test whether the microenvironment accelerates transformation. These experiments will use CRISPR/Cas9 technology to edit human fetal HSPC which have been recently established in our lab14. This will also allow us to identify leukaemogenic T21 HSPC populations. HSPC-niche interactions: We will use imaging mass cytometry to investigate the differences in the relationship between the niche and HSPC it contains, in normal vs. T21 FBM, and how it may be perturbed in leukaemia. Hyperion®, a state-of-the-art platform available at the WIMM will allow us to reveal differences in cell subpopulations and their spatial localization in normal vs. T21 FBM, and also identifying crucial molecular interactions in relation to the leukaemia and its surroundings. There is extensive expertise in Psaila lab for these techniques. In addition to the above approaches, HSPC/leukaemic blasts-niche interaction will be interrogated using a new pipeline (SCINDY package) (collaboration G Wang).

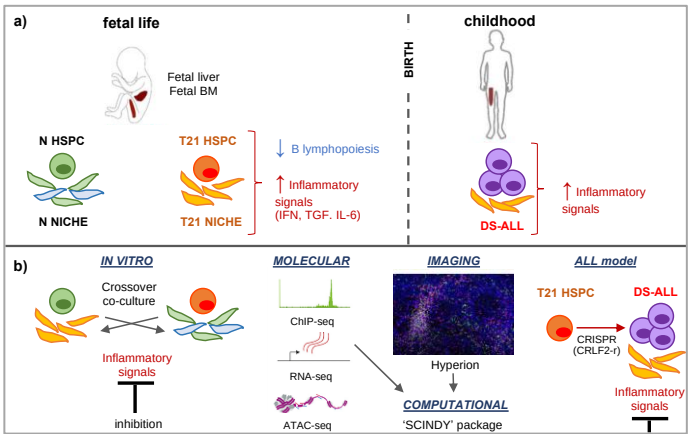

Figure 1: a) Perturbation of B lymphopoiesis and the haematopoietic niche in T21 fetal life, including increased inflammatory signature; which is also present in childhood DS-ALL. b) Key experimental approaches that will be used in the project to understand the molecular/epigenetic basis of these abnormalities.

Aim 3. Validate the role of key genes/pathways in perturbing T21 fetal B lymphopoiesis.

Experiments: (i) Based on the results generated in Aim 1 and 2, we will perform validation of promising candidate genes using CRISPR-Cas9 mediated knockdown of genes in normal or T21 fetal HSPC and/or MSC (e.g, HMGN1, IFNAR, IFNGR). The effect of gene knockdowns will be assessed using functional in vitro assays to read out the impact of gene editing on B-cell production. (ii) To determine the role of inflammation-associated signalling in T21 fetal HSPC/microenvironment, we will perform functional in vitro assays where these signalling pathways (such as IL-6, IFN and TGFβ) are blocked (collaboration S Valletta). This will allow us to establish whether the abnormal B-cell development in T21 fetal life can be rescued by dampening the inflammation associated pathways that drive the process. Similar approaches will be used for DS-ALL blasts and transformed T21 fetal HSPC to determine the importance of these inflammatory pathways in initiating and maintaining leukaemia.

Expected outcome: By elucidating the molecular mechanisms by which T21 perturbs B-lymphopoiesis and promotes transformation, we will provide important insight into DS-associated leukaemia biology.

Translational potential

This research is important because the prognosis of ALL for children with DS is inferior to children without DS, due to higher rates of relapse and treatment-related mortality, particularly infection. Therefore this project may ultimately contribute to improved treatment for children with DS-ALL by identifying molecular pathways that might be exploited for better drug design; and by helping elucidate the basis for B-cell deficiency, which likely contributes to the infective complications in children with DS-ALL.

Training opportunities

The student will be based in the Roy/Psaila labs and be embedded in the excellent scientific environment of the WIMM. He/she will be supported by experienced postdoctoral researchers in our labs, as well as collaborating labs (Milne, Valletta) to learn cutting edge functional/molecular techniques such as in vitro assays, flow cytometry, ChIP-sequencing, CRISPR-Cas9 gene editing and imaging mass cytometry and bioinformatics. All of these techniques are already in use in our labs; and additional training opportunities will be available for FACS sorting and computational analysis (via dedicated courses at the Centre for Computational Biology, WIMM). The student will be encouraged to take up the excellent training opportunities provided within the WIMM and the University of Oxford, to develop their research career.

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37. Discovery and mechanistic elucidation of small molecule inducers of myeloblast differentiation for ALL [3] – Prof. Russell

Primary Supervisor: Prof. Angela Russell
Additional Supervisors: Thomas Milne
Eligibility: Track 3 students are eligible to apply for this project.

Project Summary

The most common childhood cancer is acute lymphoblastic leukaemia (ALL), a disease which leads to the accumulation of immature lymphoid cells in the bone marrow. This is thought to be caused in part by a block in normal lymphocyte differentiation. There has been amazing progress in treating childhood ALL, but unfortunately a subset of childhood ALL continues to be refractory to treatment, especially in those patients that harbour a rearrangement (r) of the *Mixed Lineage Leukaemia* (*MLL*) gene. The most common MLLr results in a fusion protein, MLL-AF4, that is responsible for many of the poor prognosis ALL patients. In addition, even for children who are cured, conventional therapies are often toxic and can cause long lasting life-altering effects. ***Current treatments typically aim to kill abnormal cells via chemotherapy, but our goal is to establish a new paradigm in the treatment of ALL, that is to induce differentiation of ALL blasts.***
Our inspiration comes in part from the wave of new small molecule therapies for acute myeloid leukaemia (AML) that have been shown to have reduced toxicity compared to conventional therapy and function by causing AML cells to differentiate. Our hope is that by applying the concept of differentiation therapy to ALL we will be able to i) provide novel treatments for refractory ALL such as MLLr leukaemias and ii) develop novel therapies that have fewer toxic side effects than current conventional therapies. Previously, we established an *in vitro* screen to detect differentiation of AML cells using flow cytometry and used this to identify multiple classes of small molecules which can block proliferation and overcome the differentiation block in AML blasts. Our leading examples are orally bioavailable in mice and are being progressed into *in vivo* trials to determine efficacy. We have performed some preliminary time-course studies and global RNA-seq analyses to better understand the compounds' effects at a cellular level. From these data we have shown that our compounds are distinct from other known inducers of differentiation in AML cells. However, we have not yet defined their direct cellular target(s). Preliminary data also suggests that treatment of ALL cells with these compounds impairs their growth in vitro. Our goal in this project is to apply these same compounds, alongside other classes of AML drug candidates which also induce differentiation, to ALL blast cells from a novel MLL-AF4 humanized model (see Figure 1) as well as ALL patient samples to determine i) which novel compounds can disrupt ALL growth; ii) if ALL blasts can be induced to differentiate; iii) how the compound(s) impact the function of the target(s), and (iv) what downstream cellular pathways are impacted by target engagement.

Research Objectives
In this project we aim to use a combination of chemical and biological techniques to address questions (ii)-(iv) for one of these series of molecules. Two parallel approaches will be developed using an integrative approach combining existing cutting-edge expertise in Milne/Russell groups and collaborators Dr Anindita Roy and Prof Irene Roberts:
1. Analyze differentiation of ALL cells using a novel MLL-AF4 ALL model, with a combination of tools as well as novel compounds. Counterscreen for toxicity and/or B-lineage differentiation potential of normal cells (e.g. cord blood) to exclude compounds which exhibit non-specific effects.

2. Identifying compound binding partners in ALL cells through a combination of affinity and photoaffinity proteomics, candidate screening, native intact mass analysis and follow up target validation.

3. Identify key pathways controlled/impacted by compound treatment through a combination of nascent and RNA-seq, CRISPR/CAS9 screening and proteomic analysis.

The overall workflow is depicted in **Fig. 1**.

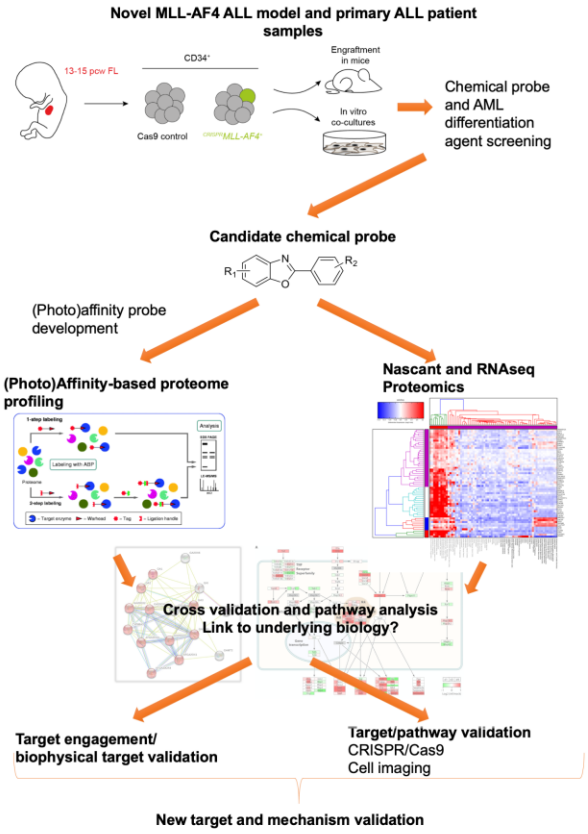

Fig. 1: Proposed project workflow.

In the environment of the chemistry research laboratories (CRL), training will be provided in chemical synthesis, analytical methods (e.g. NMR, mass spectrometry), medicinal chemistry, drug design, photoaffinity labelling, chemical biology, affinity and photoaffinity protein profiling and proteomics. These techniques are well established in the Russell group, and have been successfully mastered by several DPhil students in recent years (for example Wilkinson et al, 2020). As well as all of these techniques, training will also be provided in how to ask and answer scientific questions in medicinal chemistry and chemical biology. Similarly, a wide range of technical training will be provided in the RDM/WIMM environment including basic cell biology techniques, analytical methods (e.g. FACS), drug screening, RNA-seq and other next generation sequencing techniques, CRISPR/CAS9 screening, basic bioinformatics and dataset analysis. Training will also be provided in answering questions about biological impact and efficacy of compounds.

Professor Russell and Professor Milne already collaborate and hold biweekly joint meetings to discuss and monitor research progress. It would be expected that the student would attend these meetings as a primary means to monitor progression in the presence of both academic supervisors so as to ensure coherence and consistency of messaging – a critical factor when students have joint supervision arrangements. It would not be expected that the student would present at every one of these meetings – most likely every other one. These meetings will be critical to set overall objectives and project direction. Together, these distinct environments will provide the unique opportunity for the candidate to bring together two interdisciplinary areas of research to address and important area of unmet medical need in oncology.

Translational Potential

Accomplishing the goals of this project could potentially impact four specific areas: 1) pharmacodynamic biomarker discovery, 2) yield insights into the basic biology underpinning ALL blast cell differentiation, 3) identifying patient subsets to target in clinical trials, and 4) revealing new molecular targets for future drug discovery endeavours. As the intention with this project is to focus on a mechanistic evaluation of tool compounds this means there would be no commercial restriction or delay to publish the outcomes of the research. The sharing of new data with the scientific community in a timely fashion we anticipate will further the overall goal of identifying and developing drugs that will go into clinical trials to one day impact patient health.

References:

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38. Clinical utility and biological impact of platelet-restricted clonal haematopoiesis in cancer [1] – Dr. Shapiro

Primary Supervisor: Dr. Susie Shapiro
Additional Supervisors: Prof. Adam Mead
Eligibility: Track 1 students are eligible to apply for this project.

Project Summary

Clonal haematopoiesis (CH) is a pre-cancerous inflammatory state associated with a 10-fold increased risk of developing blood cancers, and also an increased risk of venous thromboembolism (VTE) and cardiovascular disease (CVD).1 CH can therefore be used as a biomarker for early detection of blood cancers as well as increased risk of VTE and CVD, opening up opportunities for preventative interventions. The detection of CH is additionally important for people with cancer, who are at increased of VTE, CVD and secondary blood cancers. Indeed, a number of studies have linked presence of CH to a markedly increased risk of secondary blood cancers following treatment of solid tumours,2 and with worse outcomes in people with solid tumours from both disease progression and cardiotoxicity.3,4 Long-term cancer patients are known to have increased risk of CVD, although whether or not this is associated with CH is not known.1 We have recently identified presence of platelet restricted/biased CH at a high frequency in hospital inpatients. This is a novel finding with important implications, as current techniques to detect CH only use gDNA from white blood cells and will therefore fail to identify patients with platelet-biased CH. Given the central role of platelets in thrombosis, we propose that presence of platelet-biased CH might increase the risk of VTE and CVD. In order to assess this, we will determine the prevalence of platelet-biased CH in patients with unprovoked VTE and CVD, including a cohort of patients with cancer-associated VTE, in comparison with age-matched controls. We will also investigate the functional consequences of CH on platelets and haemostasis, and explore the underlying drivers for platelet biased CH. A new strategy to detect 'pre-cancer' states in people with unprovoked VTE and CVD could lead to new strategies to reduce the risk of progression to blood cancer and to reduce the morbidity and mortality associated with thrombosis and chemotherapy. Furthermore, we anticipate that improved strategies to detect CH in cancer patients will facilitate early detection and preventative interventions to reduce cancer-associated VTE, CVD and secondary blood cancers

Research objectives

Background: Clonal haematopoiesis (CH) is defined as the presence of an expanded somatic blood-cell clone in people without other haematological abnormalities (i.e. with normal red cells, white cells and platelets). CH is usually detected by next-generation sequencing (NGS) of DNA isolated from the white blood cells in the peripheral blood. The variant allele frequency (VAF) describes the percentage of the mutant clone within a cell population. The prevalence of CH varies based on the VAF threshold and the depth of sequencing used. It also varies with age, ranging from < 1% in those younger than 40 years to >15%-20% in people over the age of 70 years. The most frequently mutated genes include DNMT3A, TET2 and JAK2.

CH provides a fascinating link between cancer, VTE and CVD. For example, TET2 and JAK2 CH mouse models show increased inflammation and atherosclerosis, heightened IL-1β/IL-6 signalling; and demonstrate inhibition of NLRP3 or AIM2 inflammasome ameliorates atherosclerosis.5,6 These observations suggest inhibiting the IL-6/IL-1β pathway may be effective in reducing CVD risk in humans with CH, with upstream targets (eg NLRP3 and AIM2 inflammasomes) differing depending on the precise underlying myeloid mutation. In the CANTOS trial, patients who received canakinumb (anti-IL-1β antibody) not only had a lower rate of cardiovascular events compared with those who received placebo,7 but patients who received higher dosages of canakinumab also had fewer incident lung cancers, lower total cancer mortality, and lower lung cancer mortality.8

Accumulating evidence shows that many long-term haematopoietic stem cells (HSCs) produce cells exclusively of the platelet lineage despite sustained multipotency,9 whereas no HSCs contribute exclusively to any other single blood cell lineage.10 Furthermore, the number of these platelet-biased HSCs expands considerably with age.11 The presence of platelet-biased HSCs has considerable implications for genomic based approaches for early detection which, in haematology, are primarily based on isolation of nucleic acid from leucocytes which would fail to detect emerging platelet-biased clones. Preliminary work undertaken by the Mead lab at the Weatherall Institute of Molecular Medicine (WIMM) on hospital inpatients over 70 years old with normal haematological parameters detected JAK2V617F CH in platelets using digital droplet PCR (Figure 1). Using the established >2% VAF definition of presence of CH, of 116 samples, platelet-biased/restricted JAK2 CH was detected in 10.4% of people which is a novel finding: 11 (9.5%) platelet clone

alone, 1 (0.9%) platelet clone greater than granulocyte clone. Furthermore, although JAK2V617F was detected in granulocytes from some patients, the clone size was in general larger in platelets than in granulocytes, with much of the JAK2V617F detected in gDNA from granulocytes at VAF <2%. Given the central role of platelets within thrombosis, it is possible that platelet-biased CH increases the risk of thrombosis to a greater degree than granulocyte-associated CH, and is potentially a more sensitive maker than 'standard' white blood cell CH for detecting CH in patients with unprovoked VTE and CVD.

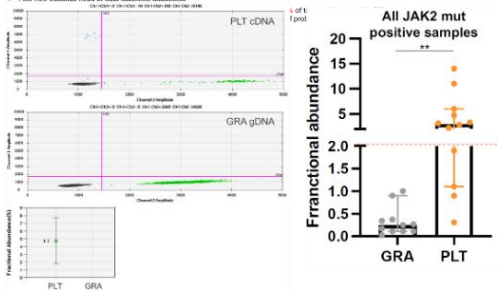
Objectives

Objective 1: To determine prevalence of platelet-biased CH in people with (A) unprovoked VTE and CVD and (B) cancer, including patients with cancer-associated VTE; compared to age matched controls.

We will screen for the presence of platelet-biased CH in a cohort of patients with unprovoked VTE and CVD. In order to do this, we have developed an NGS panel designed to detect CH-associated mutations from both gDNA and cDNA with a sensitivity to detect mutations down to a VAF of 1%. Initial pilot data are shown in Figure 2. Following consent, 100 patient samples will be processed from each of the VTE and CVD clinics in Oxford Hospitals, with 50 age matched controls. We will also screen for platelet-biased CH in 30 patients with cancer-associated VTE and 100 patients with cancer who are undergoing cytotoxic chemotherapy.

Figure 2. Next Generation Sequencing
Custom-made hybridisation panel for parallel sequencing of both gDNA and cDNA

Figure 1. JAK2 V617F digital droplet PCR assay



Objective 2: Functional studies to explore the physiological changes driving increased thrombosis risk with CH. We will carry out flow cytometry following stimulation of platelets from donors with platelet-biased CH with a range of low-dose activators using cell sorting of activated platelets to look for differential enrichment of platelets with myeloid mutations; thrombin generation with platelet-poor plasma and platelet-rich plasma and NETosis assays. These studies will allow us to determine whether platelet-biased CH is associated with aberrant platelet activation and whether this impact differs for particular CH-associated mutations.

Objective 3: Investigation of the cellular origin and drivers of platelet-biased CH. We will establish a small study to investigate the bone marrow of 5-10 patients with a substantial platelet-biased clone, in order to identify the cellular origin of the aberrant platelets and molecular signatures of these progenitor cells. Patients will undergo a detailed phenotypic and molecular analysis including single-cell TARGET-seq analysis of stem/progenitor cells from patients with platelet-biased CH, inflammatory and cytokine profiles and histological features of megakaryocytes using established machine learning approaches.12

Objective 4: To screen for novel mutations selectively associated with platelet-biased CH. Certain mutations might selectively lead to platelet-biased CH and would therefore not be included in the NGS panel which is based on known mutations identified by sequencing peripheral blood granulocyte gDNA. In order to explore this possibility, we will screen for CH mutations that are specifically associated with a platelet-restricted lineage output in a normal elderly population (50 people) by performing deep RNA sequencing of platelet cDNA.

Translational Potential

The work will be fundamental in exploring a novel finding with significant implications for detection of people at increased risk of blood cancer, VTE and CVD. Although it is well established that cancer increases the risk of VTE and CVD, there are currently no effective cancer screening strategies in place for people presenting with unprovoked VTE and early-onset CVD; screening strategies for unprovoked VTE fail to detect 30% of cancers, and do not improve overall cancer survival.[13,14] We anticipate platelet-biased CH could enable a novel screening approach in people presenting with unprovoked VTE and early-onset CVD, detecting people at increased risk of blood cancers, allowing counselling and early intervention to reduce the risk of progression to cancer. Furthermore, improved screening to look for CH in patients with solid cancers will facilitate early detection and preventative interventions to reduce cancer-associated VTE, CVD and secondary blood cancers. A key aim of the planned body of work is to develop clinical and research expertise in CH which would help support new CH clinics (already set up in USA but not in UK).[15]

Training Opportunities

This project will strengthen the collaboration between Dr Shapiro (Consultant Haematologist in Thrombosis and MRC CARP Fellow), Prof Adam Mead (Professor of Cancer Haematology, WIMM) and Professor Robin Choudhury (Professor of Cardiovascular Medicine, RDM). The student will be based in the Mead lab at the WIMM which designed the NGS panels required for this project. Structured supervision will be through weekly meetings with Dr Shapiro and Prof Mead and hands-on training in experimental techniques will be provided by experienced laboratory members. The student will be trained in techniques including RNA and DNA sequencing, NGS panels, flow cytometry and cell-sorting, thrombin generation and ELISAs. The student will be supported in data analysis, and developing skills of critical analysis, experimental design, scientific presentation and publication, and will benefit from the training and career development programme at the WIMM.

References

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39. Multimodal cell-free DNA epigenetic sequencing for early detection of pancreatic cancer [1,2,3,4] – C. Song

Primary Supervisor: Chunxiao Song
Additional Supervisors: Shivan Sivakumar
Eligibility: Track 1, 2, 3 and 4 students are eligible to apply for this project.

Project Summary

Although recent advances in cancer research offer new ways to treat cancer, early detection still represents the best opportunity for curing cancer. Early-stage treatment not only greatly improves patient survival but also costs considerably less. Circulating cell-free DNA (cfDNA) - the free-floating DNA in blood plasma originating from cell death in various healthy and diseased tissues - holds tremendous potential to develop an early cancer detection assay. Genetic information in cfDNA, such as mutations and copy-number variations (CNVs), demonstrate potential utility for monitoring cancer progression and treatment. However, genetic alterations are challenging to detect given the low fraction of tumour DNA in early-stage disease. Furthermore, genetic alterations are weakly informative about the tissue-of-origin needed to determine the location of the malignancy.

In contrast, widespread epigenetic changes such as DNA methylation of both cancer cells and tumour microenvironment occur early in tumorigenesis. Recent studies have shown cfDNA methylation to be one of the most promising biomarkers for early cancer detection, by providing thousands of methylation changes that can be combined to overcome detection limits, and tissue-of-origin information that allows cancer localisation with high confidence. Combined together, a multimodal approach that characterize the genome-wide epigenetic and genetic information in cfDNA could enable more sensitive early cancer detection. However, due to technological challenges associated with DNA methylation sequencing in low input cfDNA samples, most studies have been limited by DNA damage caused by bisulfite sequencing, or the qualitative nature of enrichment-based sequencing.

Recently, we developed TET-assisted Pyridine Borane Sequencing (TAPS), a new bisulfite-free DNA methylation sequencing method(1). TAPS uses mild chemistry to detect DNA methylation directly and showed improved sequence quality, mapping rate, and coverage compared to bisulfite sequencing, while reducing sequencing costs by half. The combination of direct methylation detection and the non-destructive nature of TAPS makes it ideal for DNA methylation analysis and also simultaneous genetic analysis in cfDNA. More recently, we optimized TAPS for cfDNA (cfTAPS) to provide high-quality, deep whole-genome cell-free methylomes. In a first proof-of-concept study, we applied cfTAPS to 85 cfDNA samples from patients with mainly early-stage pancreatic ductal adenocarcinoma (PDAC) or hepatocellular carcinoma (HCC) and non-cancer controls. From just 10 ng cfDNA (1-3 mL of plasma), we generated the most comprehensive cfDNA methylome to date. We demonstrated that cfTAPS provides multimodal information about cfDNA characteristics, including DNA methylation, tissue-of-origin, and DNA fragmentation. Integrated analysis of these epigenetic and genetic features enabled accurate identification of early PDAC and HCC (Figure), indicating that the technology holds great promise as a tool for minimally invasive early cancer detection(2).

Built on the promising results, this project will be a comprehensive study on PDAC early detection. We will improve our platform by adding new modalities from the rich information of cfTAPS, such as microbiome and nucleosome positioning, and further incorporating with simultaneous multiplex mutations detection. These additional modalities from a single cfTAPS assay would further improve sensitivity and ability to detect outliers within highly heterogeneous disease cohorts. We will apply the improved platform to multiple large cohorts of PDAC for independent validation as there is a pressing clinical need to diagnose this cancer at an earlier stage.

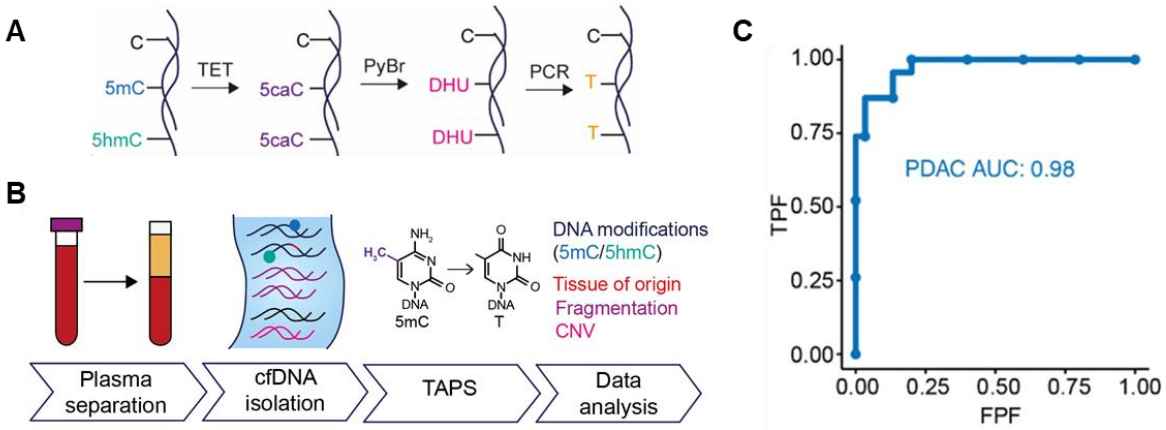

Figure. A, Overview of TAPS for direct base-resolution sequencing of DNA methylation. B, Schematic representation of the cfTAPS approach for cfDNA analysis. C, ROC curve of cfTAPS classification for PDAC and non-cancer controls (n = 53, PDAC = 23, non-cancer controls = 30).

Research objectives

Objective 1 Extracting new modalities from the cfTAPS data

Proposed Outcome A new computational pipeline will be established for the analysis of cfTAPS data.

Objective 2 Developing simultaneous multiplex mutations detection with cfTAPS

Proposed Outcome A detailed protocol on how to perform multiplex mutations detection within the cfTAPS workflow.

Objective 3 Apply improved platform on large PDAC cohort

Proposed Outcome The new improved workflow will be applied to multiple independent cohorts, each with hundreds of samples.

Objective 4 Integrated analysis to evaluate the performance of cfTAPS to separate PDAC from non-cancer controls.

Proposed Outcome Machine learning methods that integrated various genetic and epigenetic features will be developed to evaluate the sensitivity and specificity of cfTAPS in detecting PDAC with multiple independent validations.

Translational potential

PDAC has the worst survival of any human cancer, mostly due to detection at an advanced disease stage. Importantly, there is no blood test to detect or diagnose PDAC, which contributes to its late diagnosis. Therefore, novel approaches for PDAC detection are urgently needed. The TAPS technology was spun out to Base Genomics (June 2020) and was acquired by Exact Sciences for $410m (October 2020). This project is crucial in the development of comprehensive cell-free DNA epigenetic sequencing using TAPS and related methods to realize the full potential of liquid biopsy for cancer diagnostics.

Training opportunities

Training opportunities include a wide range of basic and advanced molecular biology and chemical biology techniques, clinical sample handling and study design, as well as cutting-edge next-generation sequencing techniques and bioinformatics data analysis.

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40. Galectin-3 promotes glioblastoma emergence from the subventricular zone stem cell niche [1,2,3,4] – Associate Prof. Szele

Primary Supervisor: Associate Prof. Francis G. Szele
Additional Supervisors: Prof. Eric O'Neill
Eligibility: Track 1, 2,3 and 4 students are eligible to apply for this project.

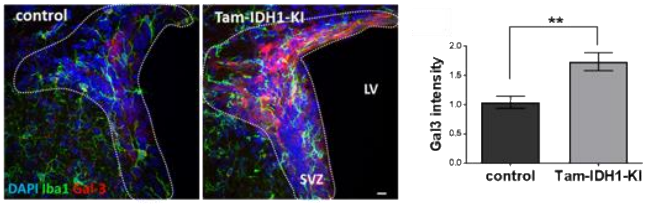
Project Summary

Galectin-3 (Gal-3) is commonly expressed by and regulates cancer, has profound roles in promoting inflammation and is uniquely expressed in the adult subventricular zone (SVZ) stem cell niche. Together with Eric O'Neill, we have found that Gal-3 regulates several molecules including Wnt and Yap/Taz signaling[1,3]. Whereas Gal-3 increases Wnt signaling in cancer cells, it decreases it in the healthy SVZ[3]. We also demonstrated in pilot work that Gal-3 regulates apical basal polarity (ABP) of human embryonic stem cell (ESC) rosette formation. Here, we will test Gal-3's role in coordinating Wnt and Yap/Taz function in ABP. ABP is a key hallmark of SVZ stem cells (apical primary cilia and basal blood vessel contact) and loss of ABP promotes gliomagenesis. Szele and colleagues at Oxford also showed that the IDH^{R132H} human cancer driver mutation promotes gliomagenesis when specifically expressed in the murine SVZ[4]. The work in this studentship will define how Gal-3 signaling pathways disrupt ABP in this translationally-relevant SVZ gliomagenesis model. The student will use established in vitro and in vivo approaches and also novel state-of-the art live imaging to visualise the generation and evolution of SVZ glioblastomas. This work will position us for future funding to use a powerful new drug screening approach which we developed with the Target Discovery Institute to find small molecule inhibitors of glioma infiltration[5]. *Thus, the student will test the novel hypothesis that Gal-3 signaling in the SVZ induces tumorigenesis via loss of ABP[2].*

Research objectives

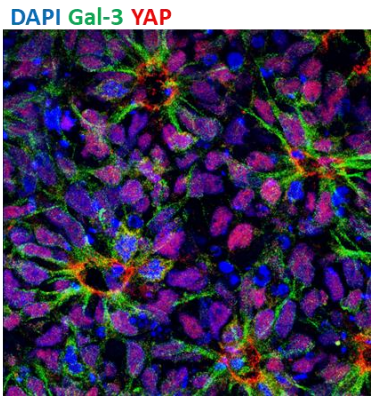
Gliomas contacting the SVZ are aggressive, are likely to spread or reappear and thus have a worse prognosis than gliomas not contacting the SVZ[6,7]. Inflammation predisposes cancer development via cytokines which can activate developmental pathways shifting the tumor toward a more undifferentiated state and increasing the number of cancer stem cells, which are characterised by loss of ABP[8]. In the brain, the SVZ is a uniquely inflammatory region that can predispose the niche to cancer development by the action of the pro-inflammatory regulator Gal-3 on stem cells[2]. Gal-3 has been linked with cancer aggressiveness, not only due to its pro-inflammatory role, but also because it upregulates several pro-tumorigenic pathways, and its expression is correlated with brain tumor grade and prognosis[9,10].

Fig. 1



In unpublished work, the Szele and O'Neill labs have shown that Gal-3 expression is increased in the SVZ during IDH1^{R132H} tumourigenesis (Fig. 1). Additionally, macrophages were activated in and around the tumour, which is interesting because they secrete Gal-3 and are chemotactic to it, causing a feed-forward cycle of inflammation[11]. Using human ES cells, we have also shown that anti-Gal-3 antibodies disrupt rosette formation and that Gal-3 and YAP are differentially expressed in the apical and basal poles of stem cells in rosettes (Fig. 2). We further showed that Gal-3 binds to b-catenin[3] and hypothesise that this regulates ABP via YAP signaling. In other work, we developed and are using intravital 3-photon (3PM) microscopy with Adam Packer (bespoke microscope in DPAG). With 3PM, we can visualise the entire SVZ in live animals at cellular resolution and will be used in this project.

Fig. 2



Workpackage 1. In vitro studies of ABP. Wnt pathways have been implicated in malignancy and "stem-ness" of gliomas, and are a possible therapeutic target[12]. Since Gal-3 modulates Wnt signaling in opposite directions in cancer compared to the healthy postnatal SVZ, malignant transformation of SVZ cells could involve altered Gal-3

function. The student will modulate Gal-3, Wnt signaling molecules and Yap/Taz in vitro to uncover their role in ABP of healthy and IDH1^{R132H} mutant SVZ stem cells. They will learn and employ the murine neurosphere stem cell assay which is well established in the Szele lab and will be used to assess ABP as well as key features of SVZ lineage progression: self-renewal, proliferation, fate choices and migration. The student will complement this with parallel functional studies in human ES cells. Rosette ABP will be examined to dissect its molecular regulation.

Workpackage 2. In vivo studies of ABP. The student will next confirm in vitro findings with in vivo approaches well-established in the Szele lab including knockdown, conditional knockout and rescue experiments of Gal-3 and downstream signaling partners in the SVZ. They will determine ABP of SVZ stem cells and loss of ABP in IDH mutant mice. They will also whether altering Gal-3, Wnt and Yap/Taz signaling in these stem cells affects cardinal features of malignancy, including self-renewing symmetric divisions, increased proliferation or emigration into surrounding tissues.

Workpackage 3. In vivo studies of inflammation. The student will assess the level of in vivo inflammation in the mice generated in WP 2 and this will be correlated with the extent of changes seen in ABP and SVZ stem cell behaviour mapped in WP 2. In other work, they will pharmacologically manipulate inflammation in mice to directly determine its effect on ABP of SVZ stem cells and tumourigenesis. We will determine macrophage infiltration into the gliomagenic lesion by carrying out adoptive transfer experiments with CD68-GFP mice (collaboration with David Greaves, Oxford), whilst monitoring the expression of inflammatory cytokines/chemokines and microglial and macroglial activation.

Workpackage 4. Imaging studies. We will next turn to imaging ABP loss and inflammation in live animals. Based on results from the other WPs we will judiciously chose functional manipulations to image. The student will learn 3P microscopy in live mice and 2P time-lapse in slices[13], (easier and will provide finer temporal and spatial resolution than 3P microscopy). We will use bespoke 3PM and 2PM time-lapse in DPAG to determine how the IDH mutation alters ABP, inflammation, cell proliferation and migration (collaboration with Adam Packer, Oxford). We will also image tumour evolution in the same mice with the small animal MRI in the South Parks Road BSB animal facility (collaboration with Jason Lerch, Oxford). All licenses for this work in place.

Translational potential

Gal-3 confers resistance to traditional treatment with chemotherapy and radiotherapy in glioblastoma[10]. Thankfully, several inhibitors of Gal-3 are in clinical trials for cancer and other diseases[14,15]. One of these inhibitors (GB0139, Selleckchem, phase IIb clinical trials for idiopathic pulmonary fibrosis) is commercially available and in parallel to this studentship, the Szele group will use it to pharmacologically block Gal-3 and its signalling pathways. Additionally, we recently published a unique medium-throughput 3D approach – the "spheroid migration assay" to measure SVZ cell migration and metastasis[5]. This assay will be used to study the effects on Gal-3 blockade on SVZ emigration. In conjunction with the Target Discovery Institute at Oxford, we plan to use molecular libraries targeted towards the most relevant pathways revealed in Aims 1 and 2. Compounds that decrease spheroid migration will be progressed in vivo to mice with the IDH1^{R132H} mutation to verify blockade of metastasis. Thus, the translational potential of this parallel project is very good as it will screen for molecules that limit glioma infiltration.

Training opportunities

The student will be trained in a rich variety of techniques including in vitro stem cell culture, in vivo electroporation of the IDH mouse, functional assays, 2- and 3-photon microscopy, small animal MRI and adoptive transfer. Szele and O'Neill will train the student in the theoretical concepts described above.

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41. Developing single-cell transcriptomics tools for PARP inhibitor resistance in BRCA1/2-deficient cells and tumours [1,2,3,4] – Prof. Tarsounas

Primary Supervisor: Prof. Madalena Tarsounas
Second Supervisor: Christiana Kartsonaki
Eligibility: Track 1, 2, 3 and 4 students are eligible to apply for this project.

Project Summary

Cells and tumours with compromised breast cancer susceptibility genes BRCA1 or BRCA2 retain the ability to proliferate, in spite of the severe genomic instability caused by accumulation of DNA lesions. This vulnerability is exploited by highly-specific therapies that enhance the susceptibility of BRCA1/2-deficient tumours to DNA damaging agents, with the poly-ADP ribose polymerase inhibitors (PARPi; e.g. olaparib) as a prominent example. In spite of clear therapeutic benefits, cure rates for BRCA1/2-mutated cancers remain low, as patients frequently develop resistance to PARPi. Several mechanisms of PARPi resistance have been reported. However, PARPi resistance remains a critical problem in the clinic, limiting sustained responses to these drugs. Here, we aim to identify transcriptional signatures associated with PARPi resistance, specifically olaparib resistance. To identify such signatures, we will perform single-cell RNA sequencing (scRNAseq) using BRCA1/2-deficient cells in culture and cells obtained from patient-derived xenograft (PDX) models that have acquired olaparib-resistance upon prolonged exposure to olaparib. We will furthermore combine scRNAseq and EdUseq data to test whether replication failure at specific genomic sites could interfere with transcription and therefore represent the underlying mechanism of the identified transcriptome alterations. In the longer term, this line of research can lead to predictive markers for patient response to olaparib, which could facilitate early patient stratification and the development of personalized treatment strategies.

Research objective

The work proposed here will help elucidate, at single-cell resolution, the relationship between the emergence of rare, tumour-initiating cells subpopulations within tumours lacking BRCA1 or BRCA2 genes and the response to the PARP inhibitor olaparib. Accordingly, we will pursue two main objectives:

a. Characterize the single-cell transcriptomic landscape of olaparib-resistant BRCA1/2-deficient cells in culture. The scRNAseq technology will enable us to generate gene expression profiles of single cells and to identify cell sub-populations with specific transcriptional signatures. To achieve this first objective, scRNAseq will be carried out in populations of olaparib-sensitive and -resistant BRCA1/2-deficient cells, already generated in Tarsounas lab. We will prepare libraries from each cell line, before and after olaparib resistance onset, to sequence between 7,000 and 10,000 cells using the standard protocol of the Chromium Single-Cell 3' gene expression profiling solution (10x Genomics). Unsupervised clustering approaches will be developed to classify cells into sub-groups with specific signatures (e.g. immune response, metastasis etc.) and to monitor cell dynamics using algorithms for pseudotime analysis. We will apply this combination of analytical approaches to the cell lines that are sensitive or become resistant to Olaparib, and anticipate that this will allow us to identify cell clusters with unique patterns of gene expression, which could Tarsounas, Kartsonaki – CRUK Cancer Centre Studentship not be resolved at the whole-cell population level. In addition, this approach will enable us to identify eventual differences between the signatures specific to BRCA1- and BRCA2-deficient cells. Lastly, the collection of signatures identified for distinct cell subpopulations selected by olaparib will be further explored in the large METABRIC and TCGA PanCancer Atlas breast and ovarian cancer cohorts (5,098 samples, among which 355 and 362 carry alterations in BRCA1 and BRCA2, respectively), specifically to assess their prognostic abiilty through univariaable and multivariable regression models.

b. Characterize the single-cell transcriptomic landscape of olaparib-resistant BRCA1/2-deficient PDX tumours in vivo. In addition to linking the transcriptomic signatures of olaparib-resistant cell subpopulations to tumour gene expression data and clinical information found in databases (e.g. TCGA, METABRIC), we will recapitulate in vivo the results obtained in vitro using cell cultures. To achieve this, scRNA-seq will be carried out in cell

suspensions prepared from BRCA1- or BRCA2-mutated (n = 3 BRCA1-/- and n = 1 BRCA2-/-) olaparib-naïve and -resistant patient-derived xenografts (PDX). In these models resistance emerged after treatment with olaparib for up to 150 days, when individual tumours regrew. These models are also already available for processing in Tarsounas lab.

This project will facilitate the collaboration between basic cell biology and bioinformatics, and the student funded here will be trained in and benefit from both types of expertise. Importantly, this collaboration will enable the co-applicant, Dr. Christina Kartsonaki, who is an early career researcher within the NDPH, to achieve scientific independence. Securing this CRUK award will enable her to co-supervise a graduate student in an area of research novel for her, and at the same time, will place her in a stronger position for further funding applications.

Translational potential

In spite of initial responses to targeted therapies such as PARPi, BRCA1/2- deficient tumours develop a resistance to these therapies. PARPi resistance often entails genomic rearrangements and mutations that trigger rewiring of the damage response pathways within the tumour so that apoptotic responses to treatment are replaced by cell survival and metastasis. Here we anticipate to identify new, robust transcriptional signatures associated with Olaparib resistance, which can be used to stratify patients for PARPi therapy. In addition, these gene expression profiles will identify vulnerabilities that can be exploited to target resistant disease. In the longer term, these approaches can be used to develop patient screening protrocols using machine learning and statistical methods

Training opportunities

The student will receive training in statistical and bioinformatics methods used in the analysis of high-throughput transcriptomic data, as well as software commonly used in such analysis, such as R, Unix and other command-line tools. Wet lab work training will include cell culture, qRT-PCR and western blottihng techniques necessary to validate any candidate genes and pathways.

42. Imaging metabolism in cancer and the heart to assess efficacy and safety of mitocans [2,3] – Dr. Timm

Primary Supervisor: Dr. Kerstin Timm
Second Supervisor: Prof. Adrian Harris, Prof. Lisa Heather
Eligibility: Track 2 and 3 students are eligible to apply for this project.

Project Summary

Targeting mitochondrial metabolism is a promising new strategy to treat cancer, especially chemotherapy-resistant tumours. However, cancer drugs targeting mitochondria, called mitocans, may have toxic side effects on the heart, due to its reliance on mitochondrial energy metabolism. Hyperpolarized magnetic resonance imaging (MRI) is an exciting new technology that can measure tissue metabolism in real time in vivo. Hyperpolarized MRI can assess tumour response to treatment as well as cardiotoxic side-effects of chemotherapy in both preclinical models and in patients by evaluating metabolic flux. This project will assess in a rat model of breast cancer the effect of different mitocans on mitochondrial function in tumours and the heart using hyperpolarized MRI. Imaging data will be validated with ex vivo high resolution respirometry and metabolomic analysis of tumour and cardiac tissue. This workflow is readily translatable into clinical trials of cancer patients at Oxford.

Background

It was long believed that mitochondrial function is impaired in cancers, and that they rely instead on aerobic glycolysis (the "Warburg effect") for energy generation and proliferation. We now know that mitochondrial metabolism is vitally important for cancer cells [1]. In fact, mitochondrial metabolism is a new key target for anti-cancer therapy, using drugs termed 'mitocans'[2]. However, drugs targeting mitochondrial metabolism in cancer could have severe metabolic side-effects, especially on the heart, which relies on mitochondrial function to fulfil its energetic requirements [3]. This could mean that an effective mitocan to treat cancer may have toxic side-effects to the heart, causing energy-deprivation and heart failure. Hyperpolarized 13C magnetic resonance imaging (MRI) is the only non-invasive imaging technique that can assess early changes in real-time tumour metabolism in response to treatment in preclinical models [4], [5] and in cancer patients [6], [7]. In addition, hyperpolarized MRI can assess early toxic side-effects of chemotherapy on the heart by assessing mitochondrial metabolic fluxes, both in pre-clinical models [8] and in patients [9]. Thus, hyperpolarized MRI can assess both tumour treatment response and drug-induced cardiotoxicity. Crucially, dual imaging of the heart and cancer simultaneously has never been done, neither in pre-clinical models nor in patients, and pre-clinical models of cardiotoxicity are performed in naïve animals rather than in tumour-bearing models. In this project, we will assess tumour treatment response and chemotherapy-induced cardiotoxicity in breast tumour-bearing rats, using hyperpolarized MRI of both the heart and tumour simultaneously. We will first assess the role of mitochondrial metabolism in response to doxorubicin (DOX), a broad spectrum chemotherapeutic known to affect mitochondrial metabolism [8]. We will then investigate new mitocans in clinical development that specifically target the electron transport chain, in comparison with the anti-diabetic complex I inhibitor, metformin, as combination therapy with DOX. We will use rats as their larger hearts yield superior imaging data compared to mouse models using hyperpolarized MRI.

Research objective

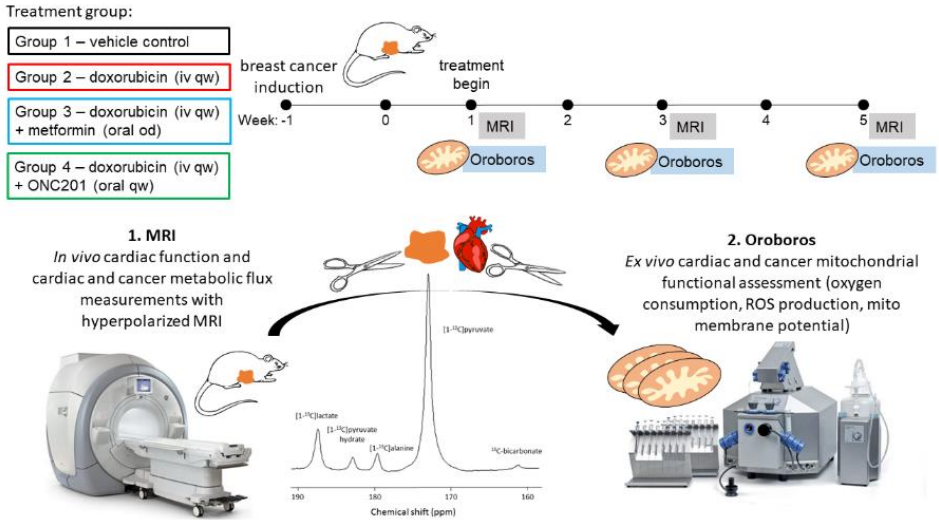

Figure 1
Outline of the general workflow for the project

Doxorubicin (DOX) is a widely used chemotherapeutic agent for the treatment of many cancers. However, DOX exhibits cardiotoxic side effects, causing heart failure (HF) in ~5-10% of patients [10]. However, there are no routine clinical tests, imaging or biomarkers, which can assess susceptibility to development of heart failure before changes in cardiac function occur. We have previously established a rat model of DOX-HF, which is representative of clinical treatment regimens. This leads to a robust model of cardiotoxicity, culminating in impaired cardiac function, characterised by a loss of mitochondria and a decrease in mitochondrial oxidative metabolism, assessed with hyperpolarized [1-13C]pyruvate MRI [8]. We now want to assess mitochondrial metabolism in response to DOX in a rat model of breast cancer, imaging both the heart and the tumour. We will use a rat breast cancer cell line (Walker 256), which is sensitive to DOX in vivo [11], to induce a tumour allograft in the mammary fat pad.

Metformin is a commonly prescribed oral anti-diabetic, which inhibits complex I of the electron transport chain [12]. In breast cancer cells, metformin prevents doxorubicin-resistance [13], while type 2 diabetic patients on metformin therapy display decreased cancer mortality compared to patients without metformin [14]. Metformin can be safely administered to cancer patients alongside chemotherapy [15]. However, metformin treatment in breast cancer patients leads to two distinct cancer phenotypes: those that respond with increased glucose uptake, and those that upregulate OxPhos and show treatment resistance [16]. Walker 256 carcinomas in rats in vivo have previously been shown to be sensitive to complex I inhibitors [17]. As hyperpolarized MRI measures oxidative metabolism, it is an ideal imaging modality to assess tumour response or resistance to treatment, while simultaneously assessing cardiotoxic side-effects. ONC201 [18] and Mubritinib [19] are mitocans in clinical development, and they impair mitochondrial respiration through complex I inhibition, thus selectively targeting cancer cells that rely on mitochondrial metabolism. We will compare the tumour treatment response and cardiotoxicity of ONC201 and mubritinib with metformin as combination therapy with DOX using hyperpolarized MRI.

To validate in vivo imaging data we will pursue two ex vivo methods: metabolomics and high resolution respirometry. Metabolomics has previously proved to underpin hyperpolarized MRI results from a DOX-cardiotoxicity model in rats [8]. The Oroboros O2k FluoRespirometer (Oroboros Instruments) is a state-of-the-art mitochondrial bioanalyzer, which has been successfully used in DOX-treated mouse skeletal muscle and rat cardiac mitochondria [20], [21]. We will also optimise high resolution respirometry in patient derived cells in this project in collaboration with Dr Simon Lord, paving the way for clinical translation of this cutting-edge workflow to assess safety and efficacy of mitocans.
Dr Kerstin Timm, the main supervisor, has extensive experience in hyperpolarized MRI, cancer and cardiac metabolism and cardiotoxicity. Prof Adrian Harris is an oncologist with research experience in cancer metabolism and Prof Lisa Heather is an expert in cardiac mitochondrial metabolism. Both will act as second supervisors on this DPhil, forming a multidisciplinary team to ensure project success

Translational potential
We will determine if non-invasive metabolic imaging with hyperpolarized MRI can detect tumour treatment response and early cardiotoxic changes simultaneously and thus discriminate between safe and toxic drugs, which could be rapidly translated to cancer patients, paving the way to personalised cancer care. The data will be validated with state-of-the-art high resolution respirometry and metabolomic analysis, which can also be performed in human samples [22].

Training opportunities
The student will receive personal licence training to conduct in vivo rat work including tumour induction, chemotherapy and hyperpolarized MRI, a cutting edge non-invasive and translatable imaging technique that has already led to fascinating clinical trials in both cancer and cardiac patients. The student will furthermore learn how perform mitochondrial function analysis with a FluoRespirometer, including in patients samples, as well as metabolomic and molecular biology analysis.

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43. Heterogeneity of macrophages in colorectal cancer: the role of IRF5 [1,2,3] – Prof. Udalova

Primary Supervisor: Prof. Irina Udalova
Second Supervisor: Holm Uhlig
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Project Summary

The intestinal immune system is a delicately balanced system between tolerance to commensal and food particles and raising an immune response upon infection. Dysregulation of the intestinal immune system can lead to inflammation, which may progress to colorectal cancer (CRC) [1]. Macrophages are playing a central role in maintenance of homeostasis, initiation of inflammation, restoration of tissue upon injury and mediation of chemoresistance in tumours. Integrating cues of their immediate tissue microenvironment, macrophages can adapt their functions according to tissue-specific needs and adapt upon change [2]. Depending on the polarisation of the tumour-associated macrophages (TAMs), cancer progression and initiation can be hindered or helped [3]. Macrophages also suppress effector T cell recruitment and function in tumours, further highlighting their pivotal role and importance in understanding the mechanisms underneath [4,5]. Various subsets of macrophages have recently been identified in different tissues using single-cell RNA sequencing (scRNA-seq), highlighting the heterogeneity and plasticity of macrophages [6–9]. Furthermore, a role for Interferon Regulatory Factor 5 (IRF5), a master transcription factor involved in regulating the transcription of pro-inflammatory mediators in shaping macrophage polarisation was identified [7,10]. IRF5 controls both acute and chronic inflammation and is protective in pathogen clearance [7,11,12]. IRF5 has also been identified as a DNA-damage sensor, highlighting a potential beneficial role in CRC [13]. Therefore, dissecting the molecular mechanisms involving macrophage polarisation and function is crucial for identification of treatment options for both inflammatory bowel disease (IBD) and CRC

Research objective

The aim of this project is to firstly assess the role of IRF5 in macrophages in resolution of inflammation using the *Helicobacter hepaticus* and anti-IL10R (Hh + aIL10R) colitis model [14]. Previous work in the lab has profiled IRF5-dependent inflammatory CD11c+ macrophages at peak of inflammation using scRNA-seq [7]. Based on this work, we aim to profile the heterogeneity of macrophages in resolution of inflammation using targeted mouse models (CX3CR1-IRF5 fl/fl and CCR2-mKate ER2 IRF5 fl/fl). We hypothesize that lack of IRF5 is beneficial for resolution of inflammation as macrophages are being polarised towards a tissue-regenerating phenotype. Furthermore, the T cell pool was shifted at peak of inflammation, which might be a result of different T cell priming by IRF5-proficient and deficient macrophages.

The comparison of macrophage heterogeneity in resolution to CRC will help identify molecular targets in shaping macrophage phenotype and directing towards resolution rather than progression of inflammation into cancer development. It also was suggested that localisation of macrophages within the tumour microenvironment might be a crucial determinant of their function [3]. Therefore, assessing the localisation of different macrophage subsets and their interaction cell-cell contacts could also provide further information about their function and potential targeting. Investigation of the differences in T cell priming of IRF5-deficient macrophages will also offer insight of the interplay of IRF5 in innate immune cells with the adaptive immune system in both resolution and CRC. The role of TAMs in CRC is yet unclear with various studies suggesting both detrimental and beneficial effects. Thus, in addition to inhibition of IRF5 (as above) we would also consider stimulating IRF5 specifically at tumour sites might improve anti-cancer immunity [8,13]. This could be achieved by targeted delivery of adenoviral vector expressing IRF5 (overexpression) or inhibition of IRF5 through phosphorylating kinase inhibition [15].

Translational potential

T cell immunity, which is beneficial in tumours, is undermined by immunosuppressive myeloid cells, of which a subset of TREM2+ macrophages have been identified as a potential target in tumours 16. Understanding the role of macrophages as pivotal cells in the resolution of inflammation as well as progression of inflammation into CRC will help shaping specific therapies targeting macrophages. IRF5 plays a crucial role in mediating differentiation of

infiltrating monocytes into pro-inflammatory macrophages during intestinal inflammation and may therefore be central during resolution and cancer development.

Training opportunities

The student will be trained in the Hh + aIL10R colitis model as well as in basic immunology techniques like flow cytometry, RT-qPCR and in vitro cultures to analyse the outcomes. Furthermore, insights and potential guided analysis of single-cell RNA sequencing as well as cutting-edge microscopy to define the localisation of macrophage subsets within the tumour microenvironment will be made available.

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44. Non-Invasive metabolic imaging of liver cancer [1,2,3,4] – Associate Prof. Valkovic

Primary Supervisor: Associate Prof. Ladislav Valkovic
Second Supervisor: Dr. Michael Pavlides, Prof Damian Tyler
Eligibility: Track 1, 2, 3 and 4 students are eligible to apply for this project.

Project Summary

Liver cancer is one of the leading causes of cancer-related mortality around the world. The effectiveness of treatment, i.e., resection or transplantation, depends heavily on the stage of liver cancer at the time of diagnosis. **Hence potential identification of early malignant metabolic changes in liver tissue would be very valuable**. Magnetic Resonance Spectroscopy (MRS) allows identification of tissue metabolism and, thanks to its non-invasive nature in contrast to biopsy, can be safely employed as a screening technique.

In this work, the metabolic profile of liver cancer stages will be identified using ultra-high field MRS. The main goals of this research are:

1. To establish a robust protocol for metabolic screening of patients with liver cancer using ultra-high field MRS.
2. To identify metabolic impairments in cancerous tissue as well as in normal appearing liver tissue in patients with hepatocellular carcinoma.
3. To compare the metabolic profile of patients with liver cancer to patients with high risk of progression towards liver cancer, e.g., patients with liver cirrhosis.

These new measures will help provide unique insight into the pathophysiology of liver cancer and help potentially identify individuals with developing liver cancer before tumour formation.

Research objective

Hepatocellular carcinoma (HCC) is the most common primary cancer of the liver, and its mortality rates parallel its increasing incidence[2]. Besides chronic hepatitis B and C, non-alcoholic fatty liver disease (NAFLD) is the most important aetiology of HCC, and effective screening and management strategies are crucial to reduce the HCC risk[3]. Early diagnosis, via non-invasive screening among persons with HCC risk factors, remains the most important strategy to identify early-stage disease appropriate for resection or transplantation, maximizing survival chance.
Contrast enhanced imaging with CT or MRI are used for HCC diagnosis, but liver biopsy is still required in some cases, particularly in small / early tumours. Alternative techniques are needed, particularly to identify those at most risk of developing HCC so that they can have more intensive surveillance. Tools that can predict or monitor treatment response are also needed.

Magnetic Resonance Spectroscopy (MRS) is a unique tool capable of liver tissue metabolism assessment non-invasively. Of particular interest is phosphorus (^{31}P)-MRS, which provides direct insight into tissue energy metabolism. In addition, the detectable phosphomonoesters (PME) and phosphodiesters (PDE) provide insight into anabolism and catabolism of the cell membrane[4]. The PME/PDE ratio (Fig 1) has been demonstrated in several tumour types to allow malignancy assessment and treatment monitoring[5, 6]. **Changes in PME/PDE could potentially identify early malignant changes in liver tissue that appears normal on conventional imaging.**

^{31}P-MRS strongly benefits from the use of ultra-high field strength MR systems, i.e., 7T, increasing the signal-to-noise ratio (SNR) and providing separation of individual metabolites that contribute to PME and PDE peaks[7]. However, due to the inherent inhomogeneities of 7T, especially with the use of surface coils, the quantification of liver metabolites is challenging and only a small portion of liver is typically covered.

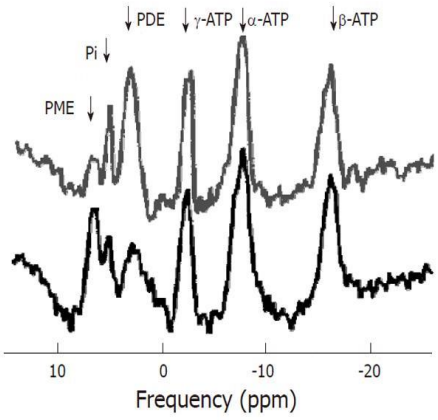

Figure 1 *In vivo ^{31}P MR spectra of human liver tissue obtained from a healthy volunteer (top) and from a patient with HCC (bottom). Note the increased PME/PDE in the HCC patient.[1]*

We have recently developed a method for absolute quantification of liver ^{31}P metabolites[8] at 7T and demonstrated that using a whole-body coil can provide metabolic information across the chest[9]. These two techniques now need to be combined in order to provide absolute quantification of ^{31}P metabolites across the whole liver. This will allow **assessment of metabolic changes beyond the carcinoma** and comparing the normal appearing tissue profile to liver metabolism of patients with high risk of hepatocellular carcinoma development, i.e., liver cirrhosis and NAFLD patients.

The aims of this research are to:

1. *Establish a protocol for assessment of metabolic changes across the liver using ^{31}P-MRS at 7T*
2. *Investigate the potential of PME/PDE to identify malignant changes in normal appearing liver tissue in hepatocellular carcinoma patients*
3. *Compare the metabolic profile of the normal appearing liver tissue in HCC patients with metabolic profile of patients with liver cirrhosis who are considered at high risk*

This will allow identification of early metabolic changes in liver tissue pre-dating carcinoma formation. Hence identifying patients with high likelihood of HCC formation early and allowing treatment with a high chance of success. In addition, development of therapies targeting metabolism is currently on the rise and this work will provide tools for safe, non-invasive treatment monitoring.

Translational potential

Hepatocellular carcinoma (HCC) is an end-stage-liver disease requiring resection or transplantation to increase survival chance. Early diagnosis and treatment are therefore vital. Since changes in tissue metabolism predate malignant tumour formation, detailed mapping of metabolic profiles in tumours, normal appearing liver tissue in HCC patients and livers in high-risk patient groups will provide screening biomarkers for early therapy start and monitoring of treatment outcome. This metabolic profiling will be also highly valuable for the assessment of efficacy signals in early phase 2 studies (or the lack thereof, i.e. de-risking metabolic drug development).

Training opportunities

The student will be trained in Magnetic Resonance Imaging and Spectroscopy, this includes sequence programming for driving the MR system. Next the student will learn about scanning protocol development and optimization, through measurements in objects called phantoms. And finally, the student will be trained in MR data acquisition, post-processing and final data analysis.

In addition, through the multi-disciplinary environment in OCMR, the student will learn how human-centred research is conducted, i.e., interacting with doctors, physicists and biological scientists to answer clinically relevant research questions.

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45. UGT8 and microcarrier signalling in the development of breast cancer [1,2,3] – Prof. Wilson

Primary Supervisor: Prof. Clive Wilson
Second Supervisor: Prof. Adrian Harris, Dr. Simon Lord
Eligibility: Track 1, 2 and 3 students only are eligible to apply for this project.

Project Summary
Intercellular signals and signalling pathways promote the development of all cancers. Traditionally, such signals are considered to be discrete molecular entities like growth factors, but recently, more complex multimolecular complexes, such as extracellular vesicles (EVs), which can simultaneously reprogramme many aspects of cell behaviour, have emerged as key mediators. Using a Drosophila prostate-like genetic model, we have discovered a new EV subtype1 and novel signalling complexes called microcarriers2 (Fig. 1A), which have a neutral lipid core and store protein signals at their surface that rapidly dissipate during mating. We recently showed that the highly evolutionarily conserved UDP Glycosyltransferase 8 (UGT8)[3,4], a ceramide galactosyltransferase, is required for microcarriers to be released from secreting cells (Fig. 1B). UGT8 is upregulated in several aggressive cancers, particularly breast cancer[5-7]. Galactosyl-ceramide is also the major glycosylated lipid that coats lipid droplets secreted from breast epithelial cells[8]. We hypothesise that breast cancer cells up-regulate UGT8 to secrete galactosylceramide-coated, neutral lipid-containing, microcarrier-like structures and that these play important intercellular signalling roles in tumour biology.

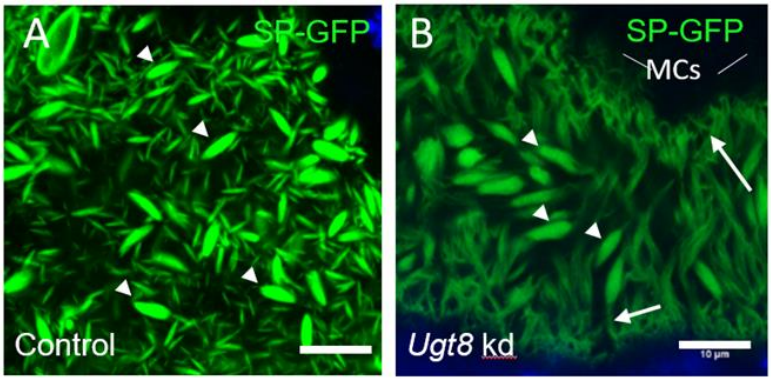

Figure 1. Microcarrier release requires *UGT8*. A. Microcarriers (arrowheads mark four large representatives) in accessory gland lumen can be visualised with a Sex Peptide-GFP fusion protein (or neutral lipid dye [not shown]). Their surface cargos control microcarrier shape and block fusion into large lipid droplets. B. When *UGT8* is knocked down in microcarrier-secreting main cells (MCs), microcarriers remain connected to MCs by projections (arrows).

Research objective
The project will have the following four research objectives and proposed outcomes, to fully test our central hypothesis:

2.1 Optimise assays for microcarriers and lipid droplets secreted from breast cancer cell lines: Initially use neutral lipid dyes (eg LipidTox) to stain small volumes of conditioned medium from 2D culture of breast cancer cell lines with high UGT8 expression[5,6]. These dyes are highly specific and do not stain other secreted multimolecular signalling structures like extracellular vesicles. Also use organoids generated from UGT8-expressing cell lines, eg. PMC42, MCF10[9], staining the lumen of fixed tissue (and extend to breast carcinoma organoids, if successful). In secreting cells, we will express a GFP protein carrying a glycosylphosphatidylinositol anchor (GFP-GPI), which associates with secreted phospholipid-coated structures and inserts into the outer coat of microcarriers in flies, to test as a vital marker for microcarriers. Confirm microcarrier identity by absence of transmembrane protein cargos, failure to stain with lipid bilayer dyes, immuno-EM and UGT8-dependency (***2.2***). Develop assays for measuring size and number of microcarriers.

Proposed outcome: Identification of microcarrier-secreting cells and development of microcarrier assays.

2.2 Characterise the role of UGT8 in microcarrier biogenesis: Block ceramide synthesis, eg with zoledronic acid[6], or knockdown *UGT8* to test roles in microcarrier biogenesis, measuring microcarrier numbers and size in conditioned medium, and analysing secreting cells and organoids for stalled microcarrier secretion.

Proposed outcome: Determine the roles of UGT8 and ceramide in microcarrier biogenesis.

2.3 Identify cargos carried by microcarriers: Pull-down GFP-GPI-labelled microcarriers or isolate by density gradients on basis of low density, and undertake proteomics analysis of cargos using several cell lines (with Dr Roman Fisher [Target Discovery Institute; collaboration letter included], with whom we already collaborate[2]). Screen for cargos previously identified in *Drosophila*, which are already implicated in UGT8 function, eg. Contactin[2,10]. Start to screen for specific cargos in patient samples, eg. organoids and blood, as the basis for grant applications to CRUK.

Proposed outcome: Identification of signalling molecules and other cargos on microcarriers *in vitro* and in patients.

2.4 Determine functions of microcarriers: Reduce microcarrier secretion by *UGT8* knockdown (or knockdown of cargos from ***2.3***) and test effects of resulting conditioned medium and control medium on breast cancer cell growth and migration, blood vessel network formation, etc, with assays guided by cargos found in ***2.3***. If time allows, undertake preliminary xenograft experiments to test effects on microcarrier secretion, tumour signalling, growth and metastasis.

Proposed outcome: Determine the functions of breast cancer microcarriers

Translational potential

Intercellular signalling plays a central role in tumour growth and interactions with the tumour microenvironment. However, studies of such signalling often focus on single molecules, even though there is increasing evidence that multimolecular complexes are involved. One explanation for this is that initially defining the basic biology of these complexes requires advanced cell biological and genetic approaches, preferably in an in vivo system. Recent studies in the fly have allowed us to identify a novel subtype of exosomes, for which we have now demonstrated a role in cancer[1]. We are now in a strong position to take a similar approach for microcarriers[2] and UGT8 function. This project will allow the first study of this new form of signalling in cancer cells, guided by the combined expertise of the collaborating supervisors (see 2.6 above). It will also provide the proof-of-principle for future patient-focused, CRUK-funded studies in this area.

Training opportunities

The student will investigate how breast cancer microcarriers and neutral lipid secretion are regulated by UGT8, define microcarrier cargos and determine their functions. (S)he will be trained in advanced cell culture techniques, cell biology, molecular genetics, high-resolution fluorescence imaging and the bioinformatics associated with proteomics analysis. (S)he will also develop expertise in cancer signalling and in bringing together ideas from clinical and basic science through the collaborative links we have established. Overall, this work has the potential to open up a new field in cancer biology, relevant not only to breast cancer, but to other cancers where high level UGT8 expression is a marker for tumour metastasis and therapy resistance[7,12].

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