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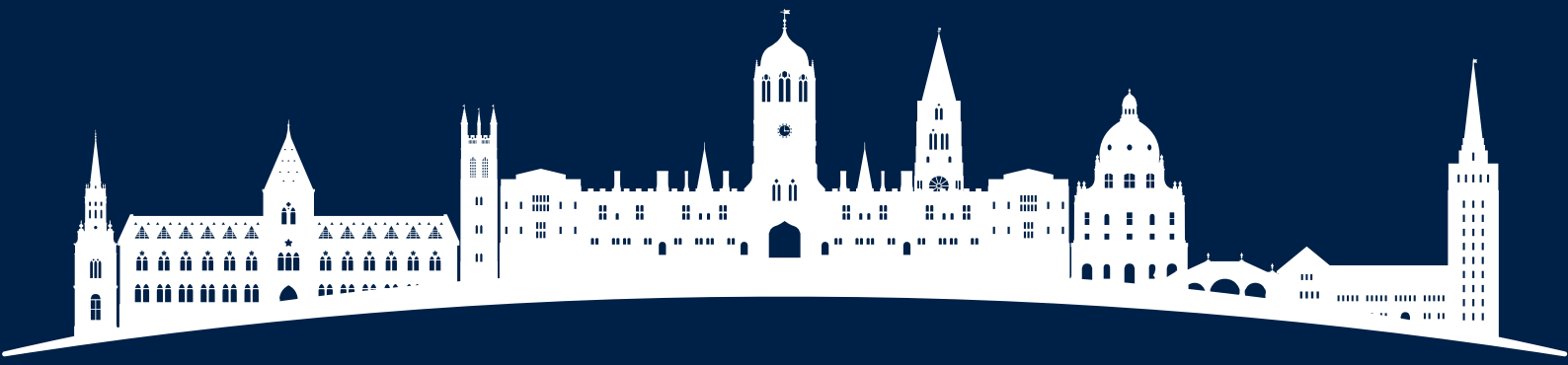
DPhil in Cancer Science

University of Oxford

2023 Intake Project Book



DPhil in Cancer Science
2023 Intake Project Book

Introduction

This handbook provides an overview for prospective students looking to study for a DPhil in Cancer Science starting in 2023 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). At entry, we will be looking for evidence of completion of at least the first two years of a primary medical qualification and achievement at the level of an upper-second or first-class honours degrees (or iBSc).

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 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 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 Tracks 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 9th December 2022. Shortlisted students will be invited to interview in January. 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. Modelling early ovarian tumorigenesis using fallopian tube organoids [1] - Prof. Ahmed

Primary Supervisor: Prof. Ahmed Ahmed
Additional Supervisors: Prof Hagan Bayley
Eligibility: Track 1 students are eligible to apply for this project.

Lay abstract

Ovarian cancer: a fatal disease where early detection can make a real difference

Worldwide, every two minutes, a woman is diagnosed with ovarian cancer: the vast majority receives their diagnosis when the cancer is already at an advanced stage and more than 55% of them will die within 5 years.

A timely diagnosis would dramatically change these statistics, because detecting ovarian cancer at stage I means a survival rate higher than 90%.

What is lacking to be able to achieve early detection in ovarian cancer?

Currently, there are no efficient screening tools that we can use in the clinic: the most renowned ovarian cancer marker is not specific enough for early detection and we are in dire need of new more efficient biomarkers.

On the research side, we now know that most ovarian cancers actually start in the fallopian tube rather than the ovary itself. We have managed to identify the most important DNA mutations causing ovarian cancer and to somewhat mimic the early stages of cancer formation in culture.

However, these systems have overlooked important aspects related to female physiology, such as the deep effects ovulation and hormones can have on cancer formation.

A new culture system to recreate the early stages of ovarian cancer

We believe that a 3D culture system where we can faithfully recapitulate what happens in the fallopian tube could be an invaluable tool for early detection.

Our plan is to build on previously established models and create a system where the fallopian tube cells are exposed to the same environmental and physiological factors naturally occurring in women.

By carefully characterising this new model, we expect to gain meaningful insight on the early stages of ovarian cancer, including the identification of biomarkers to be used for screening purposes.

Abstract

Around 7500 cases of ovarian cancer are diagnosed in the UK each year, and the majority are at stage III/IV.

Late diagnosis is one of the main factors leading to the high mortality of this cancer, which is characterised by a 10-year survival rate of only 35%.

Since the fallopian tube has been recognised as the tissue of origin of most ovarian cancers, fallopian tube biology has become crucial for research on early detection and ovarian cancer cells can be obtained by knocking out BRCA1/2, TP53 and PTEN in fallopian tube organoids or mouse oviducts.

However, these genetic approaches have so far failed to identify novel biomarkers for early detection, most likely because of their inability to fully recapitulate the complexities of the fallopian tube microenvironment.

In this project, we aim to establish a more physiologically relevant model of early ovarian tumorigenesis by adding two components: 1) the ovulatory follicular fluid, which contains reactive oxygen species and can induce DNA double-strand breaks in the fallopian tube cells; 2) tissue-resident stem cells, which recent work suggests as the putative cell of origin of ovarian cancer.

Research objectives and proposed outcomes

Background

High Grade Serous Ovarian Cancer (HGSOC) is the most common and most lethal ovarian malignancy and kills more than 4,000 women every year in the UK alone.

Early detection of ovarian cancer is hindered by the lack of both specific early symptoms and efficient screening tools. However, a significant breakthrough was achieved with the discovery that HGSOC originates from the fallopian tube (FT) epithelium: the pathway of normal FT cells, to acquiring a p53 signature, to serous tubal intraepithelial carcinoma (STIC), to HGSOC, is now well established.

FT organoids and transgenic mouse models have been developed to mimic this malignant transformation (1,2). These systems can successfully recapitulate some histologic and genomic features of HGSOC, but they have intrinsic limitations: they exclusively rely on the knockout of non-specific tumour suppressor genes (BRCA1/2, TP53, PTEN) and they do not take into account important physiological aspects of FT biology that could play a crucial “priming” role in tumorigenesis.

The most obvious is ovulation: factors that reduce the lifetime number of ovulatory cycles (such as parity, breast-feeding and oral contraceptive use) have been linked to a reduced risk of developing ovarian cancer. The rationale for this seems to be in the composition of the ovulatory follicular fluid; its reactive oxygen species, inflammatory cytokines and hormones can lead to tissue injury and DNA double strand breaks in the fimbrial epithelium (3,4,5).

The fimbrial section of the FT is the closest to the ovary and the most exposed to the follicular fluid; interestingly, this part of the FT is where STICs or precursor lesions are usually localised and is also enriched in tissue resident stem cells, which could represent the cell of origin for ovarian cancer (6).

Objectives and research plan

Our overall aim is to model early ovarian tumorigenesis using FT organoids in the most physiologically accurate way as possible; this model will then be used to identify biomarkers specific to early ovarian cancer.

We propose to achieve this goal by:

1) Establishing a FT organoid system that mimics the potentially carcinogenic effect of ovulation on the FT epithelium. Known components of the follicular fluid will be added to the culture media of FT organoids, with the expectation that this could potentially "prime" FT cells for malignant transformation. We will also explore the possibility of liaising with our colleagues in IVF to consent super-ovulating patients and obtain actual follicular fluid during egg harvesting.

2) Increasing the percentage of stem cells in the FT organoids by activating the WNT7- FRZD5 pathway, which we recently identified as a hallmark of FT stem cells (6).

3) Inducing the sequential KO of BRCA1/2, TP53 and PTEN in the primed, stem cell enriched FT organoids using an already optimised lentiviral CRISPR Cas9 system.

At each of the three stages, FT organoids will be characterised by measuring proliferation, multi-layering, changes in nuclear morphology and mitotic figures. Selected organoids will also undergo full genomic and transcriptomic characterisation using whole genome sequencing and RNAseq.

In order to have enough material to establish all the necessary FT organoids, we plan to recruit 30 patients with anticipated benign uterine conditions, who are awaiting hysterectomy with opportunistic salpingectomy. Patients will be recruited under the Gynaecological Oncology Targeted Therapy Study 01 (GOTarget 01, COREC reference number 11/SC/0014). Eligibility will be determined based on the following criteria:

Inclusion criteria:

1. Women aged ≥ 18 years.
2. Suspected benign uterine diagnosis requiring hysterectomy (e.g. fibroids).
3. Capacity to provide informed consent.

Exclusion criteria:

1. Patients with any current or previous malignant condition.
2. Patients with a history of, or current tubal disease.
3. Known carrier of BRCA1/2, PTEN, or TP53 mutations.
4. Untested individual with a family history of BRCA1/2, PTEN or TP53 mutations.
5. Previous salpingectomy.
6. Patients unable to give informed consent.

Translational Potential

Better survival rates for ovarian cancer patients will not be easily achieved unless we develop reliable and non-invasive screening tests for early detection.

We believe that this project will take us a step closer to such tests: in establishing a physiologically accurate FT organoid model which faithfully recapitulates HGSOC formation, it may be possible to study and characterise the earliest events of malignant transformation.

Transcriptome analysis of organoids could assist with the development of a protein or microRNA-based peripheral marker test. Additionally, optimised screening tests could advise which patients at risk of HGSOC should be for prophylactic bilateral salpingo-oophorectomy, but also salvage fertility by reducing unnecessary surgeries in those not at risk.

Training opportunities

The candidate will be encouraged to develop scientific independent thinking as well as ideas and hypotheses that could be tested experimentally. The Ahmed laboratory has a strong track record of collaboration over the last 10 years and established systems for co-supervision. The postdoctoral fellows and the PIs will provide daily support and supervision when needed. The student will learn established protocols for imaging, advanced sequencing, and genomic analysis from existing postdoctoral fellows. The candidate will be expected to participate in the weekly laboratory meetings and encouraged to present research at national and international meetings.

References

(1) Perets et al., 2013. Cancer Cell; (2) Lõhmussaar et al., 2020. Nature Communication; (3) Huang et al., 2015. Carcinogenesis
(4) Hsu et al., 2019. EBioMedicine; (5) Schildkraut et al., 1997. Journal of the National Cancer Institute; (6) Alsaadi et al., manuscript in submission

2. Using big data and AI to improve early cancer detection using UK Biobank[1,2,3,4] - Prof. Allen

Primary Supervisor: Prof. Naomi Allen
Additional Supervisors: Dr Ben Lacey
Eligibility: Track 1, 2, 3 and 4 students are eligible to apply for this project.

Lay Summary
When cancer is detected at the earliest stages, treatment is more effective and survival improves. Yet about half of cancers are still first detected at an advanced stage.1 Improved earlier detection of cancer could substantially increase survival rates. The latest research strategy from Cancer Research UK states that 'early detection and diagnosis is arguably the single most important way we can beat cancer'.2 Although recent advances in early detection have saved lives, further innovations and development of early cancer detection approaches are needed. General practitioners ('GPs') have a key role in diagnosing people with cancer, but this can be challenging because the initial signs and symptoms of many cancers are often vague and may be confused for other (often minor) conditions.3 This project will develop computer programmes to help GPs diagnose cancer at an earlier stage, using data from the UK Biobank study - a study of 500,000 people recruited across the UK.4 The very large amounts of data from electronic health records ('big data') in this study will be analysed using artificial intelligence ['AI'], and particularly machine learning (a type of AI that allows computers to learn and improve without specific instructions from researchers). The primary aim of this project is to assess whether the use of AI on big data from medical records might improve the early detection of cancer by GPs. It will do this by identifying those patients who (based on their medical record alone) are most likely to have cancer at an early stage. The secondary aim of this project is to assess whether there is any additional patient information that could be collected by GPs (such as genetics or the results of certain blood tests) that might further improve the performance of AI to detect cancer at an early stage.

Abstract of the project
Background: GPs have a key role in the early detection of cancer. However, diagnosis of cancer in primary care can often be challenging, in particular because the symptoms are often not specific to cancer and may be mistaken for other conditions. Some cancer types are more difficult to diagnose than others in primary care: for example, patients with lung, pancreatic and stomach cancer and myeloma are significantly more likely to pay multiple visits to their GP before being referred than patients with breast or endometrial cancer. Further adding to the complexity of cancer diagnosis is that common symptoms (such as stomach or back pain, unexplained weight loss, and indigestion) are frequently associated with more than one type of cancer. Delays in primary care can contribute to being diagnosed with cancer at a more advanced stage with potential effects on prognosis, intensity of treatment and quality of life. Numerous risk tools are now available to help clinicians predict risk of cancer.5 In theory, these tools have the potential to improve patient cancer outcomes through: enhancing the consistency and quality of clinical decision-making; facilitating equitable and cost-effective distribution of finite resources, such as screening tests or preventive interventions; and encouraging behaviour change. This project aims to improve the early detection of cancer in primary care using big data and artificial intelligence.

Methods: UK Biobank is a prospective cohort study with deep genetic and phenotypic data on approximately 500,000 individuals from across the United Kingdom, aged between 40 and 69 at recruitment. The open resource is unique in its size and scope. A rich variety of phenotypic and health-related information is available on each participant, including biological measurements, lifestyle indicators, blood and urinary biomarkers, and imaging scans of the body and brain. Detailed genomic data have also been collected on all participants, providing many opportunities for the discovery of how genetics affect health and disease. Follow-up information is provided by linking to longitudinal health records, including GP records for about half the cohort. This project will use UK Biobank data to develop a risk prediction tool for a range of tumour types, including (but not limited to) cancers that often present with vague, common and overlapping symptoms such as colorectal, gastro-oesophageal, lung, haematological, renal, pancreatic and ovarian cancer. The project will use advanced machine-learning methods and will initially use only GP data in UK Biobank. It will then explore the potential improvement in model performance of including other data collected by UK Biobank, such as genetic and blood-based biomarkers.

Research objectives and proposed outcomes
The main objectives are:

- Describe the distribution of late diagnosis by cancer type in UKB
- Assess the major determinants of late diagnosis by selected cancer type in UKB
- Develop risk prediction models for cancers using machine learning on UKB primary care data

- Assess the improvement in these models by adding genetic and other biomarker data in UKB
- Assess the potential impact of these risk prediction models on health inequalities

The proposed outcomes are:

- To develop risk prediction models to improve the early detection of cancer in primary care
- To assess whether enhanced data collection in primary care would improve these models
- To understand the impact of such models on health inequalities

Translational Potential

Risk prediction tools have the potential to improve cancer outcomes by enhancing the consistency and quality of clinical decision-making in referral for early detection of cancer; facilitating equitable and cost-effective distribution of finite resources such as screening tests or preventive interventions; and encouraging behaviour change. Although there are numerous risk tools available that predict either current or future risk of cancer, few use AI methods. Furthermore, to our knowledge, no study has directly compared risk prediction tools developed using routinely-collected primary care data with tools that use the depth of information collected by UK Biobank. In doing so, the project will assess what additional patient information might enhance cancer prediction in primary care.

Training opportunities

The student will work within the rich academic environment of the Big Data Institute, Nuffield Department of Population Health and affiliated institutions, gaining research experience and skills training in epidemiology and statistics, including machine learning. The successful candidate will have access UK Biobank and other large datasets of electronic health records. Candidates will receive professional mentorship through regular supervisory meetings, and acquire research skills by attending seminars and workshops. Candidates will work closely with other team members, and will have the opportunity to communicate their findings in international conferences and with the public.

References

1. Crosby D, Bhatia S, Brindle KM, et al. *Science* 2022; **375**(6586): eaay9040.
2. CRUK. Research Strategy: Making Discoveries, Driving Progress, Bringing Hope. 2022.
3. Emery JD, Shaw K, Williams B, et al. *Nat Rev Clin Oncol* 2014; **11**(1): 38-48.
4. Sudlow C, Gallacher J, Allen N, et al. *PLoS Med* 2015; **12**(3): e1001779.
5. Price S, Spencer A, Medina-Lara A, Hamilton W. *Br J Gen Pract* 2019; **69**(684): e437-e43.

3. Understanding the hepatic immune and molecular environment in the development of hepatocellular carcinoma.[1] - Dr. Barnes

Primary Supervisor: Dr Eleanor Barnes
Additional Supervisors: Dr. Emma Culver and Dr. Paul Klenerman
Eligibility: Track 1 students are eligible to apply for this project.

Lay Summary
Background: Cancer that begins in the liver (hepatocellular carcinoma) is one of the fastest rising and fourth commonest cause of deaths due to cancer world-wide. Liver cancer is usually associated with liver viral infections, alcohol and obesity, causing the immune system to attack the liver leading to scarring and liver nodules (liver cirrhosis). We know that changes throughout the cirrhotic liver tissue are the major risk factor for liver cancer, but we do not understand the nature of these changes, or why and when some people develop cancer and others do not. So far, little effort has been made to characterise these changes.

Aim and methods: We aim to better understand the changes within the liver that lead to cancer so that strategies for early cancer detection can be applied.

We plan to take a sample from the blood and the liver using a fine needle, from people at risk of getting liver cancer and those already with liver cancer. Fine needle sampling of the liver is considered safe and has been used to gather tissue cells for many years. We want to look at the cells in the liver to assess some of the material that is inherited (DNA) and look at chemical reactions that occur in the body that can influence how the DNA may interact with other molecules (methylation). We also want to look at the different immune cells (sequencing) and the proteins and chemicals (metabolome) in the blood and liver that have a role in inflammation and cancer development.

Outcomes: We believe that our approach will lead to a much better understanding of the biological reasons for the development of liver cancer. We hope also that in using the best combination of the most promising tests, we will be able to identify cancer at the very earliest stages so that people can be cured.

Abstract
This project builds on recent momentum at Oxford to develop strategies to detect hepatocellular carcinoma (HCC) early when curative therapies may be applied, underpinned by a CRUK HCC early detection award-DeLIVER. The incidence of HCC is increasing in the UK and is a leading cause of death globally (1). HCC usually occurs in the setting of advanced fibrosis/cirrhosis with a “field effect” change in the liver that predisposes to cancer transformation. Less often, HCC may arise de novo, particularly in fatty liver disease (FLD) (2). Factors that are known to be associated with the development of liver cirrhosis and HCC include viral infections (hepatitis B and C viruses), non-alcoholic fatty liver disease (NAFLD), alcohol induced liver disease and iron overload (haemochromatosis). Once cirrhosis is established approximately 1-4% of people/year develop HCC However, the biological pathways that lead to the development of HCC are not understood.

This proposal now focuses on understanding the changes within the liver, characterising in detail the field effect that leads to HCC transformation.

Recently, we have now established the ethical framework to recruit patients with HCC and those with cirrhosis at risk of developing HCC, with diverse underlying disease aetiologies. Now, fine needle aspiration (FNA) of non-cancerous liver in patients with and without HCC will be performed to identify the liver cellular phenotypes linked to the development of HCC. Cancerous liver tissue will also be obtained where possible in a new collaboration with the department of surgery. Blood samples from the same patients will be collected and analysed by collaborators to identify genetic/epigenetic mutations in cell free DNA from peripheral blood, and metabolites from blood and urine.

The overarching aim is to establish early data that will identify new methodologies that alone, or in combination, may be used for the early detection of HCC.

Research objectives and proposed outcomes

Although connections between some of the collaborators have been made through the DeLIVER award, this work focusing on the analysis of the liver tissue is new. The objectives are to:

a. Identify and extend a cohort of patients at Oxford with HCC (n=> 25) and recruit these patients into the DeLPHI study (Deep Liver Phenotyping): Ethics permission is granted, facilitating the use of liver fine needle aspiration (FNA) of non-cancerous liver tissue in patients with and without HCC.

b. Identify a cohort of patients with liver cirrhosis (n=25 of each disease aetiology; alcohol, NAFLD, viral hepatitis HCV/HBV), without HCC, for comparative analysis with the HCC patients.

c. FNA and flow cytometric analysis will be performed to develop immune profiles and single cell RNA transcriptomic maps (SC-RNA-seq) (3) to identify the predominant cell types (with functional and spatial resolution) in each patient (leads E Barnes/E Culver P Klenerman). Liver FNAs are considered safe and are an effective means for liver immune sampling (4) and this technique is being established as a source of sufficient liver tissue for single-cell analysis on the 10x Chromium platform within the Translational Gastroenterology Unit, where cells are isolated using protocols designed to either recover all cells or target resident lymphocytes.

d. Tumour and non-tumour liver tissue will be sought from patients having liver resections and biopsies where possible in a new collaboration with the department of surgery (collaborator Mike Silva)

e. Serum/plasma will be collected and processed for cell free (cf) DNA methylation profiling (C Song/B Schuster-Boeckler), and urine (with J Tomlinson) analysis by collaborators:

Translational Potential

This proposal has enormous translational potential. HCC is one of the fastest rising cancer types in the UK and a leading cause of death globally. Identifying biomarkers for the early detection of HCC could have enormous patient impact leading to new screening and treatment opportunities. Identifying the biological correlation of the transition from pre-cancer to cancer would support the development of new therapeutics. The project has a clear focus on understanding the liver pre-cancerous field effect-but blood analysis at the same time, means that blood markers that correlate with changes in the liver could be used in clinics for HCC early detection.

Training opportunities

This proposal is interdisciplinary, and the training opportunities are significant. These include:

a. The development of advanced clinical knowledge in hepatology and liver cancer
b. New knowledge development in immune and genetic risk in cancer development.
c. The development of new practical skills and tools including liver FNA, that will facilitate research in this proposal and also in next career steps.
d. Advanced flow-cytometry for multi-maker immune analysis using the Aurora platform to identify immune cell types in HCC and non-HCC livers.
e. Analysis of complex data sets -the focus of this may be tailored to suits the applicants interest, but could include sc-RNA seq analysis and the assessment of methylation, genetic or metabolomic profiles.
f. The integration of parametric data into new statistical models that can use for HCC early detection.

References

1. Global Burden of Disease Liver Cancer C, Akinyemiju T, Abera S, et al. The Burden of Primary Liver Cancer and Underlying Etiologies From 1990 to 2015 at the Global, Regional, and National Level: Results from the Global Burden of Disease Study 2015. *JAMA Oncol* 2017;3(12):1683-91.
2. Paradis V, Zalinski S, Chelbi E, et al. Hepatocellular carcinomas in patients with metabolic syndrome often develop without significant liver fibrosis: a pathological analysis. *Hepatology* 2009;49(3):851-9.
3. Stoeckius M, Hafemeister C, Stephenson W, et al. Simultaneous epitope and transcriptome measurement in single cells. *Nat Methods* 2017;14(9):865-68.
4. Gill US, Pallett LJ, Thomas N, et al. Fine needle aspirates comprehensively sample intrahepatic immunity. *Gut* 2018

4. Spatial resolution of the human transcriptome during gastrointestinal tumourigenesis [1,2,3,4] - Dr. Boccellato

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

Lay Summary
To predict the risk of cancer development in the gut, patients with symptoms are scanned with a camera inserted in the gut. Any area of the internal lining which does not look normal is further analysed by taking a small sample. Microscopic analysis of the sampled tissue might reveal changes in tissue shape. Some tissue changes are associated with a higher chance to develop cancer. The molecular signals that induce alterations in tissue shape in the gut are poorly understood. The aim of this project is to discover them. Small molecules and proteins that regulate the shape of tissues are called morphogens. Cells that can bind to those morphogens respond by either moving, dying, replicating or doing a specific job in digestion. Consequently, a change in the morphogens would change the configuration and the shape of the tissue.
We are using a novel technology that reveals the location of all the morphogens and all the responding cells within a microscopic image. This technology is called "spatial transcriptomics". We will use tests in cultures to verify that cells respond to the morphogens discovered with spatial transcriptomic. We predict that understanding the morphogens in the gut might have an important diagnostic value as the morphogens are altered before the tissue changes in shape. Moreover, some of these morphogens might play a role later on in cancer – e.g., by inducing uncontrolled cell proliferation. The knowledge that we generate will be useful to find new strategies for early intervention.

Abstract
The gastrointestinal mucosa is organised in invaginations called glands in the stomach and crypts in the colon. How cancer arises from these invaginations is still unclear, but before cellular transformation occurs, the analysis of early mucosal aberrations in biopsies enables the detection of pre-cancerous conditions. Tissue response to stress, toxic dietary compounds, infections and inflammation, might alter the microenvironment posing the mucosa at risk of malignant transformation. We plan to analyse pre-cancerous conditions of the gastrointestinal tract to understand why they have a different tissue configuration compared to the normal.
Growth factors and morphogens shape the tissues during embryogenesis, and they are probably responsible of its homeostasis in the adult. We hypothesize than an alteration of the morphogen signalling microenvironment is the driver for the altered configuration of the tissue observed in pre-cancerous conditions of the gastrointestinal tract. The project's main objective is to use spatial transcriptomics to map the molecular triggers dictating morphological and cellular composition changes in the gastro-intestinal diseased mucosa. We will focus on the detection of morphogens and growth factors involved in cellular regeneration and differentiation, and we plan to harness our established mucosoid cultures, an evolution of organoids, to test the different growth factors combinations in vitro.
We use fixed samples of gastrointestinal biopsies from healthy individuals and from patients with pre-cancerous conditions or lesions. We are particularly interested in the etiogenesis of oesophageal, stomach and colorectal cancer. Three patients per condition will be analysed using the GeoMX whole genome spatial transcriptomic profiling provided by NanoString®. Transcriptomic data from this experiment are analysed to extract information about the expression of morphogenic signals, their receptors and downstream target genes. The activity of morphogenic pathways is tested on mucosoid cultures using synthetic morphogens and corresponding pathway inhibitors. Upon stimulation with morphogens, cells in the mucosoids cultures can regenerate and differentiate into the different stomach lineages as they do inside the organ.
Mapping growth factors directly in the original human tissue and testing their function on relevant human-derived cultures promises to be a robust strategy to understand mechanisms of carcinogenesis

Research objectives and proposed outcomes
Aim1) Generating a spatial map of the morphogenic signals during gastrointestinal disease progression.
We will profile the transcriptome of different parallel regions of healthy and pre-cancerous gastrointestinal mucosa (eg: Barrett's oesophagus, intestinal metaplasia, colon polyps). Our clinical collaborator and co-supervisor Prof. Barbara Braden is involved in the identification of the patients for this project. Prof Fadi Issa, runs Nanostring at the department of surgical science at University of Oxford. We will focus on the detection of genes related to morphogen signalling pathways involved in cellular regeneration and differentiation. The expression of these genes, receptors and related

transcription factors will be mapped in the different region of interest of the epithelium, stroma and cell of the immune system. The comparative analysis of the transcriptomic profiles will be performed using the software "SignaLink3" [1] to identify the gene interactome and the putative pathways crosstalk. The software was developed by T. Korcsmaros, a collaborator in this project.

Aim II) Assessing the role of morphogens in driving epithelial differentiation, regeneration and proliferation.
The pre-cancerous conditions of the gastrointestinal mucosa are characterised by a different morphology but also by a disbalance in the cell population lineages. The morphogen signalling ligands identified in Aim 1 will be tested on mucosoid cultures originated from the same biopsies. Mucosoids are a patented [2] development of the organoid cultures; cells are cultivated in a monolayer forming an epithelial barrier which is very similar to the gastrointestinal epithelium [3, 4]. Cells within the mucosoids can differentiate upon stimulation [5]. By adding ligands or pathway inhibitors in the cultivation cocktail of the mucosoid cultures it is possible to determine their role in epithelial cell regeneration, proliferation and differentiation using different published functional or biochemical assays [3, 5]

Translational potential

Although there is a strong focus on understanding the microenvironment of cancer and the contribution of neighbouring non-transformed cells to the disease, little is known about the microenvironment of pre-cancerous conditions, and an unbiased approach to map all the morphogens has never been attempted. We aim to find dysregulations in specific morphogen signalling cascades that are predictive for disease progression. The gold standard for the detection pre-cancerous conditions is endoscopy and tissue imaging. Alternative serological analysis are accurate, but have a low sensitivity. A combination of ligands or proteins involved in morphogen signalling pathways could be use as surrogate of those conditions to develop diagnostic tests for pre-cancerous conditions and to predict risk of progression.

Training opportunities

Day-to-day supervision and training will be provided by Francesco Boccellato and from post-docs in the lab. The student will have the opportunity to learn cutting edge technologies such as spatial-transcriptomic and organoid and mucosoid cultures. We expect the student to become proficient into data analysis and we will support this by encouraging the attendance to bioinformatic courses. Imaging with confocal microscopies and standard biochemical assays are also part of the basic training.

References

1. Csabai, L.; Fazekas, D.; Kadlecsik, T.; Szalay-Bekő, M.; Bohár, B.; Madgwick, M.; Módos, D.; Ölbei, M.; Gul, L.; Sudhakar, P.; Kubisch, J.; Oyeyemi, O. J.; Liska, O.; Ari, E.; Hotzi, B.; Billes, V. A.; Molnár, E.; Földvári-Nagy, L.; Csályi, K.; Demeter, A.; Pápai, N.; Koltai, M.; Varga, M.; Lenti, K.; Farkas, I. J.; Türei, D.; Csermely, P.; Vellai, T.; Korcsmáros, T., SignaLink3: a multi-layered resource to uncover tissue-specific signaling networks. *Nucleic acids research* **2022,** 50, (D1), D701-d709.
2. Boccellato, F.; Meyer, T. F. Generation, proliferation and expansion of epithelial cells from primary tissue into mucosoid cultures. 2019, 2019.
3. Boccellato, F.; Woelffling, S.; Imai-Matsushima, A.; Sanchez, G.; Goosmann, C.; Schmid, M.; Berger, H.; Morey, P.; Denecke, C.; Ordemann, J.; Meyer, T. F., Polarised epithelial monolayers of the gastric mucosa reveal insights into mucosal homeostasis and defence against infection. *Gut* **2019,** 68, (3), 400-413.
4. Sepe, L. P.; Hartl, K.; Iftekhar, A.; Berger, H.; Kumar, N.; Goosmann, C.; Chopra, S.; Schmidt, S. C.; Gurumurthy, R. K.; Meyer, T. F.; Boccellato, F., Genotoxic Effect of Salmonella Paratyphi A Infection on Human Primary Gallbladder Cells. *mBio* **2020,** 11, (5).
5. Wölffling, S.; Daddi, A. A.; Imai-Matsushima, A.; Fritsche, K.; Goosmann, C.; Traulsen, J.; Lisle, R.; Schmid, M.; Reines-Benassar, M. D. M.; Pfannkuch, L.; Brinkmann, V.; Bornschein, J.; Malfertheiner, P.; Ordemann, J.; Link, A.; Meyer, T. F.; Boccellato, F., EGF and BMPs Govern Differentiation and Patterning in Human Gastric Glands. *Gastroenterology* **2021,** 161, (2), 623-636.e16.

5. Exploring the role of immune ageing upon tissue resident T cell memory and beta papillomavirus control in pre-malignant skin lesions [1,2,3] - Dr. Bottomley

Primary Supervisor: Dr. Matthew Bottomley
Additional Supervisors: Dr Graham Ogg
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Lay summary

Cancer in transplant patients

Cancer is a major cause of ill-health in organ transplant recipients. Skin cancer accounts for over half of all post-transplant malignancy. The commonest skin cancer, cutaneous squamous cell carcinoma (CSCC), is 200 times more frequent after transplant. This is partly due to the medication taken to prevent transplant rejection (immunosuppression) and may also relate to human papillomavirus (HPV). HPV is found at highest levels in pre-malignant skin lesions that can develop into CSCC. Immune cells in the skin (T cells) play an important role in controlling HPV.

Immune ageing and skin cancer

The immune system progressively changes as we age, becoming less effective at responding to new challenges, such as vaccination. We call this 'immune ageing' (immunosenescence). Kidney transplant recipients with blood markers of immunosenescence are more likely to develop more aggressive CSCC, due to alterations in the immune response to the cancer. We hypothesise age-related changes in T cell immunity to HPV in the skin may pre-date and predispose to the development of cancer.

Project outline

This project will assess the impact of immunosuppression and immunosenescence upon skin T cells responses to HPV in pre-malignant lesions. Kidney transplant recipients and non-immunosuppressed controls with evidence of pre-malignant skin lesions will be assessed for blood markers of immunosenescence. Patients will be divided into those with more and less advanced immune ageing. T cells from pre-malignant skin samples will be evaluated for changes in appearance and function, including use of a new approach to deep-profile cell behaviour in tissue sections. We will specifically gauge responses to HPV.

Impact on patient care

Understanding of the impact of immunosenescence in CSCC development may allow us to identify patients who will benefit from early interventions. It may identify new pathways we can target to reduce risk of progression to cancer.

Background

The incidence of cutaneous squamous cell carcinoma (SCC) is increasing worldwide and is the most common cancer in organ transplant recipients (1). In transplant recipients it exhibits increased aggression and metastasis risk and is frequently recurrent, leading to significant morbidity and mortality. Understanding novel risk factors for SCC may facilitate early intervention to prevent transformation from premalignant lesions, benefitting both the general population and patients who are immunosuppressed. Beta-genus HPV infection may increase risk of SCC (2): it has been suggested that the virus acts in a 'hit and run' fashion to modulate early oncogenic changes, based on data showing that viral levels are highest in pre-malignant lesions. However, no single strain has been consistently implicated and risk may relate more to overall viral load. Recent data suggests T cell responses to HPV may be cross-protective against SCC (3).

We have previously shown that immune ageing (immunosenescence) is associated with enhanced SCC risk and poorer outcomes in kidney transplant recipients (4). Our recent spatial transcriptomic evaluation of the immune infiltrate around post-transplant SCC demonstrated that immunosenescence synergises with immunosuppression leading to a failure of CD8+ T cell infiltration, associated with expansion of M2-like macrophages in the peritumour milieu, leading to suppression of T cell responses (Bottomley et al, manuscript in draft).

Research objectives

We hypothesise that these changes are also present in pre-malignant lesions and leads to a failure of HPV control with advancing immunosenescence. The proposed project aims to evaluate this hypothesis by assessing T cell responses against βPV in the setting of (a) immunosuppression and (b) immunosenescence.

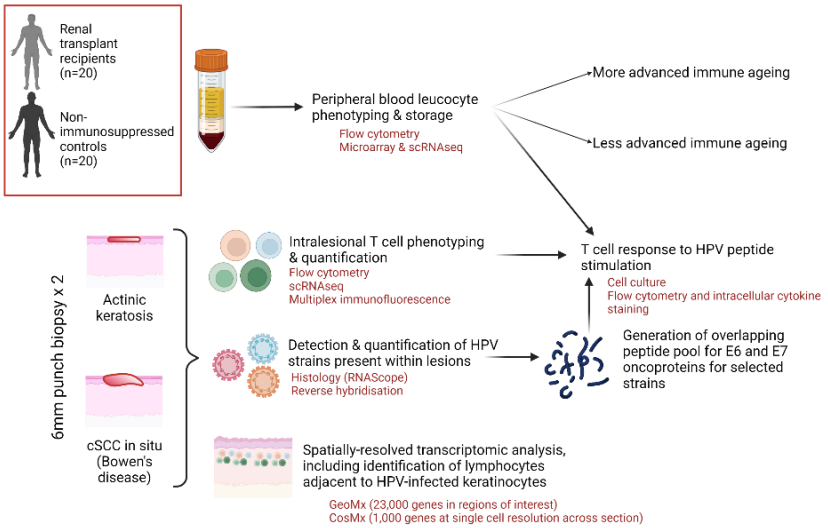


Figure 1: Summary of DPhil plan of work

This project will utilise a case-control design and will analyse peripheral blood and biopsies of pre-malignant skin lesions (actinic keratoses and SCC in situ [Bowen's disease]) taken from transplant recipients (cases) and non-immunosuppressed controls (study design summarised in Figure 1). Participants will be stratified based on markers of peripheral blood immunosenescence. Cutaneous leucocyte populations will be evaluated by a combination of flow cytometry and multiplex immunofluorescence and will be compared to peripheral blood populations. βPV strains present within the lesion will be identified and quantified by reverse hybridisation assay of skin and RNAscope of sections, respectively. Peptide pools from the E6 and E7 proteins, associated with oncogenic activity (2), from HPV strains present within the lesion will be generated and used to assess T cell function, including generation of clonal populations from T cells present within biopsies. Spatial transcriptomic analysis using the Nanostring GeoMx (of areas of interest) combined with the next-generation CosMx (allowing focused analysis of single cells of interest) platforms will allow identification of T cells in proximity to virally-infected keratinocytes in situ in lesional sections to validate in vitro and ex vivo findings.

Translational potential

Cancer-related morbidity and mortality is significantly over-represented in immunosuppressed populations. Despite their high risk, there is little in the way of risk stratification in widespread clinical use. This project will extend our previous findings to the premalignant stage and will contribute to a body of evidence that will form the basis for a future interventional trial using our risk marker. Furthermore, this study will assist in disentangling the conflicting evidence regarding the role of beta-genus HPV in cutaneous carcinogenesis. Single cell analysis of infected cells, using a combination of scRNAseq and CosMx spatial single-cell profiling, may reveal novel pathways that can be manipulated therapeutically to retard HPV replication and infection. Importantly, the findings of this project may guide risk stratification in other immunosuppressed populations

Training opportunities

Where appropriate (medical undergraduate or graduate) the student will receive training in Good Clinical Practice principles and the conduct of observational studies. From a laboratory perspective, flow cytometry, cell expansion and sorting as well as functional cellular assays will be utilised throughout the study to assess T cell phenotype and function. Hands-on immunology experience combined with training in RNA-seq and spatial transcriptomics will provide the student with experience in bioinformatic analysis. The student will have full access to the facilities, expertise, and resources available within CAMS-COI and across the broader community at the University of Oxford. By the end of the project, the candidate will be in a strong position to drive fundamental and translational clinical research in cutaneous immuno-oncology.

References

1. Madeleine MM, Patel NS, Plasmeijer EI, Engels EA, Bouwes Bavinck JN, Toland AE, et al. Epidemiology of Keratinocyte Carcinomas after Organ Transplantation. Br J Dermatol (2017) 177(5):1208-16. Epub 20171010. doi: 10.1111/bjd.15931. 2. Bandolin L, Borsetto D, Fussey J, Da Mosto MC, Nicolai P, Menegaldo A, et al. Beta Human Papillomaviruses Infection and Skin Carcinogenesis. Rev Med Virol (2020) 30(4):e2104. Epub 20200330. doi: 10.1002/rmv.2104. 3. Strickley JD, Messerschmidt JL, Awad ME, Li T, Hasegawa T, Ha DT, et al. Immunity to Commensal Papillomaviruses Protects against Skin Cancer. Nature (2019) 575(7783):519-22. Epub 20191030. doi: 10.1038/s41586-019-1719-9. 4. Bottomley MJ, Harden PN, Wood KJ. Cd8+ Immunosenescence Predicts Post-Transplant Cutaneous Squamous Cell Carcinoma in High-Risk Patients. J Am Soc Nephrol (2016) 27(5):1505-15. Epub 20151112. doi: 10.1681/ASN.2015030250.

6. Harnessing measurements of tumour metabolism and proliferation to improve the early detection of prostate cancer [1,3,4]– Prof. Bryant

Primary Supervisor: Prof. Richard Bryant
Additional Supervisors: Prof. Damian Tyler
Eligibility: Track 1,3 and 4 students are eligible to apply for this project.

Lay Summary

Men investigated for possible prostate cancer currently receive imaging of the prostate gland in the form of a magnetic resonance imaging (MRI) scan, followed by a needle-based prostate biopsy. The biopsy procedure is an invasive process, with potential complications including infection and bleeding. Whilst the adoption of prostate MRI scans has been a significant advance in clinical care over the last decade, not all clinically significant prostate cancers (i.e. tumours that require treatment) are visible on the MRI scan. Research has demonstrated that between 5 and 15% of men with an elevated prostate cancer blood test (i.e. abnormal Prostate Specific Antigen, PSA, test) but a 'normal' MRI scan have a 'falsely negative' MRI result. These patients may choose to avoid biopsy based on the MRI result, thus leading to potential under-diagnosis, or delayed diagnosis, of significant cancers, with worse clinical outcomes for those patients. Conversely, some MRI-detected abnormalities are not due to prostate cancer, leading to unnecessary biopsies and resultant side-effects in men without prostate cancer. It would therefore be a significant advance in the field to more accurately determine which men being investigated for possible prostate cancer definitely need a biopsy, and which men could perhaps safely avoid biopsy, based on advances in prostate Magnetic Resoannce Imaging (MRI).

This DPhil project focuses on the use of traditional and advanced MRI to aid the early diagnosis of prostate cancer. Advanced MRI, in particular 'Restricted Spectrum Imaging' (RSI) and 'hyperpolarised MRI', are new ways to image prostate cancer, and offer new opportunities to improve our ability to accurately detect early prostate cancer.

The DPhil student employed for this project will work with radiological and surgical research teams to apply these new imaging approaches to men undergoing investigation for prostate cancer. The project would aim to improve early detection of this cancer. Imaging results will be compared to biopsy findings, and results used to enhance the clinical pathway for diagnosing prostate cancer. If successful, these new MRI approaches will be further adopted into clinical practice to aid in early prostate cancer detection.

Abstract

Approximately 190,000 invasive prostate biopsy procedures are performed each year in the UK alone. The current standard of care recommends the use of pre-biopsy MRI scan to improve the accuracy of the prostate biopsy. Despite the success of using the '*Prostate Imaging Reporting And Data System'* (PI-RADS) (*1*) score (and similar scores such as the Likert score) in the reporting of MRI scan images ahead of targeted prostate biopsy, around 5-15% of clinically significant cancers would potentially be missed if individuals with an elevated age-specific PSA and normal clinical imaging did not receive a prostate biopsy (*2*). It is difficult to derive precise figures, but this would likely amount to several thousand men with a delayed or missed diagnosis of clinically significant prostate cancer each year in the UK alone, the clinical impact of which is currently unknown.

Whilst changes in imaging contrast due to tumour growth tend to be slow, primarily due to the inherently low sensitivity of clinical MRI, alterations in the motion of water (due to the restriction of motion by highly cellular regions of cancerous tissue) and the direct metabolic reprograming of cancerous cells to upregulate production of lactate to fuel proliferation (known as the "Warburg Effect") can be harnessed to potentially provide early detection of prostate cancer (*3*). Indeed, the first in-human trial using hyperpolarised MRI demonstrated the ability of the technique to detect oncological metabolism in the absence of conventional imaging alterations (see **Figure 1** showing cancerous metabolism in both the left and right sides of the prostate, with conventional imaging showing changes only on the right side, data from (*4*)), and Restricted Spectrum Imaging (RSI) has been shown to be highly sensitivity to changes in water motion in prostate cancer (*5*).

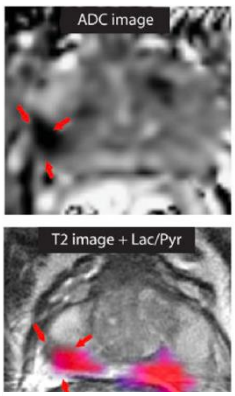


Figure 2

We have experience in advanced imaging techniques to study neurological and cardiovascular disease, and now is an opportune time to expand these techniques to benefit the oncological arena, this being an area of important unmet clinical need for prostate cancer.

The DPhil student will work with the surgical and radiological teams to recruit and image a cohort of 40 men aged between 60-69 years (as defined by power calculations from previous studies in Oxford) being investigated for possible prostate cancer. Participants will receive standard of care MRI imaging, with the functional MRI appended to the end of the clinical session. The biopsy process will be performed based on the clinical imaging, as per our current practice. Of the N = 40 men in this pilot study, 10 individuals will have a normal (PIRADS 1-2) pre-biopsy MRI, 10

individuals will have an equivocal (PIRADS 3) MRI, and 20 will have a 'suspicious' (PIRADS 4-5) pre-biopsy MRI. The inclusion of patients with either a PIRADS 1-2 or a PIRADS 3 MRI is important given that individuals in the age range of 60-69 years often have diffuse PIRADS 3 change within the peripheral zone of the prostate gland due to their young age. All 40 individuals will undergo prostate biopsy following the performance of the clinical and functional scans, with targeted and systematic biopsy cores being obtained for the N = 30 with clinical lesions (PIRADS 3-5), and systematic biopsies alone being obtained for men with a normal (PIRADS 1-2) clinical imaging, as per our current protocol. The clinical and functional MR images will then be correlated with the final prostate biopsy pathology, to test the hypothesis that some clinically significant prostate cancer cases may solely be visible on functional MRI and not on standard clinical imaging. This approach will improve sensitivity of detection – with RSI acquisitions, if shown to be beneficial in the detection of prostate cancer, ready to be run in clinical practice in every patient in our NHS trust, and beyond, and hyperpolarised MRI offered as a clinical scan in targeted patients at a later time point (2-5 years). In turn, this will benefit almost every patient being investigated for possible prostate cancer using MRI scans as part of the clinical pathway.

We currently have an ethics application going through internal review to enable this project. See **figure 2** for the workflow for this project.

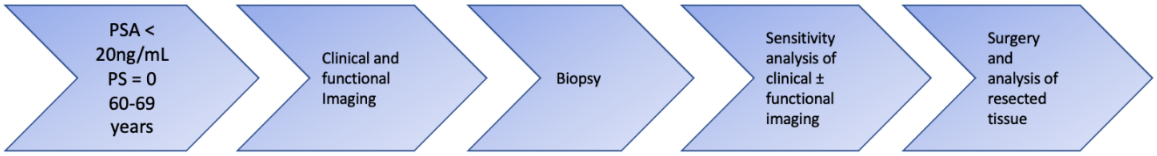


Figure 3

Research Objectives

The two key objectives for this DPhil project are:

1) To establish repeatable imaging protocols for assessing functional and clinical MRI within the prostate. A sub-cohort (N = 20, 5 from each PI-RADS group) of patients and healthy controls (N = 5) will undergo test re-test imaging on the same day in OCMR to assess for the repeatability of imaging results. **Collaborators required:** Dr James Grist, Dr Ruth MacPherson, Professor Damian Tyler. **Outcome:** Presentation of results at leading radiological conferences (European Congress of Radiology, International Society of Magnetic Resonance in Medicine), and publication of results in a leading medical imaging journal.
2) To perform a clinical study assessing the added value of functional MRI to the routine clinical protocol. The data from the full cohort of patients will be analysed to assess for the sensitivity and specificity of the clinical, functional, and combined imaging approaches for the detection of biopsy proven clinically significant prostate cancer. **Collaborators required:** Dr James Grist, Dr Ruth MacPherson, Dr Richard Colling, and Professor Ian Mills. **Outcome:** Presentation of results at leading radiological conference (Radiological Society of North America) and publication of results in a leading medical journal (for example, New England Journal of Medicine)

Translational potential

If successful, this project will enable the early detection of clinically significant prostate cancer in a cohort of patients undergoing investigation for possible prostate cancer, who might otherwise have a delayed or missed diagnosis. However, the further clinical translation and impact of this project will be felt from the incorporation of these additional functional MRI approaches into clinical practice in Oxford and beyond – thus benefitting a larger population of patients with suspected prostate cancer. However, beyond this impact there is also the potential to apply these advanced imaging methods to other cancers, to detect early therapeutic response to chemo/radiotherapy – for example paediatric brain tumours, renal cancer, and breast cancer.

Training opportunities

The student will be provided with training in clinical research and patient recruitment by Professor Richard Bryant, and will be provided with training from Professor Damian Tyler and Dr James Grist in MRI physics, data acquisition, image reconstruction and quantitative post-processing for both hyperpolarised MRI and RSI. Further training in image co-registration will be provided. Dr Ruth MacPherson will provide training in clinical image analysis and interpretation, and Dr Richard Colling will provide training in histology and will work with Professor Ian Mills to complement this with molecular phenotyping of cancerous tissue.

By the end of this DPhil project, the student will have a wide-ranging skill set that will be of great benefit to further their career in clinical research.

References 1. S. Y. Park *et al.*, *Radiology*. **280**, 108–116 (2016).2. S. S. Salami *et al.*, *JCO Precis. Oncol.*, 1–12 (2019).3. F. Zaccagna *et al.*, *Br. J. Radiol.* **91**, 20170688 (2018).4. S. J. Nelson *et al.*, *Sci Transl Med*. **487**, 109–113 (2013).5. R. L. Brunsing *et al.*, *J. Magn. Reson. Imaging*. **45**, 323–336 (2017).

7. Exploring the role of human Paneth cells in intestinal homeostasis and cancer [1,2,3,4] – Dr Buczacki

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

Lay Summary

The intestine consists of the small bowel that joins the large bowel. These two organs consist of very similar cell types. However, Paneth cells, whilst found throughout the small bowel, are rarely seen in the large bowel or bowel cancers. Paneth cells fight bacteria and support stem cell function by secreting growth factors. For unknown reasons, human bowel cancer almost never occurs in the small bowel. However, in mouse models of bowel cancer, tumours arise in both bowels. We have found mouse Paneth cells secrete high levels of key proteins that support tumour growth, whereas human Paneth cells do not. We suggest, therefore, that Paneth cells might be a key regulator of human bowel cancer development. Developing drugs that mimic Paneth cell behaviour could act as preventive medicines to decrease chances of bowel cancer developing in at-risk individuals.

We have also found that two types of rare Paneth-like cells can be found in bowel cancers, and these are strongly associated with the immune response against the cancer. An enhanced immune response to cancer improves cancer patient survival, and drugs that boost this response – known as immunotherapy - can cause some bowel cancers to melt away. Developing drugs that boost Paneth cell formation in bowel cancers could lead both to an enhanced immune response and better responsiveness to immunotherapy.

Our group has identified two proteins that, when altered, cause Paneth-like cells to form at high levels in bowel cancers and in the human large bowel. This project will explore these preliminary findings to investigate further the role of Paneth cells in the control of bowel cancer formation, and understand how Paneth cells in bowel cancers interact with other non-cancerous cells, such as immune cells. The project may provide advances in therapeutic strategies for both the prevention and treatment of bowel cancer.

Abstract

Paneth cells are rarely found in the human and mouse colon but are known to play an important role in stem cell maintenance in the mouse small intestine. Mouse small intestinal Paneth cells are known to secrete Wnt ligands supporting intestinal stem cells however emerging data suggests that human small
intestinal Paneth cells do not have this function. Strikingly, Apc mutant mouse models and the human corollary, familial adenomatous polyposis have different phenotypes in the small and large intestine. We hypothesise this may occur because of an interaction between basal Wnt gradients and the behaviour/presence of Paneth cells. We have also recently identified that good prognosis colorectal cancer is characterised by both a high lymph node yield and a high immune cell infiltrate that is independent of subtype status (Lal et al. 2022). Molecular interrogation of these tumours has surprisingly shown the presence of subsets of Paneth-like cells within them (Fig 1). This project will use state-of-the-art technologies to explore the nature of human Paneth-like cells in intestinal homeostasis and tumorigenesis. Using established protocols, the project will leverage our ready access to human intestinal surgical tissue to isolate and characterise Paneth-like cells from the human small and large intestine. This project will generate new findings characterising a novel cell-type hitherto little explored.

Research objectives

WP1 – Characterise the molecular landscape of Paneth-like cells from normal human colon and tumours. Accessing datasets and protocols present within the group, the student will learn the techniques of processing human tissue samples for fluorescence activated cell-sorting (FACS) analysis to establish a sorting strategy to isolate and molecularly interrogate Paneth and Paneth-like cells from the human small intestine, colon and colorectal cancers. Candidate cell-surface markers specific for Paneth-like cells will be tested and validated. Isolated Paneth-like cells from normal and malignant tissue will be analysed using advanced microscopy, next-generation sequencing and proteomics to identify molecular characteristics and generate hypotheses about function. Data generated will be compared to pre-existing public single cell datasets.

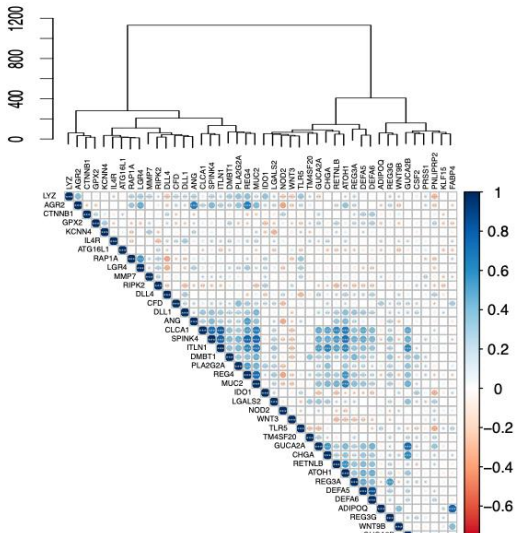


Figure 1. Correlation heat map identifying clusters of Paneth cell gene expression in colorectal cancers

WP2 – Exploring the role of Paneth-like cells during tumour initiation. To explore the hypothesis that Paneth and Paneth-like cells may regulate tumour initiation, normal colonic and small intestinal organoids will be generated and then using established CRISPR gene-engineering strategies targeted to either eliminate or enhance Paneth-like cells in their cellular makeup. Sequential introduction of driver mutations will then be performed in control and experimental organoids. Organoids will be quantified for growth rates, mutation fixation rates and cellular kinetics. In collaboration with Prof. Helen Byrne and Prof. Simon Leedham, data generated will be combined with mathematical modelling and comparative mouse organoid models to identify the relative contribution of Paneth and Paneth-like cells to tumour initiation between mouse and human.

WP3 – Quantify the interactions and behaviour of Paneth-like cells through co-cultures of colon and colorectal tumour organoids with non-epithelial cells. Having identified specific markers of Paneth-like cells from WP1, normal and tumour organoids will be quantified for Paneth-like cell presence and behaviour under homeostatic and injury environments using live cell microscopy, single-cell RNAseq and co-cultures with the different cellular components of the normal and tumour microenvironment.

Translational potential

Colorectal cancer is a common malignancy with significant mortality (~17K/annum in the UK). Despite huge investment of resources in the identification and development of precision chemotherapeutics there have been surprisingly few drugs that have made a significant advance for patients with bowel cancer. Preliminary data from our group suggest that Paneth-like cells may play a significant role in both tumour initiation and the variable host-tumour immune response seen in colorectal cancer. This project will identify novel biomarkers and therapeutic strategies that have the potential to make advances in the care of patients with colorectal cancer.

Training opportunities

The Buczacki group has a strong focus on personal development and is open and harmonious with a flat hierarchy. This CRUK/Pharsalia Trust funded group is housed in the Department of Oncology on the Old Road Campus and we mostly work on the link between stem cell biology and tumour evolution. This project will arm the student with many wet and dry lab skills to assist their development into a successful modern scientist through learning the process of hypothesis driven research. All students are closely supervised by the PI and supported in acquiring lab skills by members of the wider group. The Buczacki group is fortunate in having an experienced in-house bioinformatician who will help train the student in contemporary bioinformatic techniques. Collaborators (Leedham and Byrne) will provide exposure to complex mathematical modelling and an understanding of murine models.

Wet lab: Human tissue processing, organoid culture (normal and tumour), CRISPR-Cas9 gene engineering, NGS (RNAseq,scRNAseq), RT-PCR, western blotting, Sanger sequencing, flow cytometry and microscopy (confocal, live cell and EM).

Dry lab: Modelling, transcriptomic analysis, coding and big data handling.

References:

Lal, N., Chan, D. K. H., Ng, M. E., Vermeulen, L. & Buczacki, S. J. A. (2022). Primary tumour immune response and lymph node yields in colon cancer. Br J Cancer, 126, 1178-1185.

Buczacki, S. J., Zecchini, H. I., Nicholson, A. M. et al. (2013). Intestinal label-retaining cells are secretory precursors expressing Lgr5. Nature, 495, 65-9.

Sato, T., Van Es, J. H., Snippert, H. J. et al. (2011). Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. Nature, 469, 415-8.

8. Spatial modelling and quantification of T Cell exhaustion in the tumour microenvironment of oesophageal cancer [4] – Prof. Byrne

Primary Supervisor: Prof. Helen Byrne
Additional Supervisors: Prof. Tim Elliot
Eligibility: Track 4 students are eligible to apply for this project.

Lay Summary

Oesophageal cancer is the 7th most prevalent cancer in the world: it is responsible for approximately 500,000 deaths per year, including 8,000 in the UK. Patients with oesophageal cancer have an overall 5-year survival of only 17%, and treatment options are limited. The aim of this project is to develop new mathematical approaches to identify which immune cells are present in tissue samples from oesophageal cancer patients, where the immune cells are located, and with which neighbouring cells they communicate. This is important because immunotherapy of oesophageal cancer is not yet very effective, and we think that is because the beneficial effects of killer T cells, a type of immune cell, are being neutralised in the tumour by other, immunosuppressive cells. Simply counting the numbers of these different types of cells in tumour biopsies does not provide enough information to predict whether a patient is likely to respond to immunotherapy: we need a new way to approach the question. We think that the answer lies in the composition of the cellular neighbourhood, or environment, within which potentially beneficial killer T cells are located. Further, new mathematical tools will enable us to classify a patient's cancer according to its so-called "spatial phenotype" score. This score will be used in the future to improve the accuracy of diagnoses and help physicians to decide on the best course of treatment for a given patient quickly following diagnosis.

Abstract

There is growing evidence that increased levels of tumour-infiltrating T cells and their colocalisation with immunosuppressive cancer associated fibroblasts (CAFs) contribute to poor clinical outcomes for patients with oesophageal adenocarcinoma (OAC). In this project we will combine computational modelling with spatial analysis of multiplexed images of OAC in order to understand how the spatial distributions of different T cell populations and CAFs influence the effectiveness of the immune response to OAC. We will develop a hybrid, agent-based model (ABM) that simulates interactions between critical cell populations in OAC, extending and specialising an existing ABM of tumour-immune interactions [2]. We will use the ABM to understand the mechanisms by which regulatory T cells and CAFs suppress the cytotoxic activity of CD8+ T cells in OAC. We will use spatial statistics to describe and quantify the spatial patterns that the ABM generates, and the patterns observed in multiplexed immunohistochemistry (mIHC) images of OAC. We will compare ABM outputs with these images to identify spatial patterns, and associated metrics, which will improve diagnostics, prognostics, and immunotherapeutic strategies.

Research objectives

Background. Recently, immunotherapy of OAC with immune checkpoint inhibitors has been shown to improve depth and durability of therapeutic responses for a significant minority of treated patients [1]. Successful control and elimination of a cancer by the immune system requires the trafficking and infiltration of activated tumour antigen specific cytotoxic T lymphocytes into tumours, followed by recognition and killing of cancer cells, as described in the cancer-immunity cycle [2]. This process can be disrupted at multiple points, leading to breakdown and prevention of effective lymphocyte-driven destruction of cancer. Prominent among these is the influence of a suppressive microenvironment that can inhibit tumour-specific, activated, cytotoxic CD8+ T lymphocytes from expanding, migrating to the tumour, and killing cancer cells; and the impact of lymphocyte exhaustion. These immunological "checkpoints" prevent an anti-tumour response from developing and frustrate immunotherapeutic activation of the anti-tumour response. Thus, identifying the mechanisms underlying primary resistance to targeted immunotherapies is vital for progress. Multiple immunosuppressive networks have been described, including interactions with regulatory T cells (T-regs) and cancer associated fibroblasts (CAFs). In OAC, bulk counts of T cell infiltration correlate poorly with clinical outcomes, and CAFs are strongly implicated in cytotoxic T cell suppression. Together, these observations suggest that spatial relationships between different types of T cells and CAFs are important for understanding immunosuppression in OAC. The aim of this project is to analyse and quantify spatial domains involving immune cells from patients with OAC in order to develop new metrics, based on spatial statistics, that will improve diagnostics, prognostics, and immunotherapeutic strategies.

Objectives. The main project objectives are to develop a hybrid ABM that simulates interactions between CAFs, T-regs, and CD8+/CD4+ T cells in OAC, and then to use it to understand i) how crosstalk between these cells drives

immunosuppression, and ii) how disruption of specific immunological checkpoints influences the observed spatial patterns and immunosuppressive behaviour. We will use spatial statistics to compare model outputs with mIHC (Vectra) images of OAC and identify spatial patterns prognostic for immune suppression. Images will be provided by the Elliott lab using bespoke panels optimised for analysis of T cell functional (and exhaustion) subsets.
Approaches. We will develop a spatially-resolved, multiscale computational model describing the in vivo growth of OAC and its interactions with CAFs and different T cell subtypes, adapting an existing model [3]. Subcellular variables will represent each T cell's level of exhaustion as a continuous value, which will determine its efficacy for killing OAC cells. In turn, T cell exhaustion will be altered through interactions with CAFs and T regs and immunotherapy.
Simulation outputs will be described quantitatively using a suite of spatial statistical analysis tools. These tools will also be applied to mIHC images of OAC, generated by the Elliott lab, with an existing panel optimised for CD8+ subsets, Tregs and CAFs. Direct quantitative comparison will therefore be possible between biomedical images and ABM simulations. We will identify combinations of spatial statistics [3,4,5] which distinguish between simulations generated via different parameters, and apply these to IHC samples to predict patient outcomes and responses to therapy.
Proposed outcomes. The project will deliver a versatile multiscale computational model that simulates interactions between immune cell subsets and OAC, and that generates synthetic spatial data for comparison with mIHC images. The model will provide new mechanistic understanding of processes driving T cell exhaustion and immunosuppression within OAC and also serve as a tool for identifying potential new immunotherapies. By comparing model outcomes with mIHC data, we will identify statistical descriptions of cell colocalization which act as imaging biomarkers and which can distinguish patients who would benefit from immunotherapeutic treatments from those who would not.
Academic Value of the Research and Funding Justification: Funding for a DPhil student will enable us to take the very significant next step forward in transforming our understanding from a fundamental experimental level into a clinically viable application. This will involve addressing a number of new challenges, and require a multidisciplinary team working together closely. The funds will cover the costs of a graduate student who will carry out research in the MI.
Collaborations: This project will initiate a new collaboration between Professor Byrne's group at the MI and Professor Elliott's lab at the NDM. The student will make frequent visits to the Elliott lab where they will interact with lab-based students and postdocs investigating mechanisms of immunotherapeutic T cell activation and suppression. This will allow the student to learn the relevant tumour biology while contributing to experimental design. PI Byrne and Collaborator Bull will meet weekly with the student, while the entire multidisciplinary team will meet monthly.

Translational potential

This project aligns with the Cancer Big Data and Immuno-Oncology themes, of which Elliott and Byrne are Cancer Centre Theme Leaders. It will demonstrate how multidisciplinary working can accelerate research. It will allow us to suggest enhancements of prognostic, diagnostic and immunotherapeutic strategies based on cell phenotype and spatial measurements in the tumour micro-environment and how to modify the micro-environment to enhance treatment efficacy.

Training opportunities

The student will be trained in mechanistic modelling using differential equations, hybrid agent-based models and related computational techniques, and also in spatial statistics and image analysis. The student will attend relevant Masters-level lectures and join the WCMB's large mathematical oncology group), which has weekly group meetings during term. The WCMB also meets weekly for research skills training sessions, and to present research, and formal weekly seminars are given by external expert speakers. The student will also join the Elliott lab for meetings. In this way, the student will receive a broad training in mathematical modelling in immune-oncology and its clinical implications, and methods for analysing multiplexed histology images of biological tissues.

References

[1] RJ Kelly et al (2021). New England J Medicine 384: 1191-1203. DOI: 10.1056/NEJMoa2032125
[2] DS Chen, I Mellman (2013). *Immunity* 39. DOI: 10.1016/j.immuni.2013.07.012
[3] JA Bull and HM Byrne (2022). *bioRxiv preprint* 2022.05.26.493564. DOI: 10.1101/2022.05.26.493564
[4] JA Bull et al. (2020). Scientific Reports 10: 18624. DOI: 10.1038/s41598-020-75180-9
[5] O Vipond et al (2021). PNAS 118 (41): e2102166118. DOI: 10.1073/pnas.2102166118

9. Understanding STING regulation in cancer and the crucial role of ubiquitination in the ER [1,3] – Prof. Christianson

Primary Supervisor: Prof. John Christianson
Additional Supervisors: Dr. Eileen Parkes
Eligibility: Track 1 and 3 students are eligible to apply for this project.

Lay Summary
In order for cancers to grow and spread, they need to avoid activating immune responses which would eradicate the cancer. Some cancers appear to do this by hijacking an immune pathway, the central hub of which is a protein called STING. This STING pathway normally activates in response to bacterial or viral infection, and is needed for normal immune activity. However, in cancer, this STING pathway instead appears to help cancers to grow and spread. At the moment, we don't know exactly how STING changes its behaviour in cancer. We know that the location of proteins within the cell can change their behaviour and function. Previously, we have found a mechanism which controls the movement of STING within the cell and subsequently how well STING can activate an immune response. How STING moves within the cell could be key in controlling STING activation in cancer, and understanding this could identify new ways to target STING in cancer. In this project, we want to build on our previous findings and further investigate how the cellular location of STING, and therefore its activity, is controlled in cancer. This could identify new ways to restore the immune-activating function of STING and uncover a new strategy for immune targeting in cancer as a future anti-cancer treatment.

Abstract
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-labelling 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 signalling. 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 Signalling By S-Phase-Specific DNA Damage in Breast Cancer. J Natl Cancer Inst . 2017;109(1). doi:10.1093/jnci/djw199.

10. Genetic and functional characteristics of novel immune escape mutations in DNA mismatch repair deficient cancer [1,3,4] – Dr. Church

Primary Supervisor: Dr. David Church
Additional Supervisors: Prof. Tim Elliot, Dr Nicole Ternette
Eligibility: Track 1, 3 and 4 students are eligible to apply for this project.

Lay Summary
Cancer arises as a result of errors in DNA which in turn cause abnormal proteins. While these abnormal proteins drive cancer growth, they also represent a vulnerability if they are recognised by the immune system, as this often results in killing of the cancer cells. Consequently, cancers use lots of ways to escape from immune destruction, including inactivating the ways by which abnormal proteins are 'shown' to the immune system, and switching off the killing function of immune cells themselves. Drugs targeting the latter have proven very effective against some cancer types, though many patients fail to benefit. We have recently identified two new alterations in cancer, which may help cancers escape immune destruction, and potentially from the effects of such immune-targeting drugs. We propose to test this in a collaborative project spanning several groups using different methods. If successful, our project will refine use of these expensive and potentially harmful agents, and may identify novel strategies to treat cancers carrying these alterations.

Abstract
Defects in DNA mismatch repair (dMMR) occur in many cancer types, where they cause increased tumour mutation burden (TMB), microsatellite instability (MSI) and enhanced immune response1. However, the acquisition of immune escape mutations enables cancers to elude immune destruction, and become resistant to immunotherapy. Our unpublished analysis of cancers from the Genomics England 100,000 Genomes Project (100KGP) has identified two novel candidate immune escape mutations which occur commonly in dMMR cancers. 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

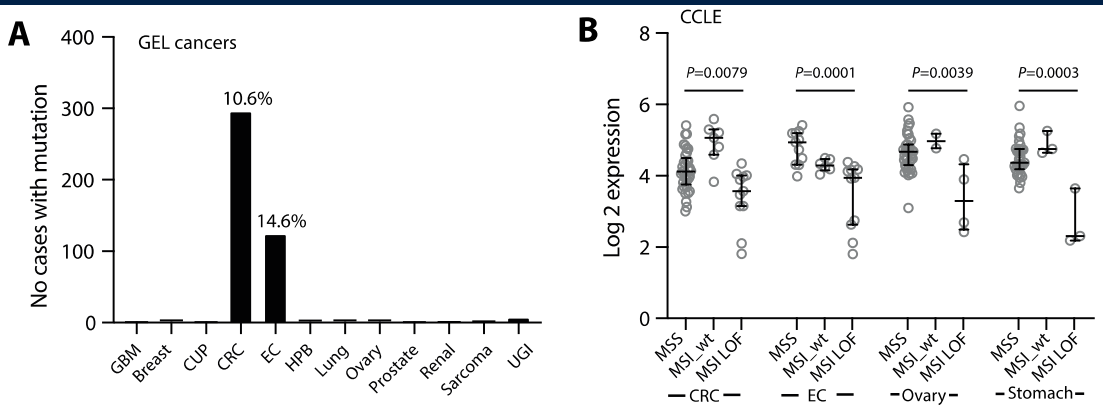


Figure 1. (A) Frequency of novel LOF immune escape mutation in Genomics England cancers by tumour type. (B) Association of MSI and immune escape gene LOF mutation status with expression in Cancer Cell Line Encyclopedia (CCLE)

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 collaboration[2]. 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.

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 spectrometry[3]. 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. ***Academic value and collaborations:*** The results will be of substantial academic value as the first demonstration of the impact of previously uncharacterised and common immune escape mutations in common cancers. The work will help consolidate an exciting collaboration between tumour genetics and functional immunology between the Church, Elliott and Ternette labs.

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.

References

1. Domingo, E. et al. Lancet. Gastro & Hepatol 1, 207-216 (2016). 2. Horeweg N et al. Can Immunol Res 8, 1508-1519 (2020). 3. Paes, W. et al. Proc Natl Acad Sci U S A 116, 24748-24759 (2019).

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11. Exploring the value of big data for access to treatment and quality of care for patients with blood cancer in the UK [1,4] – Dr. Dhiman

Primary Supervisor: Dr Paula Dhiman
Additional Supervisors: Prof. Simon Stanworth
Eligibility: Track 1 and 4 students are eligible to apply for this project.

Lay Summary

Blood cancer is the fifth most common cancer in the UK and includes cancers such as myelodysplastic syndromes (MDS) and acute myeloid leukaemia (AML). MDS is a blood disorder where bone marrow does not produce enough blood cells. Patients with MDS are often older; suffer from anaemia, infection, bleeding, and fatigue; and have poor quality of life. MDS can progress to AML, but AML usually affects younger people. This aggressive cancer requires intensive chemotherapy and stem cell transplants. Much MDS/AML research uses limited and manually collected information from patients in single hospitals and geographical areas. MSD/AML clinical trials tend to recruit younger patients, so are less generalisable to older patients. Black patients with blood cancers are also underrepresented in clinical trials and don't survive as long as white patients. We need studies using real-world, anonymised and routinely collected patient data that represents all UK blood cancer patients.

Hospitals collect data from patients as part of their care, such as routine blood tests. Many hospitals now allow researchers to use this routinely collected data. Combining data from different hospitals can help us understand treatment pathways and improve patient care and health outcomes. We plan to use anonymised patient data from many hospitals to study patients with blood cancers like MDS/AML. We will describe patients' clinical characteristics, treatment regimens, and health outcomes, such as disease severity, blood transfusion frequency, infection, survival, and clinical trial access. We will investigate how care and treatment differ with geography and ethnicity, indicating healthcare inequity. Many MDS/AML patients need frequent blood transfusions. We will look at transfusion patterns and bad reactions to blood and see if hospital protocols for transfusion support can be improved.

Abstract of the project

Blood cancer is the UK's fifth most common cancer, most common childhood cancer, and third biggest cancer killer.1 Common blood cancers such as leukaemia, lymphoma, myeloma, and myelodysplastic syndromes (MDS) have variable survival and growth rates and can change over time. MDS is a haematopoietic stem cell disease characterised by ineffective haematopoiesis, myeloid dysplasia, and low blood counts. Life expectancy varies from months to several years, and 30% of patients progress to acute myeloid leukaemia (AML). About 20% of AML cases evolve from a bone marrow disorder such as MDS. It is characterised by rapidly proliferating immature myeloblasts replacing healthy blood cells and is fatal without treatment. AML occurs in all adults, whereas MDS is more common in the elderly.2,3. For both diseases, chemotherapy can be used to control progression. Allogeneic stem cell transplant, the only curative option, is intensive and usually limited to younger, fitter patients, so is used more often in AML. Access to clinical trials and survival rates differ by age, geography and ethnicity, creating equity concerns. A 2021 study found Black AML patients in the US had worse survival than white patients4. AML is thought to be more refractory to treatment in older patients, yet this age group is under-represented in trials7,8.

Supportive care, such as blood transfusion with or without palliative chemotherapy, is the main treatment for many MDS and AML patients, particularity older patients. Around 150 blood donors support blood transfusions for one MDS/AML patient undergoing all required rounds of chemotherapy5. Given transfusion's importance in treatment and significant resource implications, optimal strategies and thresholds should be explored. For example, more frequent transfusion could minimise haemoglobin swings and improve quality of life, but must be balanced with alloimmunisation risks. Nearly all trials of red blood cell transfusion therapies have involved patients with acute hospitalised anaemia (e.g., after surgery), not patients with outpatient-based transfusion-dependent cancers.

The NHS Long Term Plan to gain insights and drive improvements in care relies on high-quality, big, routinely available data for analysis6. Routine data will enable more accurate evaluation of how patient factors such as age, co-morbidity, and ethnicity affect outcomes. The EU Low Risk MDS study suggested that trends in routine blood results can indicate early disease progression9. However, it only used low-risk cases and manual data capture. Common data collection methods such as cancer registries, biobanks, and randomised trials are limited by enrolment bias, reliance on human-coded data, and outcome data collected at set times.

The NIHR set up the Health Informatics Collaboration (HIC) to more efficiently capture routine clinical data. This collaboration of now 30 NHS trusts has developed validated pipelines for prospective studies using secondary care data. An HIC theme on transfusion-dependent anaemia has been set up and ethics approval received for a central database. We

will work with the HIC theme to develop a comprehensive, dynamic data repository of MDS/AML patients in the UK and assess their real-world outcomes. We will plan different studies (e.g., cohort studies) and apply advanced statistical methods (e.g., logistic and Cox regression analyses), to address the research objectives outlined below.

Research Objectives

a. Describe AML/MDS patients' characteristics, resource use (e.g., hospital visits, treatment, timing and frequency of blood transfusion), prognosis, and health outcomes (e.g., infection rates, cardiac outcomes, survival time in and out of hospital, alloimmunisation rates) by disease severity, co-morbidities, geographic location, hospital type, socioeconomic status, and ethnicity
b. Assess equality of care and treatment decisions
c. Use and collate information on pre-diagnosis blood count trends in blood cancer patients to explore and inform referral, diagnostic, and prognostic pathways; enable early disease and progression detection; and inform treatment regimen selection after diagnosis

Our existing collaborators with the National Cancer Research Institute (NCRI) Working Groups for MDS and AML (Dr Mehta/Dr Coats), and Supportive Care/Transfusion (Dr Stanworth) and the NIHR HIC theme for transfusion-dependent anaemia (Dr Wang, UCLH, co-ordinating site lead) all support the project. We will collaborate with the new NIHR blood transfusion research unit on data-enabled transfusion practice (NHS Blood and Transplant and NCRI, Dr Stanworth). This project and its outcomes have the potential to deliver transformative change in AML/MDS treatment and allow us to address care and resource equity.

Translational potential

The work will provide real-world data on treatment patterns, clinical trial access, and outcomes for all AML/MDS patients. There is little current data on how these patients' outcomes and survival are affected by geography, socioeconomic status, and ethnicity. Many of these patients consider outcomes other than survival important. We need to understand how co-morbidities and treatment approaches affect quality of life measures that can be collected and assessed in real-world data, such as admissions, day-case appointments, ICU admission rates, and transfusion. Patients needing repeat transfusion and their carers face complex, hugely disruptive care pathways. Understanding transfusion complexities (e.g., transfusion patterns, screening frequency, and ethnicity effects on alloimmunisation rates) will help to optimise these care pathways. We plan to use data to develop and inform evidenced-based personalised pathways for first-line AML/MDS treatment, to improve blood cancer patients' survival and quality of life.

Training opportunities

Training will be provided in relevant related research methodology. Attendance at formal training courses will be encouraged, including Advanced course in epidemiological analysis (London School of Hygiene & Tropical Medicine), Introductory analysis of linked health data (Swansea University), and Statistical methods for risk prediction and prognostic models (Keele University). The student will have access to free writing and statistics courses through the Medical Science Division. They will present data regularly to the department, research groups, and at external conferences. We will support additional on the job training opportunities

References

1. Blood Cancer UK. *Facts and information about blood cancer.* bloodcancer.org.uk/news/blood-cancer-facts/.
2. Miranda-Filho A *et al.* Epidemiological patterns of leukaemia in 184 countries: a population-based study. *Lancet Haematol* 2018;5:e14-e24.
3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin* 2020;70:7-30.
4. Bhatnagar B *et al.* Poor survival and differential impact of genetic features of black patients with acute myeloid leukemia. *Cancer Discov* 2021;11:626-37.
5. Dawson MA *et al.* Blood transfusion requirements for patients undergoing chemotherapy for acute myeloid leukemia how much is enough? *Haematologica* 2007;92:996-7.
6. NHS England. *NHS Long Term Plan*. www.england.nhs.uk/long-term-plan/.
7. Hutchins LF *et al*. Underrepresentation of patients 65 years of age or older in cancer-treatment trials. *NEJM* 1999;341:2061-7.
8. Lewis JH *et al.* Participation of patients 65 years of age or older in cancer clinical trials. *JCO* 2003;21:1383-9.
9. de Witte T *et al.* Novel dynamic outcome indicators and clinical endpoints in myelodysplastic syndrome; the European LeukemiaNet MDS Registry and MDS-RIGHT project perspective. *Haematologica* 2020;105:2516-23.

12. Improving chimeric antigen receptors for B cell acute lymphoblastic leukaemia [1,2,3] – Dr. Dushek

Primary Supervisor: Dr. Omer Dushek
Additional Supervisors: Dr. Ronjon Chakraverty
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Lay summary
T cells are white blood cells that search the body for abnormal cells. They detect these cells using their T cell antigen receptors (TCRs) that recognise molecules called 'antigens' on their surface. T cells are remarkably sensitive: they can respond to abnormal cells that present only a single antigen. An exciting treatment for cancer is to re-programme T cells to target a patient's cancer. This is done by expressing chimeric antigen receptors (CARs) on T cells. CARs have a part that binds an antigen and a part derived from the TCR that send an activating signal into the T cell. They allow a patient's T cells to recognise and kill their cancer cells. This therapy is very effective for leukaemias and lymphomas expressing high levels of certain antigens. However, many patients relapse when cancer cells emerge that have lower levels of antigen on their surface. One reason that this escape is possible is that CARs are much less sensitive than TCRs and so are unable to 'see' these new cancer cells. There is an urgent need to increase the sensitivity of CARs to prevent these relapses.

We have recently completed a study that identified why CARs are less sensitive than the TCR. We knew that other, accessory, receptors contributed to the high antigen sensitivity of the TCR. By testing different accessory receptors, we found that one called CD2 improved the antigen sensitivity of the TCR but not CARs. In this project, we will produce new CARs that can use CD2 to achieve high sensitivity and test them against B cell cancers. These more sensitive CARs will not only reduce relapse in existing therapies, but would also allow CAR-T cells to be used in treating a wider variety of cancers.

Abstract
T cells patrol the body in search of antigens derived from infectious organisms or cancer cells. They use their T cell antigen receptors (TCRs) to recognise peptide antigens on majorhistocompatibility-complexes (pMHC). T cells have remarkable antigen sensitivity; they can become activated when recognising only a single pMHC. This high sensitivity is important because infectious organisms and cancers deploy evasion mechanisms to reduce the amount of antigen presented to T cells. T cells are now engineered to recognise cancer antigens using chimeric antigen receptors (CARs). This therapy is approved to treat B cell cancers. However, many patients relapse with B cells that express low levels of the target antigen. It is now clear that CARs have a profound defect in antigen sensitivity; CARs require 100-1000-fold more antigen than the TCR to activate T cells. There is an urgent need to increase the sensitivity of CARs to prevent these relapses. The high antigen sensitivity of the TCR is partly a result of their adhesion receptor CD2 binding to its ligand CD58. In recent work, we have found that CARs fail to efficiently exploit the CD2/CD58 adhesion interaction. Here, we will use our basic understanding of how CD2 functions to generate novel CARs that efficiently exploit CD2 and test them using in vivo relapse models of B cell acute lymphoblastic leukaemia (B-ALL). This will improve existing CAR-T cell treatments for B cell cancers and should allow for new treatments to eliminate cancers expressing low levels of target antigens.

Background
The T cell antigen receptor (TCR) has remarkable antigen sensitivity; it is able to recognise even a single peptide antigen on target cells (1). It achieves this high sensitivity in part by exploiting the T cell adhesion receptor CD2 binding to its ligand CD58. Recent studies have shown the CD2/CD58 interaction to play critical roles in T cell function, including recognition of cancerous and infected cells (1). Adoptive cell transfer (ACT) of genetically engineered T cells expressing Chimeric Antigen Receptors (CARs) is a clinically approved cancer therapy for haematological malignancies (2). CARs are synthetic receptors that are generated by the fusion of an antibody-derived, antigen-binding single-chain variable fragment (scFv) with intracellular signalling motifs from the cytoplasmic tails of the TCR complex. Although CAR-T cells targeting the B cell surface antigens such as CD19 are initially highly effective, a large fraction of patients relapse when malignant cells emerge with reduced expression the target antigens (2). It is now appreciated that CARs are relatively

insensitive, requiring 100 to 1000-fold higher antigen densities to induce T cell activation compared to the native TCR (2). However, the mechanism underlying their defect in antigen sensitivity is presently unknown. There is now an urgent need to improve the antigen sensitivity of CAR-T cells in order to treat patients with relapse and to avoid relapse in the first place

Research objectives

In recently completed work, we have identified that the mechanism underlying the antigen sensitivity of CARs is their inability to efficiently exploit the CD2/CD58 interaction (3). We found that CD2 increased the antigen sensitivity of the TCR by 125- fold but only <5-fold for CARs. Here, we will use our basic understanding of how CD2 improves antigen sensitivity of the TCR to produce novel synthetic antigen receptors that efficiently exploit CD2.

Objective 1 Produce a highly sensitive synthetic antigen receptor targeting CD19.

Objective 2 Use pre-clinical models of B-ALL relapse to benchmark the in vivo activity of our novel synthetic receptor (see Objective 1).

Objective 3 Use in vivo findings (Objective 2) to inform on improved design (Objective 1).

The Dushek and van der Merwe laboratories have a long-standing history of studying antigen recognition by the T cell receptor, including understanding the role of accessory receptors such as CD2. Recently, they have developed an experimental system to analyse the antigen sensitivity of CARs relative to the TCR. They will lead on producing highly sensitive CARs targeting CD19 (Objective 1). The Chakraverty laboratory is focused on using murine models to understand the mechanisms of relapse to approved CD19 CARs. They will lead on in vivo testing (Objective 2) and have recently establish a model in their laboratory based on a previous report (4). There are now examples where the surface expression of CD19 is not only reduced but is completely abolished. If synthetic antigen receptors with higher sensitivity are insufficient to avoid this cancer evolution process, the study of relapse can identify preserved surface proteins that can be targeted at the outset alongside CD19 (Objective 3).

Translational potential

Currently, CARs underlie approved cancer treatments and a large number of promising treatments (e.g. using not only T cells but NK cells and macrophages). It follows that fundamental improvements to their antigen sensitivity can immediately be adopted by the large community of academic and industrial researchers.

Training opportunities

T cell isolation, tissue culture, synthetic design and genetic modification (CRISPR, Lenti-transductions), flow cytometry, mathematical modelling (to understand mechanism), pre-clinical models of B cell cancers.

References

1. Siller-Farfán, J. A., & Dushek, O. (2018). Molecular mechanisms of T cell sensitivity to antigen. Immunological Reviews, 285(1), 194–205. https://doi.org/10.1111/imr.12690

2. Majzner, R. G., & Mackall, C. L. (2018). Tumor antigen escape from car t-cell therapy. Cancer Discovery, 8(10), 1219–1226. https://doi.org/10.1158/2159-8290.CD-18-0442

3. Burton, J., Siller-Farfán, J. A., Pettmann, J., Salzer, B., Kutuzov, M., van der Merwe, P. A., & Dushek, O. (2021). Inefficient exploitation of accessory receptors reduces the sensitivity of chimeric antigen receptors. BioRxiv (Preprint).

4. Jacoby, E., Nguyen, S., Fountaine, T. et al. CD19 CAR immune pressure induces B-precursor acute lymphoblastic leukaemia lineage switch exposing inherent leukaemic plasticity. Nat Commun 7, 12320 (2016). https://doi.org/10.1038/ncomms12320

13. Interrogating and targeting metabolic plasticity in the tumour-bone microenvironment [1,2,3] – Associate Prof. Edwards

Primary Supervisor: Associate Prof. Claire Edwards
Additional Supervisors: Associate Prof. Karl Morten
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Lay Summary
Once prostate cancer spreads to bone (bone metastases) there is no cure. To develop new ways to combat this fatal stage, we need to understand exactly how it grows and spreads. Prostate cancer cells need energy to grow. We think specific changes in the energy needs of prostate cancer help prostate cancer grow within bone, and that bone cells talk to prostate cancer cells to cause these changes. Within cancer cells, mitochondria are the powerhouses that make the energy needed for the cells to grow. We believe that using new drugs that specifically target the cell's energy source will be a powerful way to stop prostate cancer from spreading to and growing in bone. We will investigate whether these drugs, either alone or in combination with current clinical approaches, are effective in reducing tumour burden and bone disease. We will grow human prostate cancer cells in contact with bone cells, mimicking the situation in the human body, and we will use in vivo (mouse) models to investigate energy disruption in prostate cancer bone metastases. We will also develop a new method to detect these changes in energy needs in patients with prostate cancer, to be able to better predict those patients at greatest risk of prostate cancer spreading to their bones and most likely to respond to this treatment approach. This project will uncover the function and potential of targeting the mitochondria as a novel treatment for bone metastatic prostate cancer.

Abstract
This proposal combines one of the most recently identified hallmarks of cancer with arguably the most fatal tumour microenvironment to investigate and disrupt the metabolic symbiosis between tumour cells and the bone microenvironment. The Edwards lab have recently used transcriptomic and metabolomic profiling to identify a new metabolic mechanism underlying prostate cancer bone metastasis and a new target with which to prevent the progression to advanced disease. The Morten lab have demonstrated the power of targeting metabolism, using novel mitochondrial metabolism disruptors to induce cancer cell death, however their potential in prostate cancer or in the tumour-bone microenvironment is unknown. The current proposal will exploit and synergise the expertise of the Edwards and Morten labs to investigate the effect of novel mitochondrial metabolism disruptors, studying effects on tumour burden and bone disease using state of the art in vitro and in vivo approaches. Finally, we will build upon preliminary studies from the Morten lab using Raman microspectroscopy to define a metabolic signature correlating with MMD efficacy, investigating the potential for Raman microspectroscopy to define a metabolic signature associated with prostate cancer bone metastasis and/or treatment response.

Research objectives
- Target metabolic dysfunction in the prostate cancer-bone microenvironment using novel mitochondrial metabolism disruptors
- Interrogate metabolic plasticity in treatment-resistant subsets of prostate cancer cells
- Define a mitochondrial signature specific for prostate cancer bone metastasis

Background & Rationale:
Prostate cancer metastasis to bone is almost always fatal, driven by the reciprocal relationship between prostate cancer cells and the bone microenvironment promoting tumour growth, drug resistance and bone disease. As such, a greater understanding of the key mechanisms driving progression to advanced disease is paramount to develop new effective therapeutic approaches. By interrogating the metabolic tumour-bone relationship at multiple levels and in multiple systems we have demonstrated the extent of metabolic perturbation occurring within the prostate cancer-bone microenvironment, so supporting the potential for metabolic targeting as an effective approach to block prostate cancer bone metastasis (1, 2). Novel mitochondrial metabolism disruptors (MMDs), currently under preclinical and clinical development for cancer treatment including NBS037 and Atavaquone, have been found to have minimal toxicity and to effectively block mitochondrial function, resulting in elevated oxidative stress and enhanced response to chemo-and radiotherapy (3-6). As such, MMDs represent an exciting opportunity for the treatment of prostate cancer bone metastasis.

Approaches:

The project will employ a powerful combination of in vitro cellular and molecular biology, preclinical models of prostate cancer bone metastasis and primary samples from patients with bone metastatic prostate cancer. MMDs will be studied both alone and in combination with drugs currently used for the treatment of prostate cancer. We will use a comprehensive panel of prostate cancer cell lines, state-of-the-art coculture systems allowing for high-throughput analysis of the prostate cancer-bone microenvironment and 3D organoids. To mimic conditions within the bone niche experiments will be carried out under a range of glucose and oxygen conditions. We will not limit our studies to cycling tumour cells but will also investigate metabolic disruption in distinct subsets of cancer cells most associated with drug resistance and metastatic progression, including polyploid giant cells, senescent cells and dormant cells. Effects on tumour cell biology and metabolic plasticity will be determined, with mechanistic studies employing transcriptomic and metabolomic interrogation. Preclinical models of prostate cancer bone metastasis will be utilised, enabling the study of MMDs in vivo on both tumour growth and bone disease. A novel approach to effective metabolic profiling in the tumour-bone microenvironment will be developed, employing Raman microspectroscopy to detect a metabolic signature predictive of disease progression using both primary and bone marrow samples from patients with bone metastatic prostate cancer.

Outcomes:

We anticipate that this DPhil project will (i) demonstrate that the disruption of mitochondrial metabolism is an effective approach to combat bone metastatic prostate cancer and (ii) develop a new approach to defining a metabolic signature associated with progression to prostate cancer bone metastasis and treatment response, in order to identify those patients at greatest risk and most likely to benefit from such metabolic intervention.

Translational potential

The translational potential of our project is extremely high. There is an urgent need to develop better approaches to combat advanced prostate cancer, complicated by the inextricable dependency of tumour cells on the bone microenvironment to drive both tumour growth and bone disease. The proposed study will investigate a new approach to prevent bone metastatic prostate cancer, using both patient-derived material and preclinical models to ensure clinical translatability. Our studies will uncover not only a new approach to treat this final fatal stage of prostate cancer, but also identify novel metabolic indicators of prognosis and/or therapy response.

Training opportunities

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

References:

1. Whitburn J, Rao SR, Morris EV, Tabata S, Hirayama A, Soga T, Edwards JR, Kaya Z, Palmer C, Hamdy FC, Edwards CM. Metabolic profiling of prostate cancer in skeletal microenvironments identifies G6PD as a key mediator of growth and survival. Sci Adv. 2022;8(8):eabf9096.
2. Whitburn J, Edwards CM. Metabolism in the Tumour-Bone Microenvironment. Current osteoporosis reports. 2021;19(5):494-9.
3. Ashton TM, Fokas E, Kunz-Schughart LA, Folkes LK, Anbalagan S, Huether M, Kelly CJ, Pirovano G, Buffa FM, Hammond EM, Stratford M, Muschel RJ, Higgins GS, McKenna WG. The anti-malarial atovaquone increases radiosensitivity by alleviating tumour hypoxia. Nature communications. 2016;7:12308.
4. Coates JTT, Rodriguez-Berriguete G, Puliyadi R, Ashton T, Prevo R, Wing A, Granata G, Pirovano G, McKenna GW, Higgins GS. The anti-malarial drug atovaquone potentiates platinum-mediated cancer cell death by increasing oxidative stress. Cell Death Discov. 2020;6:110.
5. Cochrane EJ, Hulit J, Lagasse FP, Lechertier T, Stevenson B, Tudor C, Trebicka D, Sparey T, Ratcliffe AJ. Impact of Mitochondrial Targeting Antibiotics on Mitochondrial Function and Proliferation of Cancer Cells. ACS Med Chem Lett. 2021;12(4):579-84.
6. Stoker ML, Newport E, Hulit JC, West AP, Morten KJ. Impact of pharmacological agents on mitochondrial function: a growing opportunity? Biochemical Society transactions. 2019;47(6):1757-72.

14. Enhancing the Efficacy of Immune Checkpoint Inhibitors in Colorectal Cancer using Ultrasound-activated Cavitation Agents and Image-guided Drug Delivery[1,2,3,4] – Prof. Elliot

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

Lay Summary
Colorectal cancer (CRC) is a common and deadly cancer, and new approaches to its treatment are desperately needed. Our immune system plays a crucial role in detecting and destroying cancer cells. This ability, however, can be masked in the later stages of the disease as some aggressive cancer cells can mutate in such a way that they can evade destruction by the immune system. A recent successful treatment to boost this anti-cancer immunity is to use drugs to re-activate the immune system, thereby allowing the immune cells to see and attack the cancer cells. These drugs are known as immune checkpoint inhibitors (ICIs). Boosting anti-cancer immunity using ICIs can be even more effective if the environment within the tumour is made more inflammatory with immune cells. Unfortunately, CRC has not responded well to ICIs because CRC tumours are densely packed with irregular blood vessels and protective barriers, hence delivery of ICIs into and throughout CRC tumours is not effective. Ideally a safe, non-invasive, targetable and inexpensive drug delivery system could be used to push ICIs from the bloodstream into and throughout CRC tumours. Ultrasound is traditionally used for imaging tissues, but we have now developed technologies which allow ultrasound to activate bespoke nanoparticles within patients, so they create cavitation events near tumours. These cavitation events can act as micropumps to propel drugs into tumours, make the tumours more inflammatory and may even boost anti-cancer immunity. We can even monitor cavitation-mediated drug delivery using ultrasound imaging, thereby giving a map of where ICI delivery is taking place during treatment. This project will optimise combinations of ultrasound, cavitation nanoparticles and ICIs to achieve the best enhancement of anti-cancer immunity. Findings from this DPhil study performed on preclinical models will ultimately be translated to improve patient treatment.

Project Summary
Immune checkpoint inhibitors (ICI) targeting the programmed cell death-1 receptor (PD-1) and its ligand (PD-L1) are routinely used in clinic for treating solid tumours[1]. However, about 96% of colorectal cancer are immunologically 'cold', microsatellite stable and DNA mismatch repair proficient tumours that do not currently benefit from immunotherapy[2]. Treatment failure is often due to factors e.g., low neoantigen burden, loss of MHC-I, dysfunctional antigen processing and presentation, lack of tumour-infiltrating lymphocytes and local immunosuppression. Furthermore, the presence of dense stroma and dysregulated tumour blood vessels can serve as physical barriers to drug delivery[3]. There is an unmet need for the development of therapeutic and image-guided approaches to improve the delivery of therapeutics into and throughout tumours and transform a 'cold' immune-excluded tumour into a 'hot' immune-inflamed tumour. Focused ultrasound has emerged as a promising approach for immuno-modulation of tumours through thermal or mechanical perturbation[4]. Ultrasound-mediated cavitation using novel, biocompatible nanoparticles has been used to enhance the delivery of anti-cancer agents e.g. chemotherapy and oncolytic viruses into tumours[5–7] (Figure 1).

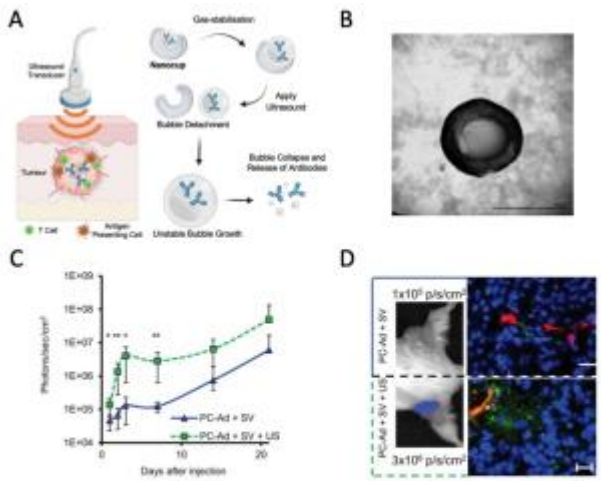

Figure 1. Ultrasound-activated nanoparticles for delivery of drugs. (A) Schematic diagram for ultrasound-guided delivery of nanoparticle encapsulated treatment antibodies. (B) TEM of novel ultrasound-responsive solid particles manufactured using immunostimulatory proteins. (C) and (D) Improved delivery of oncolytic adenoviruses ('PC-Ad') using microbubbles ('SV') and ultrasound. Green (virus), red (blood vessel), blue (DAPI). Images were adapted from [5] and [7].

Ultrasound itself is a relatively low-cost system used in clinical imaging of soft tissues, tissue stiffness and tumour blood flow, and can be used for real-time monitoring of drug delivery. The Elliott Group at the Oxford Centre for Immuno-oncology investigates the mechanisms of antigen processing and presentation involved in T cell response to cancer and during immunotherapy using biochemical, computational, and physical science methods e.g. imaging. The Biomedical Ultrasonics, Biotherapy and Biopharmaceuticals Laboratory (BUBBL) at the Institute of Biomedical Engineering specialises in the development of novel ultrasound-responsive microbubbles (MB) and solid sonosensitive particles (SP) for targeted drug delivery. This multidisciplinary project is a partnership between immuno-oncology, imaging, and biomedical engineering.

Research objectives

The aim of this project is to develop and validate ultrasound-activated SP and MB cavitation agents to actively transport and enhance the distribution of ICI in tumours via mechanical cavitation. The treatment efficacy of ultrasound-activated cavitation agents as propellants or vehicles for ICI delivery and the mechanistic effects of cavitation on antigen presentation, T cell avidity and function will be investigated. Preclinical colorectal cancer models, already comprehensively characterised by the Elliot group will be used[8,9].

Work Package 1: Development of ultrasound-activated cavitation formulations for ICI delivery

PD-1 and/or PD-L1 monoclonal antibodies of different doses will be co-delivered with SP or formulated into SP or MB. The physicochemical properties of the SP and MB formulations, doses of PD-1/PD-L1 monoclonal antibodies delivered with, or formulated into, each cavitation agent and the acoustic parameters for tumour delivery will be optimised, and correlated to the tumour volume, microvessel density and stromal thickness. For microscopic evaluation of the drug spatial distribution, PD-1 and/or PD-L1 monoclonal antibodies will be bioconjugated with amine-reactive reagents for fluorescent labelling with near infrared dyes.

Work Package 2: Establishment of a preclinical colorectal cancer model for ultrasound-activated and image-guided delivery of PD-1/PD-L1 antibodies

The BALB/c mouse model of colorectal carcinoma, CT26 is a microsatellite stable and DNA mismatch repair proficient tumour which shares molecular features with aggressive, undifferentiated, and refractory human colorectal cancer. CT26 is one of the most extensively investigated syngeneic tumour model in preclinical studies and has been used to validate most immunotherapeutics currently in the clinic or under clinical trials, with >500 studies in literature. SP and MB formulations with optimised PD-1 or PD-L1 doses will be administered into mice bearing subcutaneous CT26 tumours and actively delivered into the tumours via ultrasound-induced cavitation. Tumour growth monitoring will be conducted to determine treatment efficacy and response classification into progressors or regressors. Tumours and secondary lymphoid organs will be harvested at the study endpoint for functional analyses e.g. flow cytometry, Figure 1. Ultrasound-activated nanoparticles for delivery of drugs. (A) Schematic diagram for ultrasound-guided delivery of nanoparticle encapsulated treatment antibodies. (B) TEM of novel ultrasound-responsive solid particles manufactured using immunostimulatory proteins. (C) and (D) Improved delivery of oncolytic adenoviruses ('PC-Ad') using microbubbles ('SV') and ultrasound. Green (virus), red (blood vessel), blue (DAPI). Images were adapted from [5] and [7]. 2 transcriptomics, multiplex immunofluorescence. In particular, biomarkers related to MHC Class I antigen presentation, antigen processing e.g. tapasin and calreticulin, CD8+ and CD4+ T cell, antigen-presenting cells such as dendritic cells, immunosuppressive cells, cancer-associated fibroblasts, extracellular matrix remodelling, vascular inflammation e.g. ICAM-I and VCAM-I and tumour hypoxia will be examined closely. Experiments will be designed to distinguish the immunological effects of cavitation alone from the effects of enhanced antibody delivery instigated by cavitation.

Work Package 3: Dissecting the effects of ultrasound-activated cavitation agents and PD-1/PD-L1 blockade on the spatiotemporal dynamics of antigen-specific T cell function

The mechanical and immuno-modulatory effects of the optimised cavitation agent anti-PD-1/PD-L1 formulation on the migration and cellular kinetics of antigen-specific T cells across physical barriers (tumour stroma and vasculature) will be evaluated using live imaging techniques. Antigen-specific T cells of high versus low functional avidity will be fluorescently labelled and adoptively transferred into CT26 mice prior to treatment with the SP or MB formulation. Non-invasive imaging of tumour stiffness using ultrasound elastography and vascular permeability and perfusion using contrast-enhanced ultrasound will be conducted before and after treatment to derive clinically-relevant imaging biomarkers of response to treatment. For more detailed examination of the cellular processes at the preclinical level, two-photon imaging will be performed on vibratome-sliced tumours in perfusion chambers with intravascular dyes injection prior to sacrifice to highlight the tumour blood vessels and second harmonic generation imaging of the stromal collagen fibres at the tumour invasive margin. Image processing and analysis of the fluorescent T cell tracks, velocities, and spatial confinement within different tumour compartments (peritumoral versus Intratumoral), T cell spatial distribution and distance to tumour cells, tumour stroma and blood vessels will be conducted.

Translational potential

This work will develop and validate novel ultrasound-activated SP and MB agents for therapeutic and image-guided delivery of ICI in preclinical colorectal cancer. The aim is to investigate whether drug penetration can be optimised using this approach and better understand its mechanistic effects on antigen presentation, T cell avidity and function in tumours. An Oxford spin-out company, OxSonics Therapeutics, in which a similar cavitation technology ('Nanocups') is based, has already reached clinical-stage and is currently in Phase I/II clinical trial for ultrasound-guided delivery of anticancer agents in metastatic colorectal cancer patients. The difference in the present proposal is the introduction of a sonosensitive particle that is capable of not only enhancing antibody delivery and distribution, but also in promoting the local immune response through the introduction of immune-

stimulatory proteins adjacent to the cavitation process. A long-term plan for this project would be to translate the technology and discoveries into human cancers to optimise the efficacy of immunotherapeutics.

Training opportunities

The project is ideally suited for non-clinical candidates with a background in pharmacology, biomedical sciences or bioengineering and is also suitable for clinical candidates wishing to gain research experience in preclinical immunotherapy modelling and novel drug delivery in an interdisciplinary setting. Research training in chemical engineering, tumour immunology and the use of therapeutic and diagnostic imaging ultrasound, as well as two-photon microscopy will be provided. More general research, communication, teaching, innovation, and career development skills training will be given by the Medical Sciences Division.

References

1. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. Science. 2018;359(6382):1350-1355.
2. Ganesh K, Stadler ZK, Cercek A, et al. Immunotherapy in colorectal cancer: rationale, challenges and potential. Nat Rev Gastroenterol Hepatol. 2019;16(6):361-375.
3. Lu G, Fakurnejad S, Martin BA, et al. Predicting Therapeutic Antibody Delivery into Human Head and Neck Cancers. Clin Cancer Res. 2020;26(11):2582-2594.
4. Joiner JB, Pylayeva-Gupta Y, Dayton PA. Focused Ultrasound for Immunomodulation of the Tumor Microenvironment. J Immunol. 2020;205(9):2327-2341.
5. Kwan JJ, Myers R, Coviello CM, et al. Ultrasound-Propelled Nanocups for Drug Delivery. Small. 2015;11(39):5305- 5314.
6. Grundy M, Bau L, Hill C, et al. Improved therapeutic antibody delivery to xenograft tumors using cavitation nucleated by gas-entrapping nanoparticles. Nanomedicine. 2021;16(1):37-50.
7. Carlisle R, Choi J, Bazan-Peregrino M, et al. Enhanced Tumor Uptake and Penetration of Virotherapy Using Polymer Stealthing and Focused Ultrasound. JNCI J Natl Cancer Inst. 2013;105(22):1701-1710.
8. James E, Yeh A, King C, et al. Differential Suppression of Tumor-Specific CD8 + T Cells by Regulatory T Cells. J Immunol. 2010;185(9):5048-5055.
9. Sugiyarto G, Prossor D, Dadas O, Arcia-Anaya ED, Elliott T, James E. Protective low-avidity anti-tumour CD8+ T cells are selectively attenuated by regulatory T cells. Immunother Adv. 2021;1(1).

15. Germline genetic variation and immunotherapy: personalised predictors of response and toxicity[1,2] – Dr. Fairfax

Primary Supervisor: Dr. Benjamin Fairfax
Additional Supervisors: Prof. Paul Klenerman
Eligibility: Track 1 and 2 students are eligible to apply for this project.

Lay Summary
Background: Our immune systems protect us from infection and also act to prevent cancer. Avoiding detection by immune cells is therefore very important for cancers to grow- indeed, by the time cancers are large enough to require treatment they have usually escaped the immune response. Cancer immunotherapy consists of drugs which help the immune system 'see' the cancer again. Though we know these drugs work well across groups of patients, we can't predict how patients will respond at an individual level in terms of their tumours shrinking or whether they will develop serious side-effects. Predicting this before starting treatment, or very early on in treatment, may help guide the use of immunotherapy. Recent research shows differences in patients' genetics may impact differences in response to treatment. Changes in white blood cell numbers might show this happening and help modify treatment as patients are treated. The same research showed there might be a new role for B-cells, a type of immune cell not previously considered important in immunotherapy, in generating the immune response.

Objectives: We want to see if changes in types of white blood cell numbers during early treatment can predict response to immunotherapy in lots of cancers and treatment combinations. We will look to see if these changes are controlled by individual variation in patient genes. We will also look at whether B-cells are very important in the response to immunotherapy and whether the individual gene changes are part of this.

Translational potential: If we know about gene changes pre-treatment that can predict side-effects or response to treatment, or see responses to immunotherapy early in treatment, we can use these to guide choices about timing and intensity of immunotherapy treatment in cancer. If we know why some people don't respond as well to immunotherapy, it may also help guide new treatments to boost immunotherapy.

Abstract
Background: Immune checkpoint blockade (ICB) causes durable responses in multiple cancers but with unpredictable individual patient response and toxicity. Predicting variations in clinical outcome could guide intensity of treatment in both adjuvant and metastatic settings. A genome-wide association study demonstrated three independent loci are associated with severe toxicity, the most significant being at IL7. We have independently validated this observation in an Oxford based melanoma cohort, indicating the effect is mediated via B-cell expression of IL-7. We additionally note a response biomarker identified through changes in absolute lymphocyte count – termed the lymphocyte stability index (LSI).

Objectives: This project will examine the applicability and utility of LSI to clinical outcomes and the development of toxicity across a range of ICB-treated cancers including those of the upper GI tract. It will also explore the role of B cells and IL-7 in this response, exploring B cell interactions across treatments at the immunological and molecular level.

Translational potential: This project could identify novel personalised medicine approaches to ICB therapy including biomarkers of response, resistance and toxicity, choosing intensity of treatment, and identifying novel targets for treatment.

Background
Immune checkpoint blockade (ICB) has dramatically changed clinical outcomes across a range of cancers, with durable responses in metastatic disease. However, there remains very significant heterogeneity in responses between disease types and also at the level of individual patients. Treatment with ICB can induce severe immune-related adverse events (irAEs), particularly when combination ICB (anti-CTLA-4 & anti-PD1) is given. Conversely, irAEs are correlated with better outcomes, suggesting a common mechanistic basis for both. Elucidating the details of interaction of ICBs with the host immune system is therefore a vital area of research.
There is increasing evidence to demonstrate that germline genetic variation both influences patient responses to ICB, as well as propensity to irAE development. A recent genome-wide association study (GWAS) has identified a non-coding, single nucleotide polymorphism (SNP) in the gene IL71 which encodes the cytokine IL-7, crucial to lymphocyte

proliferation and differentiation. We find that this IL7 risk allele is similarly associated with toxicity in patients treated in Oxford and have proceeded to demonstrate that patients carrying the risk allele have high IL-7 expression prior to treatment in their B-cells, with evidence for resulting regulation of CD8+ T-cell clonal expansion. Changes in absolute lymphocyte count during ICB therapy after 1 cycle of ICB therapy, deemed lymphocyte stability index (LSI), were also associated with overall survival, offering a novel and readily available biomarker discernible from a full blood count. Understanding the relationships between LSI and specific immune cell subsets has the potential to provide greater insights into this key observation and should permit a more personalised approach to immunotherapy.

Research objectives

The primary aims of this project will be to determine the cellular correlates and determinants of LSI. This will be measured in terms of peripheral immune cell gene expression, cytokine level and flow cytometry markers. In addition, we have access to the primary tumour from a subset of patients and we will explore the relationship between LSI and tertiary lymphoid structures. We will collaborate with groups nationally to perform additional exploration of genetic determinants of LSI. We will also address LSI in other non-melanoma solid tumours, including assessing the relationship to clinical outcome as well as comparative analysis with melanoma.

Translational potential

Outside of radiological response, we currently are unable to assess likely response to ICB in patients on treatment. The results of this project have the potential for identifying novel response, resistance and toxicity biomarkers for patients treated with ICB therapy. Indeed, LSI is highly translatable to the clinical setting – potentially to guide escalation treatment in patients with poor early responses to single agent anti-PD1. Similarly, predictors of toxicity could guide treatment choices in scenarios with higher risk to benefit ratios, such as adjuvant therapy. Given this, it is imperative to understand the molecular underpinnings of LSI. Furthermore, elucidating the role of B-cells ICB response may also ultimately point to novel, synergistic treatment targets.

Training opportunities

The analysis will be based across a large immunological, genetic and transcriptomic dataset developed from a cohort of patients (n=270+) who have received ICB for cancer in Oxford. The student will be involved in a number of clinical and laboratory techniques during their DPhil, including but not limited to DNA and RNA extraction, genotyping, flow cytometry, single-cell RNA sequencing, computational biology, large dataset statistical analysis, and patient counselling. The initial analytical techniques will involve statistical analysis to develop hypotheses that can be then tested in separate cohorts and with alternative tumour types. There will also be opportunities to explore ethical use of patient data for research within the NHS. The student will work within the Department of Oncology at the Weatherall Institute of Molecular Medicine, with opportunities to interact with other researchers focused on immunology across a range of diseases.

References

1. Groha, S., Alaiwi, S. A., Xu, W., Naranbhai, V., Nassar, A. H., Bakouny, Z., Adib, E., Nuzzo, P. v, Schmidt, A. L., Labaki, C., Zarif, T. el, Ricciuti, B., Alessi, J. V., Braun, D. A., Shukla, S. A., Keenan, T. E., van Allen, E., Awad, M. M., Manos, M., … Gusev, A. (2022). Germline variants associated with immunotherapy-related adverse events. *MedRxiv*, 2022.04.10.22273627. https://doi.org/10.1101/2022.04.10.22273627
2. Fairfax, B., Taylor, C., Watson, R., Tong, O., Ye, W., Nassiri, I., Gilchrist, J., Aires, A. V. de los, Sharma, P., Koturan, S., Cooper, R., Woodcock, V., Jungkurth, E., Shine, B., Coupe, N., Payne, M., Naranbhai, V., Groha, S., Emery, P., … Gusev, A. (2022). *Genetic variation at IL7 provides mechanistic insights into toxicity to immune checkpoint blockade*. Research Square. https://doi.org/10.21203/rs.3.rs-1531341/v1

16. A spatially resolved 3D multi-omic Atlas for cancer analytics in the human brain[1,3] – Associate Prof. Fischer

Primary Supervisor: Associate Prof. Roman Fischer
Additional Supervisors: Associate Prof. Olaf Ansorge
Eligibility: Track 1 and 3 students are eligible to apply for this project.

Lay Summary

Glioblastoma multiforme (GBM) is a diverse, devastating and highly invasive brain cancer that can result in death after six months or less following diagnosis. The median survival is under two years with 40% of patients surviving year one and only 17% year two after diagnosis/treatment – a consequence of inevitable recurrence post-surgery and lack of any targeted disease modifying therapies. The genes, proteins and metabolites that drive GBM recurrence, invasion and growth especially in context of their localisation within the tumour are poorly understood, highlighting a clear need for improving understanding of the molecular mechanisms involved with the aim to improve targeted therapy and drug delivery at personalized level. The spatially resolved mapping of immune response, drug delivery and drug target distribution will facilitate the development of disease modifying therapies specifically targeted to the individual cancer.

Technologies that allow the spatially resolved detection of biomolecules are rapidly evolving with spatial proteome analysis in particular promising to allow molecular characterisation of an individual cancer and advancing drug/target discovery. In this project we will use novel spatial proteomics technology to map the deep proteome (>5000 proteins) and lipidome in GBM patient tissue down to single cell/type resolution, in order to establish the local molecular dependencies in GBMs at protein and lipid level. The spatial analysis of proteins and lipids in GBMs will support the mapping of new disease markers and drug targets in dependence of their localisation in the fine structure of the tissue, map areas in which the immune system is active against the tumour and find new ways to trigger immune response and deliver targeted drugs to areas where they are needed.

The successful candidate will work at the interface of clinical research and pathology and work hands on current state-of-the-art mass spectrometry platforms for lipidomic and proteomic analysis. This includes sampling of brain tissue, data generation (spatial proteomics and lipidomics) and data analysis including spatially aware tools for multi-modal data integration in this young field of omics research.

Data generated in this project will help discovering new drug targets and therapies in addition to establish novel technologies towards digital pathology and will provide the basis for machine learning approaches in drug development.

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), which will be provided in close collaboration with Prof. Ansorge (NDCN, Glioma Neurosurgery Group).

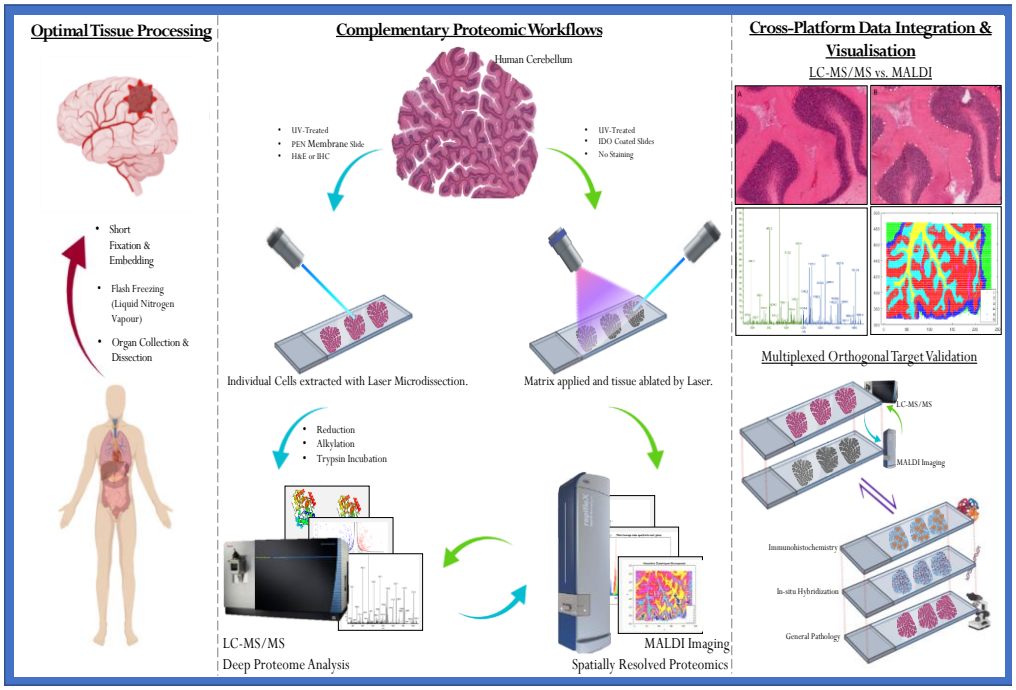

Figure 1 We have developed a novel liquid nitrogen vapour (LNV) freezing method and short-fixation protocol (**left**). Laser micro dissection combined with state-of-the-art mass spectrometry and MALDI imaging provide complementary data for mapping tumour biology at molecular level (**centre**). Integration 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 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.

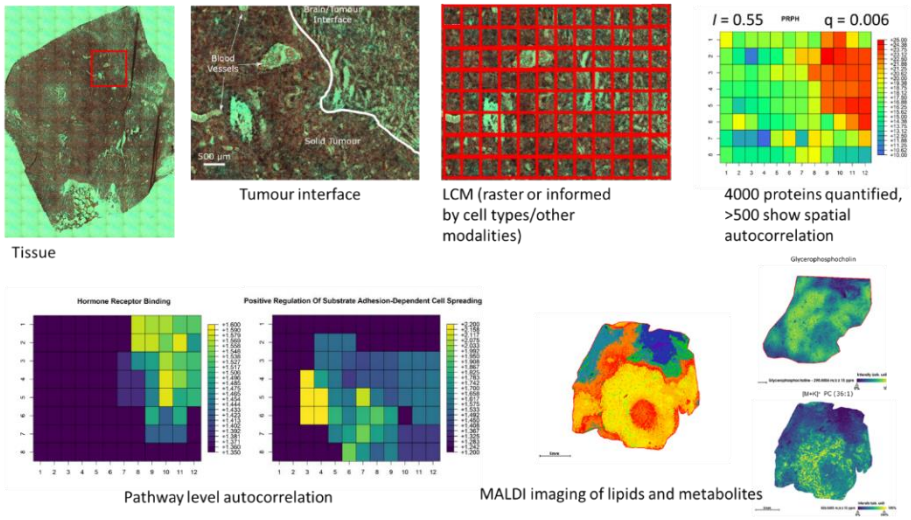

Figure 2 Established workflows for state-of-the-art proteomics and MALDI imaging reveal the spatial resolution of the cancer proteome in an AT/RT brain tumour and discover localized phenotypes at molecular and pathway level. This project aims to further advance this methodology for 3-dimensional visualisation 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:

1. Bi J, Chowdhry S, Wu S, Zhang W, Masui K, Mischel PS. Altered cellular metabolism in gliomas - an emerging landscape of actionable co-dependency targets. Nat Rev Cancer. 2020;20(1):57-70.
2. Yong C, Stewart GD, Frezza C. Oncometabolites in renal cancer. Nat Rev Nephrol. 2019.
3. Davis S, Scott C, Ansorge O, Fischer R. Development of a Sensitive, Scalable Method for Spatial, Cell-Type-Resolved Proteomics of the Human Brain. J Proteome Res. 2019;18(4):1787-95.
4. Niehaus M, Soltwisch J, Belov ME, Dreisewerd K. Transmission-mode MALDI-2 mass spectrometry imaging of cells and tissues at subcellular resolution. Nat Methods. 2019;16(9):925-31.
5. Rozenblatt-Rosen O, Regev A, Oberdoerffer P, Nawy T, Hupalowska A, Rood JE, et al. The Human Tumor Atlas Network: Charting Tumor Transitions across Space and Time at Single-Cell Resolution. Cell. 2020;181(2):236-49.

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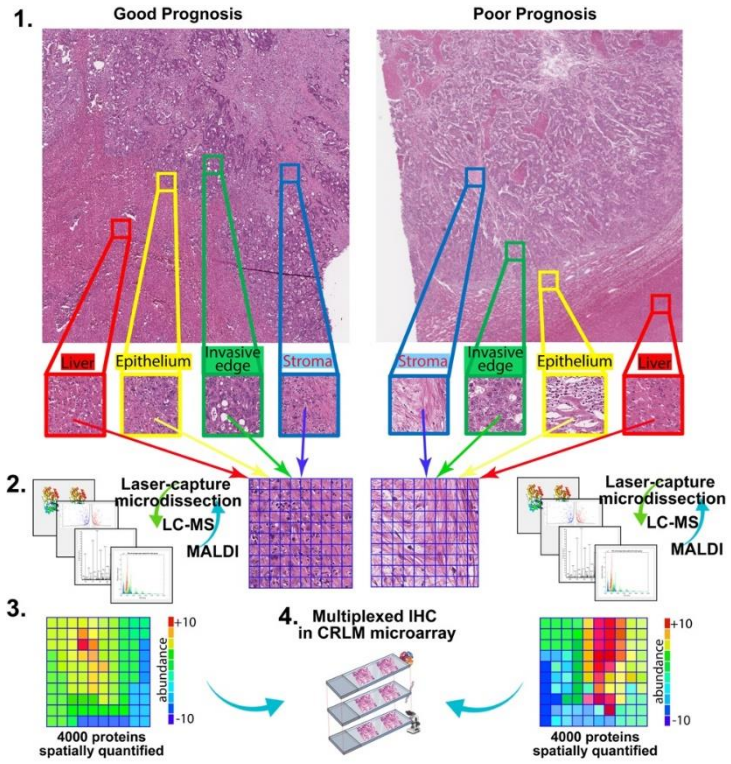
17. Multiparameter Spatial Profiling of the Metastatic Colorectal Cancer Microenvironment [1,2,3,4] Dr. Gordon-Weeks

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

Lay Summary
There are approximately 43,000 new bowel cancer diagnoses a year in the UK and up to 50% of these patients develop metastatic (secondary) cancer in the liver (CRUK statistics). Whilst cancer confined to the bowel is curable, liver metastasis treatment is more complex and significantly less likely to be successful. Surgery is the only curative treatment, but most patients have inoperable disease and recurrence after surgery occurs in about 75% of patients. As a result, liver metastasis is the most common cause of death from bowel cancer, so enhanced understanding of the biology of liver metastasis is crucial for the identification of effective treatments.

This project will use a new technology to measure the level of thousands of proteins in different cells within the liver metastases. This will enable us to draw detailed maps of protein expression in different cell types, providing a unique way of understanding how the cancer cells interact with their environment and with nearby tissues. By comparing these maps in metastases from patients who experienced recurrent disease and those that went on to survive their cancer, we will identify novel biological features linked to prognosis. This could include cell-specific or secreted factors linked to disease progression and ultimately new therapeutic targets.

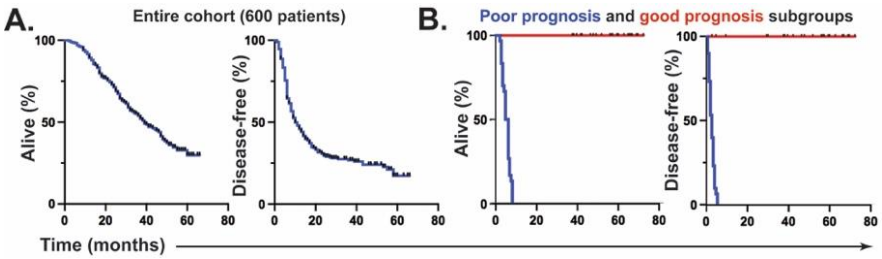


Graphical Abstract.
Regions of interest (ROIs) are identified in good and poor prognosis colorectal liver metastases (CRLM) H&E samples (1). ROIs are spatially sampled with laser-capture microscopy (LCM) and micro-dissected specimens analysed using ultra-deep LC-MS/MS and MALDI mass spectroscopy imaging (2). Proteomics is then spatially resolved back to the original ROI enabling the quantification of 4000 proteins at subcellular resolution (3). Protein quantifications are compared between good and poor prognosis CRLM and findings validated through multiplexed IHC (Cell-Dive) of interesting proteomic hits alongside relevant cellular markers on serial sections and a CRLM tissue microarray (4).

Introduction
CRLM occur in 50% of colorectal cancer patients and the median survival is 12 months without surgery. But, most patients have inoperable disease at the outset and post-surgical recurrence is common. Identification of biological features that drive disease progression is the foremost challenge to improving survival.
Although post-surgical recurrence is common and often fatal, there is immense heterogeneity in oncological outcome.

In a large patient cohort, the 5-year post-surgical survival was under 25% (Fig. 1). But, 5% of patients were alive and recurrence-free ≥5-years post-resection, whilst the opposite 5% of the prognostic spectrum experienced recurrence

and death within a year (Fig. 1). Comparison of commonly used clinicopathological prognosticators failed to identify differences between these groups (Table 1).



These data indicate that there are as yet unidentified biological differences underlying CRLM outcome. Identification of these differences will enable a greater understanding of the fundamental mechanisms through which metastasis develop and will uncover features responsible for aggressive disease biology. This is a crucial step in the development of novel therapeutics.

Figure 1. Good and poor prognosis cohorts post CRLM resection. A) Survival of 600 patients following CRLM resection. **B)** Survival of the poor and good prognosis cohorts from (A). These patients make up the study population.

	Early recurrence (30)	No recurrence (30)	*P*
Age*	66.7 (±1.7)	64.7 (±1.8)	0.42
Number of metastases#	2 (1-4)	2 (1-4)	0.78
Largest metastasis (mm)*	48 (±4.5)	42 (±6.1)	0.40
Neoadjuvant chemotherapy, n (%)	18 (60)	14 (47)	0.44
Right-sided primary, n (%)	6 (20)	2 (7)	0.25
Synchronous primary, n (%)	15 (50)	19 (63)	0.43
Node-positive primary, n (%)	20 (67)	18 (60)	0.79
Lung metastasis at presentation, n (%)	5 (17)	3 (10)	0.71
Bi-lobar liver metastases, n (%)	12 (40)	13 (43)	0.71

Table 1. Clinicopathological variables of patients from the groups in fig. 1 demonstrating lack of robust features with which to distinguish these divergent outcomes. *mean (±SE), #median (IQR).

Training Opportunities

The candidate will acquire transferable skills in the following high-end technologies:

Mass spectrometry, proteomics, metabolomics, data analytics/integration ***(Roman Fisher laboratory)***[1,2] Multiplexed IHC performance and image analysis ***(Prof Mark Coles laboratory)***

Based on the findings from Objectives 1 and 2 and dependent upon good progress, there will be scope for the candidate to develop skills in 3D organotypic culture, extracellular matrix generation, co-culture and CRISPR-Cas9 techniques in the supervising laboratory ***(Alex Gordon-Weeks and Simon Buczacki laboratories)***[3,4]. This would be with a view to providing a mechanistic context to the observational experiments highlighted.

References

1 Davis S *et al*.. *J Proteome Res*. American Chemical Society; 2019 Apr 5; **18**: 1787–1795.

2 Davis S *et al.* Deep topographic proteomics of a human brain tumour. bioRxiv; 2022.03.21.485119. https://www.biorxiv.org/content/10.1101/2022.03.21.485119v1

3 Gordon-Weeks AN *et al*. *Hepatology*. 2017; **65**: 1920–1935.

4 Yuzhalin AE et al. *Nat Commun*. 2018 14; **9**: 4783.

18. Mechanisms of resistance to DNA damage response inhibitors induced by the tumour microenvironment [1,2,3] – Prof. Hammond

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

Lay Summary

Most cancer therapies work by damaging the DNA of cancer cells which leads to the cells dying. However, all human cells, cancerous and normal, can repair their DNA using a set of specific tools (proteins) including one called PARP. If the treated cancer cells repair their DNA after treatment, they are less likely to die. Drugs which stop PARP working (PARP inhibitors) have been developed and these stop cancer cells repairing their DNA during cancer treatment and make sure the treated cells die. While a very promising form of cancer therapy, there have been some problems with resistance to PARP inhibitors i.e., tumours with cells in that do not die. The problem we plan to address is that while PARP inhibitors work very well in the lab conditions where they were developed and tested, we have found that these conditions are very different to those found in actual tumours. One of these key differences is the environment in which tumours grow in people's bodies. Often this environment (called the tumour microenvironment) includes areas of low oxygen, this is called hypoxia. Regions of hypoxia (low oxygen) occur in tumours because cancer cells are unable to grow a proper blood supply and so must rely on an inefficient delivery of oxygen to the tumour through the blood. Regions of hypoxia is very common in human tumours. Our work has shown that the amount of oxygen available to cancer cells varies and determines whether they will die or survive when a PARP inhibitor is added. The aim of this project is to try and understand why the level of oxygen has such a dramatic effect on response to PARP inhibitors and also to find out if there are ways we can reverse this drug resistance. We will investigate this problem by using sophisticated equipment that allows us to carry out experiments on human cancer cells in oxygen conditions which closely mimic those found in tumours. This equipment is already present in the lab and we have a lot of experience in this type of work. Understanding how oxygen levels impact response to PARP inhibitors would help identify those patients likely to benefit from this treatment and allow more widespread use of PARP inhibitors in the clinic.

Abstract

Approximately 50% of ovarian cancers and 10-20% of breast, metastatic prostate, or pancreatic cancers harbour mutations in homologous recombination (HR) factors, making these tumours candidates for PARP inhibitor (PARPi) therapy. However, despite the enthusiasm for the addition of PARP inhibitors to the clinical landscape of HR deficient tumours, not all patients benefit and many of whom initially respond to therapy develop resistance. Deciphering the mechanisms of resistance to PARPi is therefore essential in improving their clinical efficacy. **While past studies have identified cancer cell intrinsic mechanisms driving PARPi resistance, we have focused on the mechanisms by which the hypoxic tumour microenvironment promotes PARPi resistance in both HR deficient and proficient tumour cells**. We found that HR proficient and resistant tumour cells exhibit an oxygen-dependent response to PARPi. PARPi kills oxic tumour cells, but not hypoxic tumour cells in xenograft models. PARPi-induced DNA damage is reduced in hypoxia and hypoxia mediated PARPi resistance is associated with low reactive oxygen species (ROS) production. **Our work demonstrates that the response to PARPi is oxygen-dependent, with sensitivity seen at extreme levels (<0.5% O_2) and resistance in more commonly found moderate hypoxia (1-2% O_2), and that this resistance can be overcome by eliminating hypoxic tumour cells.** The significance of our proposal is that it will provide a mechanistic understanding of how different levels of oxygen affect the response of tumour cells to PARPi and lead to more widespread and personalised use.

Research objectives

A key unanswered oncology question, critical to the use of PARPi is the determination of the role of oxygen in predicting patient response. Enthusiasm for the use of PARPi to target hypoxic tumours came from the Hammond and Bristow labs description of "contextual synthetic lethality" which refers to severely hypoxic conditions (<0.5% O_2) where repression of HR leads to increased sensitivity to PARPi. However, recent data from the Giaccia lab demonstrate that cells experiencing more common milder levels of hypoxia are resistant to PARPi. Importantly, *in vitro* studies

demonstrating that hypoxic (2% O_2) cells are resistant to PARPi are supported by *in vivo* studies. To further increase the physiological relevance of their study, the efficacy of Olaparib in a range of breast cancer patient-derived tumour xenografts (PDTX) was determined. A previously validated, robust hypoxia signature was used to reveal a strong inverse correlation between hypoxia score of the tumours and their sensitivity to Olaparib. PDTXs with higher hypoxic scores were the most resistant to PARPi therapy based on changes in tumour growth. Thus, both tumour cell derived xenografts as well as PDTXs indicate that hypoxia was linked with decreased PARPi efficacy. Finally, data from collaborator, Dr Jeff Chang (MD Anderson), demonstrates that tumour hypoxia correlates with a resistance to PARPi in human tumour RNA samples treated with Olaparib. These data underpin the proposed studentship which will focus on the following objectives:

1. Rigorous investigation of the role of ROS in the oxygen-dependent response to PARPi. This aim will benefit from novel probes to measure ROS available through collaboration (redOx-KCL). This approach will test the hypothesis that ROS levels determine response to PARPi.
2. Identification of tumours with the levels of hypoxia likely to respond to PARPi. Using bioinformatic analysis of patient samples we plan to generate a predicative tool for response to PARPi.
3. Increasing PARPi sensitivity through manipulation of oxygen consumption. Using agents including atovaquone in collaboration with Prof Geoff Higgins.
4. Combination of PARPi with FLASH radiotherapy to reduce normal tissue toxicity. Current studies are investigating the combination of PARPi and radiotherapy, but normal tissue toxicity could be limiting. We hypothesise that FLASH radiotherapy, which significantly reduces normal tissue toxicity by inducing transient hypoxia, would be efficacious when combined with PARPi alone or potentially in combination with hypoxia modifying therapy.

Translational potential

The translational potential of this work is the extended use of PARP inhibitors clinically. This potential includes both a personalised approach to identifying who would and would not benefit from the addition of PARPi and the identification of novel strategies to use PARPi in combination with other therapies, for example radiotherapy.

Training opportunities

The supervisors and collaborators listed are all experienced DPhil supervisors and have a strong track record of working together to deliver translationally focused projects with the potential to impact patient care.

References

Identification of biomarkers of response to preoperative talazoparib monotherapy in treatment naïve gBRCA+ breast cancers. Liu X, Ge Z, Yang F, Contreras A, Lee S, White JB, Lu Y, Labrie M, Arun BK, Moulder SL, Mills GB, Piwnica-Worms H, Litton JK, Chang JT. NPJ Breast Cancer. 2022 May 10;8(1):64.

Eliminating hypoxic tumor cells improves response to PARP inhibitors in homologous recombination-deficient cancer models. Mehibel M, Xu Y, Li CG, Moon EJ, Thakkar KN, Diep AN, Kim RK, Bloomstein JD, Xiao Y, Bacal J, Saldivar JC, Le QT, Cimprich KA, Rankin EB, Giaccia AJ. J Clin Invest. 2021 Jun 1;131(11):e146256.

Contextual synthetic lethality of cancer cell kill based on the tumor microenvironment. Chan N, Pires IM, Bencokova Z, Coackley C, Luoto KR, Bhogal N, Lakshman M, Gottipati P, Oliver FJ, Helleday T, Hammond EM, Bristow RG. Cancer Res. 2010 Oct 15;70(20):8045-54.

19. One-Step Adaptive Radiotherapy Planning using Deep Reinforcement Learning[4] – Prof. Hawes

Primary Supervisor: Prof. Nick Hawes
Additional Supervisors: Prof. Katherine Vallis
Eligibility: Track 4 students are eligible to apply for this project.

Lay Summary
The goal of radiotherapy (RT) is to maximise the dose of radiation delivered to target regions in the body, whilst minimising the dose to surrounding areas and organs at risk (OAR). A radiotherapy plan is a comprehensive, machine-specific specification of the prescribed radiotherapy to be delivered to a given patient. A patients' radiotherapy plan requires many considerations and, critically, adherence of radiotherapy delivery to pre-defined criteria has been demonstrated to have a significant beneficial impact on the patient's outcome. Currently a multi-step workflow is used to produce an individualised RT dose plan for a patient, a process that can take days to several weeks from the time of their "simulator" CT/PET scan. The workflow consists of a time-consuming sequence of semi-automated or manual processes performed on the initial scan: delineation of relevant structures including target tumour volumes, RT plan generation, RT plan adjustments and approval/ clinical sign off. Delays persist between the different specialists who perform each task. This convoluted process eventually produces a deliverable RT plan considered adequate relative to institutional or national RT protocols. Auto-contouring systems based on artificial intelligence (AI) are popular across clinics to save clinician's time when contouring images. They have succeeded in providing proof-of-concept that AI can learn representations to automatically target specific regions in CT/PET scans, such as organs at risk (OARs) and, to a limited extent, tumours.

This interdisciplinary project will deliver a step change in how radiotherapy planning is performed - compressing the process into a single step that can be achieved in hours not weeks. Computer vision and machine learning will be used to develop methods that can automatically predict deliverable and optimal RT plans directly from a patient's planning CT scan, without the need for segmentation, significantly reducing the time between "decision to treat" and the first radiotherapy fraction. Radiotherapy planning will be formulated as a "decision making under uncertainty" problem, where an AI agent can train itself to predict RT plans, receiving a reward training signal depending on the quality of the plans it produces – a form of "self play". This will shift the focus of AI from image segmentation systems (which still requires manual input by clinicians to produce the final plan), to fully-automated RT planning. This will significantly reduce the delay between initial scan and dose delivery, from weeks to hours, resulting in reduced patient anxiety and better patient outcomes.

Research objectives
The main project objective is to deliver a novel method for radiotherapy planning directly from images, which we refer to as "no-contour RT". There has been considerable effort to automate contouring of CT scans using neural networks[3], predict the ideal RT dose directly [7], and also enhance images from low-dose CT to more high resolution images. However, there is little-to-no research connecting the entire radiotherapy workflow with an autonomous decision-making agent capable of learning from images through self-play [8]. Using state-of-the-art advancements in AI and ML this project will present a new research direction for radiation oncology planning, resulting in academic value with interdisciplinary collaborations and publications at leading radiotherapy, medical physics and machine learning venues, and high potential for knowledge transfer.

Objective 1: The primary objective of this project is to develop no-contour RT methods using machine learning directly from 3D CT images. This DPhil project will specifically consider how to incorporate human-in-the-loop learning into the self-play in order to learn human preferences and share autonomy [9].

Objective 2: Multi-objective and constrained learning methods that would allow oncologists to modify a target dose, inputting additional preferences after training, and for rapid re-computation to the new constraints.

Objective 3: On Interpretability: Investigate methods to make such a system interpretable and able to describe its predictions to clinicians, i.e., by additionally predicting auxiliary information, such as the contours or regions of interest.

Translational potential
This project is a novel collaboration and aligns within the "cancer big data" scientific theme of the CRUK Oxford Cancer Centre's research strategy. The aim is to use previously captured patient data to develop methods to rapidly predict a patients' personalised RT plan through AI and ML. This will significantly reduce the time a patient is likely to wait between

their initial CT scan and their radiotherapy dose delivery. The major benefits of this approach are: 1) Reducing the likelihood of progressive cancer while waiting to start radiotherapy, (2) reducing patient anxiety, (3) reducing workload in the oncology department, (4) allowing the radiotherapy team to allocate more of their time and resources to the most complex cases and other tasks.

Training opportunities

This is an interdisciplinary project between the Departments of Engineering Science and Oncology. The DPhil student will have the opportunity to work closely with ML experts in Engineering and with radiation oncology clinicians and physicists.

The supervisory team will consist of Prof Hawes, Oxford Robotics Institute, Department of Engineering Science, who brings experience in AI, planning under uncertainty, human-in-the-loop methods, and extensive experience mentoring DPhil students; and Prof Vallis, Oxford Institute for Radiation Oncology, who brings clinical knowhow to the project and who will ensure the student has close ties to the Department of Oncology, Oxford University Hospitals NHS Foundation Trust. Senior PDRA, Dr Duckworth, additionally brings ML, reinforcement learning and computer vision experience to the supervision team. The student will be expected to attend relevant seminars within the department, and Masters and CDT level MPLS lectures will be available and recommended, alongside weekly reading groups and supervision meetings. Further specialist training can be achieved via close links to Clinical Oncology, Oxford University Hospitals NHS Trust, and AI summer schools and departmental activities

References

1. B. O'Sullivan et al. Critical Impact of Radiotherapy Protocol Compliance and Quality in the Treatment of Advanced Head and Neck Cancer: Results From TROG 02.02. J. Clin. Oncol. 2010.
2. T. Bortfeld. IMRT: A Review and Preview. Article In Phys. Med. Biol. 51 (2006) R363–R379.
3. DCL Expert AI, Mirada Medical Ltd. Date Accessed 2022. https://mirada-medical.com/product/mirada-dlcexpert-ai-autocontouring
4. M. Trimpl, et. al. Beyond Automatic Medical Image Segmentation – the spectrum between fully manual and fully automatic delineation. Published by IOP Publishing Ltd. May 2022
5. A. Kolobov, "Planning with Markov decision processes: An AI perspective." Synthesis Lectures on Artificial Intelligence and Machine Learning 6, no. 1: 1-210, 2010.
6. R. Sutton and A. Barto, Reinforcement Learning: An Introduction. Second edition. c 2014, 2015. A Bradford Book. The MIT Press.
7. J. Yang et al. Auto-segmentation for Thoracic Radiation Treatment Planning: A Grand Challenge at AAPM. In Med Phys. 45(10): 4568–4581. 2017
8. Jarrett et al. Applications and Limitations of Machine Learning in Radiation Oncology. Br J Radiol.2019;92(1100):20190001
9 M. Rigter, B. Lacerda, & N. Hawes. A Framework for Learning from Demonstration with Minimal Human Effort. IEEE Robotics and Automation Letters (RA-L) 2020.

20. Utilising MR linear accelerator technology to develop spatially fractionated (lattice) radiotherapy [1] – Prof. Higgins

Primary Supervisor: Prof. Geoff Higgins
Additional Supervisors: Dr. Kristoffer Petersson
Eligibility: Track 1 students are eligible to apply for this project.

Lay Summary
Magnetic Resonance Linear Accelerators (MRL) represent state of the art radiotherapy machines. They have the unique ability to image and identify tumours, whilst simultaneously delivering highly accurate radiation therapy. This project seeks to utilise these features to assess whether MRLs are able to advance radiotherapy delivery beyond their current capabilities. In particular, we propose to assess whether MRLs have the technical capability to treat small sub-regions inside the tumour with extremely high doses of radiotherapy, without increasing the radiotherapy dose to the rest of the tumour. Side-effects from radiotherapy are caused by healthy tissues which surround the tumour unavoidably receiving a dose of radiation. Importantly, this new technique would not increase the dose of radiotherapy that the healthy tissues receive. The number of radiotherapy treatments given would also be unaltered compared with conventional treatment.

This technique is called Lattice RT and has been associated with improved clinical outcomes. The reasons for this are unclear, but it has been suggested that the high radiation doses given to small regions within the tumour might initiate an immune response against the cancer. In addition to developing Lattice RT, we propose to see whether the MRL is able to detect regions of the tumour that have very low levels of oxygen (hypoxia) using a new technique called oxygen-enhanced MR imaging (OE-MRI). Hypoxia frequently occurs in most cancers, and is associated with profound resistance to radiotherapy treatment. Being able to effectively identify hypoxic, radiation resistant regions of the tumour with OE-MRI, and then using Lattice RT to deliver very high radiation doses to the hypoxic regions would extend the capabilities of MRL machines beyond their current uses and would be expected to lead to potential benefits for cancer patients receiving radiotherapy.

Abstract
Conventional fractionated radiotherapy involves the homogenous delivery of radiation across the entire tumour in 2 Gy fractions. Recently, there has been significant interest in delivering non-uniform “spatially fractionated” radiotherapy (SFRT) particularly for bulky, locally advanced tumours that usually do not respond well to conventional radiotherapy, without increasing the side-effects of treatment. SFRT typically involves delivering extremely high doses of radiotherapy (up to 20 Gy) to multiple, small spherical subregions within the tumour, whilst ensuring that the dose to the periphery of the tumour, and to the surrounding organs at risk are within standard limits. It has been postulated that the high tumour control rates that have been reported with SFRT might be due to anti-tumour immune responses, triggered bystander effects, or changes in the tumour vasculature caused by the peak radiation doses.

The advent of Magnetic Resonance Linear Accelerators (MRL) has increased the ability to accurately deliver radiotherapy to moving tumours and facilitate real time treatment adaptation. This proposal seeks to implement SFRT using a MRL machine. In addition, we will seek to further develop the capabilities of MRL technology by assessing whether low field strength MR imaging is able to effectively identify regions of tumour hypoxia, using a relatively new technique called oxygen-enhanced MR imaging.

Research objectives
The Oxford Institute for Radiation Oncology has a well-established partnership with GenesisCare that provides access to a clinical Viewray MRL which combines a 0.35 Tesla MR scanner with a 6MV photon linear accelerator and offers a potentially ideal opportunity to accurately implement SFRT. This project will seek to assess the capability of delivering SFRT to bulky tumours using the Viewray MRL (Fig 1). In addition, we will investigate the functional imaging capability of the MRL in detecting hypoxic regions within the tumour using oxygen enhanced MR imaging (OE-MRI). This technique has been developed as a non-invasive approach to measure the dynamics of tumour oxygenation. Previous attempts at boosting the dose of radiotherapy to hypoxic regions have not been able to obtain real time assessment of tumour hypoxia, and have only been able to deliver very modest increases in radiotherapy dose. The potential to utilise modern MRL delivery techniques to cause a far greater increase in radiation doses might represent a step change in our ability to overcome hypoxia mediated radioresistance.

The first part of the project will involve radiotherapy planning studies to develop the optimal methodology to deliver MRL based SFRT and will include analysis of the position, size and dose of high dose "spheres".

Subsequently, we will open a clinical study combining SFRT and OE-MRI in patients with locally advanced disease that cannot be treated with radical radiotherapy. This would be expected to include patients with large volume non-small cell lung cancer who are currently treated with high-dose palliative radiotherapy (39 Gy in 13 fractions). This study will also incorporate exploratory imaging biomarkers of early response to treatment, in addition to circulating markers of inflammation, immune activation and tumour response.

This project will centre on an academic collaboration between experts in radiation oncology (Geoff Higgins) and medical physics (Kristoffer Petersson) and will involve detailed radiotherapy planning studies and clinical implementation.

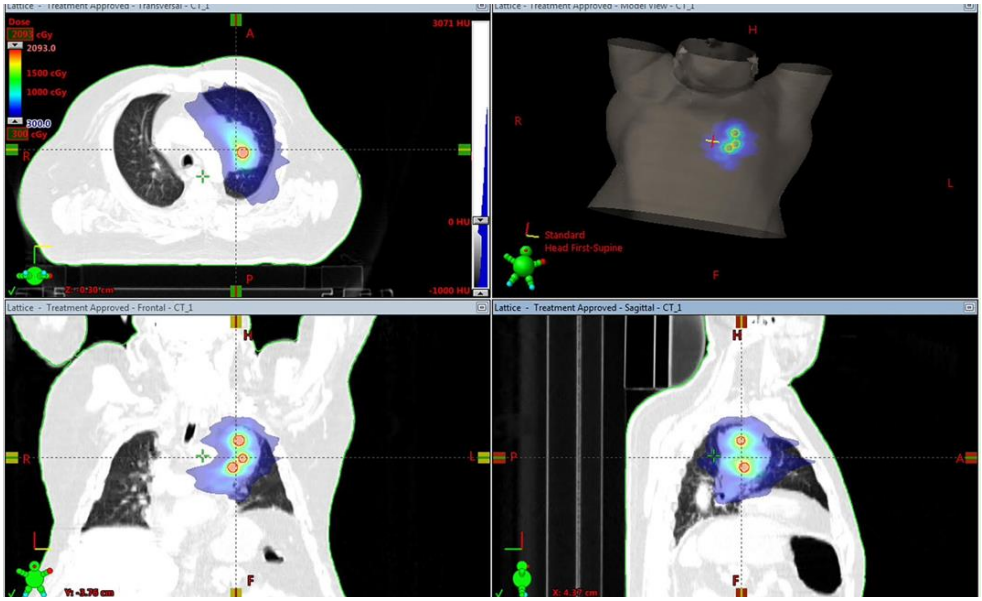


Fig. 1. Illustration of a SFRT treatment plan. Clockwise from top left; axial, model, coronal and sagittal views of colour wash dose distribution of 3 Gy around the tumour periphery and 18 Gy in the three 1.5 cm diameter dose peak spheres within the tumour. Adapted from (3).

Translational potential

The development of MRL machines has advanced the capability to deliver radiotherapy in ways that have not previously been possible. SFRT has the potential to improve radiotherapy outcomes for patients with large tumours that are not amenable to conventional radical radiotherapy and is particularly suitable for MRL based delivery. Combining SFRT with an OE-MRI approach to target hypoxic regions might result in improvements for cancer patients treated with radiotherapy.

Training opportunities

This project is suitable for a clinical oncology trainee interested in pursuing a career as a clinical academic. In addition to the successful candidate gaining expertise in MR guided radiation therapy delivery, they will also be taught advanced radiotherapy planning methodology, functional MR image analysis and clinical trial experience.

References

1) Yan et al. Spatially fractionated radiation therapy: History, present and the future. CTRO 20: 30-38 (2020). **2)** Wi et al. The Technical and Clinical Implementation of LATTICE Radiation Therapy (LRT). Radiat Res 194(6): 737-746 (2020). **3)** Amendola et al. Improved outcome of treating locally advanced lung cancer with the use of Lattice Radiotherapy. CTRO 9: 68-71 (2018). **4)** Salem et al. Oxygen-enhanced MRI Is Feasible, Repeatable, and Detects Radiotherapy-induced Change in Hypoxia in Xenograft Models and in Patients with Non-small Cell Lung Cancer. Clin Can Res 25(13): 3818-3829 (2019). **5)** O'Connor et al. Oxygen-Enhanced MRI Accurately Identifies, Quantifies, and Maps Tumor Hypoxia in Preclinical Cancer Models. Cancer Res. 76(4): 787-95.

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21. Vesicle transport of cancer invasion-promoting proteinase to the leading edge: a crucial mechanism of cancer invasion [1,2,3] – Associate Prof. Itoh

Primary Supervisor: Associate Prof. Yoshifumi Itoh
Additional Supervisors: Associate Prof. Marco Fritzsche
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Lay Summary

Cancer invasion is a fundamental cause of metastasis, a life-threatening feature of the disease. Usually, cell migration between the different tissue compartments and organs is prevented by complex tissue components called extracellular matrix (ECM), a physical barrier for cell migration. However, invasive cancer cells effectively degrade ECM by using an enzyme called MT1-MMP, creating a migration path and eventually metastasising to other organs1. When cancer cells invade, they always position the MT1-MMP to the invasion front of the cell, but it has not been understood how. We have recently discovered that MT1-MMP is brought to the invasion front by trafficking inside of the cells and identified two proteins responsible for this process2. Abolishing these two proteins to act on MT1-MMP effectively inhibits cancer invasion2. Therefore, we propose to further elucidate the molecular mechanism of these two proteins. Achieving this project will deepen our understanding of cancer invasion and contribute to developing a novel “anti-invasion therapy” in the future.

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

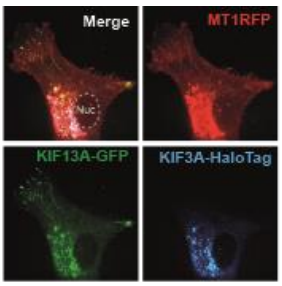


Fig. 1. Both KIF3A-HaloTag and KIF13A-GFP colocalise with MT1-RFP vesicles at perinuclear area, while only KIF13A-GFP-positive MT1-RFP were found at cell periphery

MT1-MMP localisation at the leading edge, a crucial process for cancer cell invasion. Therefore, the objectives of this DPhil project are following:

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.

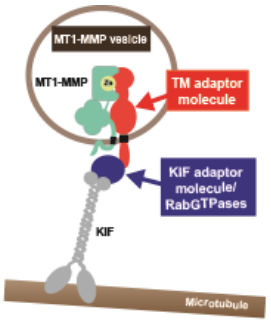


Fig. 2. Possible involvement of a trans-membrane (TM)-adaptor molecule and a KIF adaptor molecule(s) for MT1-MMP-specific vesicle transport

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 no drug is available targeting the invasion process. The cancer cell invasion ability relies on ECM degradation, which allows cancer cells to migrate and modify their microenvironment to their liking. MT1-MMP is a crucial proteinase that promotes invasion, but it is still unclear how MT1-MMP localises to the leading edge for invasion. The outcome of this project would help us understand the invasion process of soft tissue sarcoma that we use as a model in this project and 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 a novel therapeutic intervention.

Training opportunities

We are based at the Kennedy Institute, a world-renowned research centre with a state-of-the-art 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 are expected to participate in 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 in-depth experience and knowledge of cell biology, proteomics, molecular biology, super-resolution and high-resolution live cell imaging microscopies and analyses.

References

(1) Gifford, V.; Itoh, Y. MT1-MMP-dependent cell migration: proteolytic and non-proteolytic mechanisms. *Biochem Soc Trans* **2019**, *47* (3), 811-826, Review Article. DOI: 10.1042/BST20180363.

(2) Gifford, V.; Woskowicz, A.; Ito, N.; Balint, S.; Lagerholm, B. C.; Dustin, M. L.; Itoh, Y. Coordination of two kinesin superfamily motor proteins, KIF3A and KIF13A, is essential for pericellular matrix degradation by membrane-type 1 matrix metalloproteinase (MT1-MMP) in cancer cells. *Matrix Biol* **2022**, *107*, 1-23. DOI: 10.1016/j.matbio.2022.01.004.

(3) Sears, R. M.; May, D. G.; Roux, K. J. BioID as a Tool for Protein-Proximity Labeling in Living Cells. *Enzyme-Mediated Ligation Methods* **2019**, *2012*, 299-313. DOI: 10.1007/978-1-4939-9546-2_15.

22. Adipocytes as a source of nutrition for breast cancer cells [1,2,3,4] – Associate Prof. Kriaucionis

Primary Supervisor: Associate Prof. Skirmantas Kriaucionis
Additional Supervisors: Dr Simon Lord, Prof. Robin Klemm
Eligibility: Track 1, 2, 3 and 4 students are eligible to apply for this project.

Lay Summary

The human breast is predominantly made up of fat cells (adipocytes) and interest is growing in the role of these cells to provide the nutrients needed for breast cancer growth. If we can understand these interactions, there is the possibility of blocking these processes as a treatment for breast cancer. In this study we shall grow breast cancer cells next to fat cells in the laboratory so that we can see how the character of both the fat and tumour cells change when grown together compared to when they are grown in isolation. We shall also use a special technique called mass spectrometry which can allow us to trace how fat from the fat cells might be used as a building block for tumour cell growth. Differences in the genetic character of fat cells from patients will be investigated by taking samples at surgery and analysing their genetic make-up. Here, fat cells in the breast with the cancer will be compared with those taken from the uninvolved breast to see if there are differences in the character of fat cells that are near the cancer and those that are not.

Abstract

Adipocytes are one of the primary stromal cells in many tissues and adipocyte-cancer cell crosstalk has been shown to enhance tumour progression in preclinical models. There is potential to target these interactions for therapeutic benefit following greater characterisation of these complex relationships. We hypothesise that breast adipocytes within the tumour microenvironment display an altered phenotype that enables extraction of nutrients by tumour cells. To investigate this supposition, we plan to characterise adipocytes from the tumour microenvironment and use matched 'normal' control adipocytes extracted from the non-involved breast (both taken during breast surgery). The Kriaucionis laboratory has already established single cell sequencing techniques and primary cell culture techniques using human breast cancer and breast adipose samples, alongside mass spectrometry techniques to trace fatty acid utilisation as a carbon source for anabolic synthesis of macromolecules. Working with the Robin Klemm group (DPAG) we shall use co-culture of fat from patient breast tissue (both involved and control non-involved breast from the same patient) with breast cancer cell lines to assess phenotypic/transcriptomic changes in both cell lines and adipocytes. Mass spectrometry will be used to trace carbon transfer from free fatty acids derived from adipocytes treated with 13C-labelled fatty acid substrates to macromolecules in tumour cells including nucleotides. Complementary work will use single cell RNASeq to characterise the transcriptome of adipocytes in the tumour microenvironment and compare with adipocytes derived from a paired 'normal' fat sample from the non-involved breast. Genetic silencing approaches and isogenic cell lines, based on the single sequencing data described as above will be used to identify phenotypic differences in tumour cells in obesity that may alter adipocyte behaviour and transfer of free fatty acids to aid tumour growth. Primary culture of breast tumours will be used to validate the experiments in cell lines.

Research objectives

1. **Co-culture of adipocytes and breast tumour cells**
   Here, the student will investigate how breast tumour cells may utilise lipid from adipocytes in the tumour microenvironment. Working with Robin Klemm's group in DPAG the student will develop co-culture models of fat from patient breast tissue from paired involved (ipsilateral) and non-involved (contralateral) breast from the same patient with breast cancer cell lines. The student will use RNASeq approaches to assess the phenotypic / transcriptomic changes in both cell lines and adipocytes. Mass spectrometry will be used to trace carbon transfer from free fatty acids derived from adipocytes treated with 13C-labelled fatty acid substrates to macromolecules in tumour cells including nucleotides. Genetic silencing approaches and isogenic cell lines, in part informed by the single sequencing data derived as described below will be used to identify phenotypic differences in tumour cells that may alter adipocyte behaviour and transfer of free fatty acids to aid tumour growth. Primary culture of breast tumours will be used to validate the experiments in cell lines. In particular, comparisons will be made between differences in gene expression between primary culture samples when co-cultured with adipocytes sampled from the ipsilateral and contralateral breasts from the host patient.

2. **Single cell sequencing of adipocytes from oestrogen receptor positive breast cancer samples**
   Here, the student will investigate the transcriptomic signature of adipocytes in the tumour microenvironment. Single cell sequencing provides far greater phenotypic resolution than bulk sequencing and the Kraucionis laboratory has established and already piloted a protocol using a microfluidic technique that allows capture of nuclei from adipocytes from clinical breast tumour samples. The student will use single cell RNASeq to provide characterisation of adipocytes of the tumour microenvironment and compare with adipocytes derived from a fat sample from the contralateral breast. Ten obese patients and five of normal weight undergoing surgery for primary breast cancer will have samples collected (oestrogen receptor positive and HER2 negative tumours from postmenopausal women). Detailed anthropometric measurement of patients will include body composition analysis using gold standard dual-energy X-ray absorptiometry scans. The breast tumour samples will then be prepared in real time for single cell RNA sequencing alongside matched 'normal' controls using adipocytes extracted from biopsy material taken from the contralateral breast. Focus will be placed on determining expression of genes encoding fatty acid transporters and regulatory checkpoints of fatty acid oxidation in epithelial cells and enzymes regulating lipolysis in adipocytes.

Translational potential
This project will interrogate novel mechanisms of tumour progression with potential therapeutic application. using human samples already being collected in an ethically approved study. Overtures to pharmaceutical industry entities are already in play with a view to long-term partnership in this field.

Training opportunities
The student will be mentored via weekly one-to-one meeting with the scientific supervisor. They will regularly present their results at the Kriaucionis lab meetings and will benefit from a range of cutting-edge bioinformatics and laboratory tools and training. They will have the opportunity to gain skills in basic laboratory skills, primary tissue culture, interpretation of mass spectrometry data, and single cell sequencing. Additionally, the student will have the opportunity to develop clinical study protocols and use of human samples under supervision of Dr Lord. The candidate will spend time in the laboratory of Robin Klemm, to develop an understanding of novel adipocyte assays and additional knowledge of adipocyte biology (https://www.dpag.ox.ac.uk/team/robin-klemm).

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

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

Lay Summary

Cancers often have defects in processes that repair damage to their genetic material and survive by becoming 'addicted' to mechanisms that compensate for loss of these pathways. This is an Achilles heel of the tumour because drugs that interfere with these compensatory mechanisms kill cancer cells. Principal to this strategy for cancer treatment is the use of medicines that target enzymes called PARPs. PARPs are critical for the survival of cells with DNA defects in breast and ovarian cancer genes, so PARP inhibitors can be used to kill these tumours.
Unfortunately, the effectiveness of PARP inhibitors differs between patients and tumours eventually become resistant to this treatment. The reasons for this are not well understood. To address this, our goal is to identify molecular indicators that will let doctors know whether a tumour will respond to PARP inhibitors, in addition to other enzymes to inhibit to kill tumours that are resistant to PARP inhibitors. To this end, we discovered that PARP inhibitors kill cancer cells by disrupting mechanisms that repair damaged DNA when it is being copied. We will extend these studies to decipher how this process works. Also, building on our work that identified enzymes that are controlled by PARPs, we will investigate which of these become over-active in tumours to cause PARP inhibitor resistance.

These approaches will provide critical information to improve cancer treatments by identifying markers to predict whether a patient will respond to PARP inhibitors, in addition to enzymes that when inhibited will kill PARP inhibitor-resistant tumours.

Abstract

Tumours often lose DNA repair mechanisms and PARP inhibitors (PARPi) are being used to treat BRCA1/2-deficient tumours with defects in homologous recombination (HR). However, the response of tumours to PARPi is variable and multiple resistance mechanisms have been identified. Understanding how PARPs regulate DNA repair to maintain survival of HR-defective cells will identify biomarkers to predict PARPi sensitivity and alternative pathways to target in PARPi-resistant tumours. The overall goal of this research is to address these important questions by characterising novel pathways and factors regulated by PARPs in cancer cells. By combining our expertise in studying PARP biology[1-6] with cutting edge genome editing and proteomics, we identified substrates ADP-ribosylated by PARP1/2 that promote survival of BRCA1-deficient cells by regulating replication fork recovery by break-induced replication (BIR). Building on these key conceptual advances we will define the mechanistic basis of how BIR maintains viability of BRCA-defective cells, in addition to site specific ADP-ribosylation (ADPr) events that are upregulated and synthetic lethal in BRCA-deficient cells. These studies will improve detection/diagnosis and refine cancer treatments by identifying biomarkers to predict PARPi sensitivity and factors to target in PARPi-resistant tumours.

Research outcomes

Background: The current paradigm for synthetic lethality between PARPi and HR is that inhibition of PARPs results in unrepaired SSBs, or trapping PARP1 at these lesions. Collision of the DNA replication machinery with these structures causes replication fork stalling/collapse that requires HR-mediated repair[7]. More recently, the findings that PARPi induce ssDNA gaps in *BRCA*-deficient cells due to an inability to facilitate nascent strand maturation has led to another model to explain PARPi toxicity in BRCA-deficient cells[8]. HR-mediated replication repair mechanisms are central to both these models. However, other replication fork recovery pathways are utilized in the absence of HR, including Rad52-dependent break induced replication (BIR)[9-11]. How these processes are controlled, whether PARP1/2 contributes to this regulation, and how this integrates into models of synthetic lethality with PARPi is poorly understood.

Our recent data address these key conceptual questions by illustrating PARP1/2 are required for survival of BRCA-deficient cells by regulating Rad52-dependent BIR. Additionally, by performing cutting-edge mass spectrometry to identify ADPr targets induced in response to replication stress, we uncovered a critical role for ADPr of PolD3 in this

process. Together, these data not only inform how PARPs regulate replication fork recovery in BRCA1/2-defective cells, but also provide a valuable resource to identify novel synthetic lethal interactions with HR-deficiency. The overall goal of this research is to exploit these resources to define how PARPs regulate replication fork recovery and survival of HR-defective cells. This will be achieved by:

1. Assessing the requirement for BIR in maintaining viability of BRCA1/2-defective cells
2. Characterising the role of site-specific ADPr events in replication fork recovery and survival of BRCA1/2-defective breast cancer cells

1. Assessing how BIR maintains cell viability of BRCA1-defective cancer cells: Having established that PARP1/2 maintain viability of BRCA1-deficient cells by regulating site specific ADPr of PolD3, we will now establish whether this interaction is driven through an inability to repair stalled/damaged replication forks, or defective post-replicative gap-filling repair. To achieve this we will exploit *BRCA1*^{SMASh} cells in which we have inserted a SMASh degron[12] onto the *BRCA1* gene to allow chemical depletion of BRCA1 in different genetic backgrounds. High-content imaging of DNA damage (e.g. γH2AX) combined with cell cycle markers will establish whether loss of BRCA1 and PolD3-ADPr mutants in combination induces DNA damage during DNA replication. In parallel, DNA fibre analysis will establish replication fork speeds and whether cells display increased stalled forks, reflecting an inability to recover from replication stress. Using modified DNA fibre assays to detect post-replicative ssDNA gaps, we will assess whether PolD3-ADPr mutants induce replication associated DNA gaps and if this is exacerbated on depletion of BRCA1. Cell viability assays will determine whether increased gap formation correlates with Rad52-toxicity in these cells. The *BRCA1*^{SMASh} cells will allow a rapid and well controlled system to identify key concepts that underpin the synthetic lethal interactions between BIR and HR that we will then explore in a more clinically relevant context, including a variety of BRCA1/BRCA2 cancer cell lines.

2. Characterising the role of ADPr in replication fork recovery and survival of BRCA1/2-defective cells
Given toxicity of PARPi is driven by replications stress, our identification of PARP1/2 substrates targeted in response to replication fork stalling/collapse provides us with a unique resource to screen for ADPr events that are upregulated to promote survival of BRCA-deficient cells. We will perform a secondary screen to verify whether the top hits from our analysis are ADPr in response to replication stress. The ability to affinity purify ADPr proteins from HU treated extracts and western blotting using antibodies against putative targets will allow us to screen for multiple targets relatively easily. Targets that come through this screen will be further validated by assessing HU-induced ADPr of endogenous or lentiviral expressed epitope-tagged proteins by IP and western blot using reagents that detect mono- or poly-ADPr. Using this technology, we will also test whether ADPr of targets is upregulated in a variety of BRCA-deficient cells. Validated PARP targets that are upregulated will be assessed for a synthetic lethal interaction with BRCA1/BRCA2 using siRNA in *BRCA1*^{SMASh} and BRCA-deficient cancer cells. We will also determine whether any synthetic lethal interactions identified extend to the site-specific ADPr of the protein by assessing whether expressing the relevant mutants is toxic in *BRCA1*^{SMASh} or BRCA-deficient cancer cells. In the longer term, the mechanistic basis of this interaction will be determined using approaches above (Section 1).

Translational potential
This work will provide key information to underpin improved detection/diagnosis and treatment of tumours by: 1) identifying biomarkers that predict PARPi sensitivity; 2) Establishing novel synthetic lethal targets to treat HR-defective and PARPi-resistant tumours.

Training opportunities
Training will be provided in a variety of techniques including genome editing, high resolution/content microscopy and cell-based assays to assess DNA replications and repair.

References
1. J. Brustel, et al. *Nature Communications* 13: (2022). **2**. G. E. Ronson, et al. *Nat Commun* 9: 746 (2018). **3**. A. L. Kolb, et al. *Nucleic Acids Res* 45: 10056-10067 (2017). **4**. A. Rakhimova, et al. *Sci Rep* 7: 43750 (2017). **5**. C. A. Couto, et al. *J Cell Sci* 126: 3452-61 (2013). **6**. C. A. Couto, et al. *J Cell Biol* 194: 367-75 (2011). **7**. A. Ashworth and C. J. Lord. *Nature Reviews Clinical Oncology* 15: 564-576 (2018). **8**. M. P. Dias and J. Jonkers. *Molecular & Cellular Oncology* 8: (2021). **9**. S. K. Sotiriou, et al. *Mol Cell* 64: 1127-1134 (2016). **10**. D. Lemacon, et al. *Nat Commun* 8: 860 (2017). **11**. S. Mijic, et al. *Nat Commun* 8: 859 (2017). **12**. H. K. Chung, et al. *Nat Chem Biol* 11: 713-20 (2015).

24. Spatial interrogation of low grade prostate cancer to identify genomic events responsible for driving indolent not aggressive disease[1,2,3,4] – Dr. Lamb

Primary Supervisor: Dr. Alastair Lamb
Additional Supervisors: Prof. Ian Mills
Eligibility: Track 1, 2, 3 and 4 students are eligible to apply for this project.

Lay Summary
Prostate cancer is not like some cancers that are universally dangerous. Many men with prostate cancer do not need treatment. We are increasingly confident that there are a group of men with low grade cancer that do not need treating. But we think that some men with higher grade cancer could also be managed in this way. It would be useful to undertake a detailed genetic analysis of low grade cancer to identify whether there are some genes that are activated (or turned off) which are able to keep the cancer in check. If so, we could look for these genes in higher grade cancer to select men for conservative management. Until recently, techniques made it hard to tease apart the information from low and high grade cancer cells as all the tissue was analysed in bulk. But we now have new 'spatial genomic' techniques for analysing the genetic changes in small numbers of cancer cells while still in situ. This is therefore a good time to do this project.

Abstract
Background: Over the past decade we have increasingly accepted that prostate cancer classified as "low risk" (Gleason Grade Group 1 (Gleason Score 3+3 = 6), PSA <10, Stage T1-T2a) are indolent and do not progress.[1] This has led to a recommendation for most men with this type of prostate cancer to undergo active surveillance.[2,3] Indeed, opinion leaders in prostate cancer are increasingly calling for Grade Group 1 prostate cancer to cease being called "carcinoma".[4,5] However, we know little about the genetic composition of such low grade tumours, largely because research has focussed on higher grade disease, but also because we have lacked the spatial genomic technologies to carefully interrogate discrete selected areas of prostate tissue. Those technologies are now available[6,7] and have been developed to a point where the clonal copy number composition of epithelial regions can be defined,[7,8] although they still lack spatial epigenomic analyses.
In our organscale study of a prostate removed at radical prostatectomy which had multifocal prostate cancer, we identified an area of Gleason Grade Group 1 prostate cancer which lacked most of the defining somatic mutations of higher grade disease (e.g. chr8q gain corresponding to well-known prostate cancer oncogene c-Myc[9], or chr10p loss corresponding to tumour suppressor gene PTEN[10]; **Figure 1**). This raises the possibility that low grade cancer is fundamentally distinct from higher grade disease at a genetic level. We also found that areas of non-transformed 'benign' epithelia contained areas of greater genomic risk than low grade cancer. Perhaps low grade cancer is therefore 'safer' than benign epithelia which still retains the potential to undergo somatic mutations that develop aggressive disease.

Questions: Why do low grade Gleason pattern 3 prostate cancers seem not to progress? How can we better differentiate "good" from "bad" pattern 4 disease? Can we drive "bad" pattern 4 disease to indolence?

Hypothesis: Low grade prostate cancer clones harbour genomic alterations which pre-determine indolence; when present in higher grade clones, such alterations differentiate high grade disease with good prognosis.

Research objectives

1. Consolidate: To undertake clonal siCNV analysis[7] on a selection of men with pure Gleason pattern 3 prostate cancer (n=5; from archive as we no longer operate on these men) extending the findings of our recent paper in Nature, Erickson et al[7], to identify "indolence factors" when benchmarked against pattern 4 & 5 [tissue handling; spatial transcriptomics; bioinformatics]
2. Extend: To track clonal composition of Gleason pattern 3 and 4 cancer in serially sampled MRI-targeted biopsies from men on active surveillance for "low" and "intermediate risk" prostate cancer (n=5 each) [spatial transcriptomics; DNA sequencing; epigenomics; bioinformatics]
3. Establish phenotype: To genetically modify cell-line models of aggressive PCa (e.g. PC3, DUCaP) to upregulate indolence factors as a pre-clinical basis for pharmacological manipulation [cell culture; cloning; lentiviral transduction; functional assays]

Translational potential

The data produced could provide the evidence our field is looking for to underpin a redefining of low grade prostate cancer, as well as helping us understand what makes some pattern 4 prostate cancers so much worse than others. This could help a proportion of men who currently undergo radical therapy (currently approx. 25,000 pa in UK) to avoid the side-effects of such treatment.

Training opportunities

The project incorporates a range of wet and dry lab techniques (see [] brackets above) and can be tailored, given our overall lab skill sets, to someone with an interest in biology or computational work. We will also train the individual, whatever their background, in our overall approach to translational surgical science with opportunities to work with surgical oncologists, pathologists and radiologists as well as biologists and data scientists.

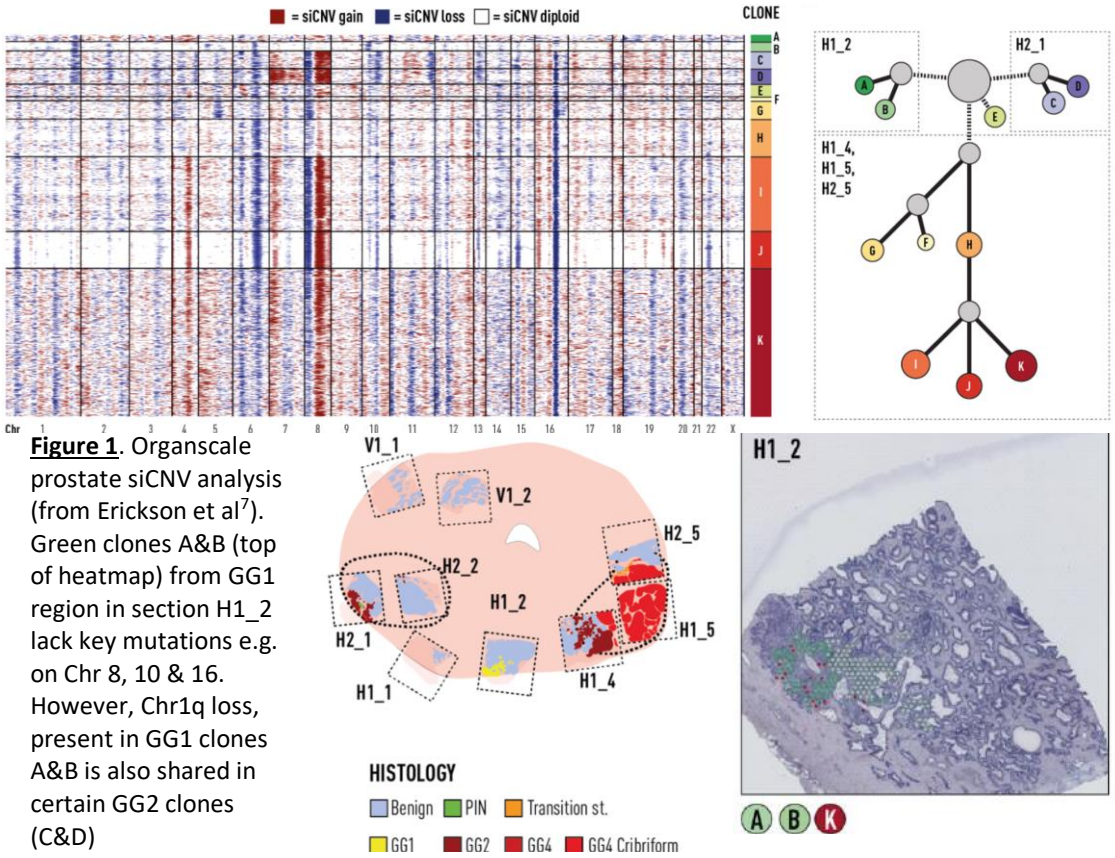


Figure 1. Organscale prostate siCNV analysis (from Erickson et al[7]). Green clones A&B (top of heatmap) from GG1 region in section H1_2 lack key mutations e.g. on Chr 8, 10 & 16. However, Chr1q loss, present in GG1 clones A&B is also shared in certain GG2 clones (C&D)

References:

1. Ross HM, Kryvenko ON, Cowan JE, et al. Do adenocarcinomas of the prostate with Gleason score (GS) </=6 have the potential to metastasize to lymph nodes? *Am J Surg Pathol* 2012;36(9):1346-52. doi: 10.1097/PAS.0b013e3182556dcd [published Online First: 2012/04/26]
2. Eastham JA, GBA, DAB, et al. Clinically Localized Prostate Cancer: AUA/ASTRO Guideline, Part I: Introduction, Risk Assessment, Staging and Risk-Based Management. *J Urol* 2022 doi: doi.org/10.1097/JU.0000000000002757
3. Lowrance WT, Breau RH, Chou R, et al. Advanced Prostate Cancer: AUA/ASTRO/SUO Guideline PART I. *J Urol* 2021;205(1):14-21. doi: 10.1097/JU.0000000000001375 [published Online First: 2020/09/23]
4. Chappidi MR, Bell A, Cowan JE, et al. The Natural History of Untreated Biopsy Grade Group Progression and Delayed Definitive Treatment for Men on Active Surveillance for Early-Stage Prostate Cancer. *J Urol* 2022;207(5):1001-09. doi: 10.1097/JU.0000000000002420 [published Online First: 2022/01/05]
5. Labbate CV, Paner GP, Eggener SE. Should Grade Group 1 (GG1) be called cancer? *World J Urol* 2022;40(1):15-19. doi: 10.1007/s00345-020-03583-4 [published Online First: 2021/01/13]
6. Stahl PL, Salmen F, Vickovic S, et al. Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. *Science* 2016;353(6294):78-82. doi: 10.1126/science.aaf2403
7. Andrew Erickson EB, Mengxiao He et al, Alastair D Lamb, Joakim Lundeberg. Spatially resolved clonal copy number alterations in benign and malignant tissue. Nature volume 608, pages 360-367 (2022) https://www.nature.com/articles/s41586-022-05023-2
8. Erickson A. Spatial iCNV 2021 [Available from: https://github.com/aerickso/SpatialInferCNV.
9. Ramos-Montoya A, Lamb AD, Russell R, et al. HES6 drives a critical AR transcriptional programme to induce castration-resistant prostate cancer through activation of an E2F1-mediated cell cycle network. *EMBO molecular medicine* 2014;6(5):651-61. doi: 10.1002/emmm.201303581 [published Online First: 2014/04/17]
10. Jurmeister S, Ramos-Montoya A, Sandi C, et al. Identification of potential therapeutic targets in prostate cancer through a cross-species approach. *EMBO molecular medicine* 2018;10(3) doi: 10.15252/emmm.201708274 [published Online First: 2018/02/14]

25. Understanding and interpreting cell-to-cell interactions in colorectal cancer[1,4] – Prof. Leedham

Primary Supervisor: Prof. Simon Leedham
Additional Supervisors: Prof. Helen Byrne, Prof. Steve Taylor
Eligibility: Track 1 and 4 students are eligible to apply for this project.

Lay Summary
Solid cancers comprise cancer cells, connecting tissue cells and immune cells, all embedded together in a complex system. Although we can use single cell biology to measure these different cell types within a cancer, this process requires separating out the different tissues, so that we are unable to see how the cells interact in the disease setting. Spatial biology is a new field, looking to exploit the development of advancing imaging technologies that can be applied to tissue sections to understand how different cells spatially relate to each other across different disease states. This requires the development of a new range of computational tools to analyse cell types, define cell coordinates and statistically test for non-random cell associations. In Oxford, a multidisciplinary team of biologists, clinicians, data scientists and mathematicians are working together to develop the pipelines needed to permit image data analysis and improve disease insight and biological interpretation from complex image files. Spatial image analysis will allow us to understand how cells interact in a disease setting and explore the signalling pathways that regulate these interactions. This will lead to drug development to target the pathways that are clinically important.

Abstract
Cellular Pathology is a key, cross-cutting medical science that stands on the brink of a giant leap forward in the evolution of the speciality. Despite the complexity of the genetic, transcriptional and post-translational repertoire, single cell technologies and improving spatial analyses have generated insight into a limited and measurable number of distinct cell phenotypic states, that can be defined by representative cell markers. Driven by advances in multiplex imaging, spatial omics, digital and molecular pathology and machine learning, the synergistic combination of morphological technologies and molecular diagnostics could underpin a revolution in disease classification. Alongside the historic characterisation of disease through the aggregation of symptoms, or the pathological processes involved (e.g. inflammation, infection, cancer), we believe that future taxonomic reclassification of disease will be driven by mapping and understanding cell-to-cell interactions in a tissue and condition-specific context Although these advances are disease agnostic, understanding cell interactions in cancer represents a huge opportunity, as the importance of regulating cell crosstalk has been highlighted by cancer immunotherapy which harnesses the power of the immune system to control cancer cell fate. This project will employ multiplex imaging technologies, and cutting edge ecological, statistical and mathematical techniques to determine and interrogate cell interactions in a spatial context and define functional cellular neighbourhoods in colorectal cancer (CRC) and other solid cancers.

Research Objectives
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. Cell interactions within this ecosystem are determined by neo-antigen generation, the expression of secreted cell signalling pathways and cytokine and chemokine production. Determining and understanding the intercellular rules of engagement will highlight shared and disease-specific signalling pathways, de-code intercellular communication, improve the identification of novel drug targets and provide evidence to support cross-disease drug repurposing. The student will join a multidisciplinary team containing clinicians, biologists, data scientists and mathematicians working together to develop pipelines for the analysis of imaging data. Biological interpretation of the data emerging from advancing spatial biology technology is at the cutting edge of this exciting science and the student will have the opportunity to develop and apply completely novel tools to these data. Multiple biological questions can be posed, and the underlying project questions could be adapted to suit the student's interests and expertise. For example, the focus could be at an architectural level, and involve comparing and contrasting cellular neighbourhoods in CRC and other solid tumours (such as pancreas or prostate) or assessing the evolution of cell interactions as colorectal adenomas progress to carcinoma. Once cell associations are determined, more granular questions can be answered such as the signalling networks that regulate stromal and immune cell interactions within discrete cell clusters. Data acquisition and analysis consists of 5 key steps, and the student will have the opportunity to learn each of these, through application to their own discrete tissue set.

1. **Data acquisition** – Oxford has a range of spatial biology imaging capability including multiplex IHC (Vectra Polaris, GE CellDIve, CODEX, imaging mass spectrometry), and spatial transcriptomics platforms (Nanostring GeoMx and 10x Visium). The student will learn the advantages and disadvantages of each technology and apply the most suitable technique for their tissue set and biological question.

2. **Cell Segmentation** – The student will undertake cell segmentation using both commercially available digital pathology platforms (HALO) and developed in-house software. Cell segmentation translates image data into cell X, Y coordinates within a tissue section, providing the data substrate for subsequent ecological and statistical analysis.
3. **Cell clustering** - The student will work with in house software to undertake QC and cluster annotation.
4. **Spatial analysis** – With Helen Byrne and Josh Bull, the student will develop and apply novel spatial analysis tools to statistically test cell associations, and define tissue cellular neighbourhoods (Bull et al, 2020). These will be mapped back onto tissue sections and to correlate with section histological features (e.g tumour invasive edge). This will include the application of spatial statistics and network theory to understand which cells are non-randomly associated with other cells. We will also use the emerging field of topological data analysis (TDA) to perform complementary, multiscale analyses of tissue samples to quantify structural features (eg immune deserts) present in the data images which are difficult to discern visually.
5. **Mathematical modelling** – The student will also have the opportunity to develop spatially-resolved computational models that simulate the time evolution of different cell neighbourhoods and account for interactions between stromal, immune and cancer cell populations. These computational models will also serve as a source of synthetic data with which to assess the utility of the analytical methods developed in step 4. (Bull et al, 2022; Vipond et al, 2021)

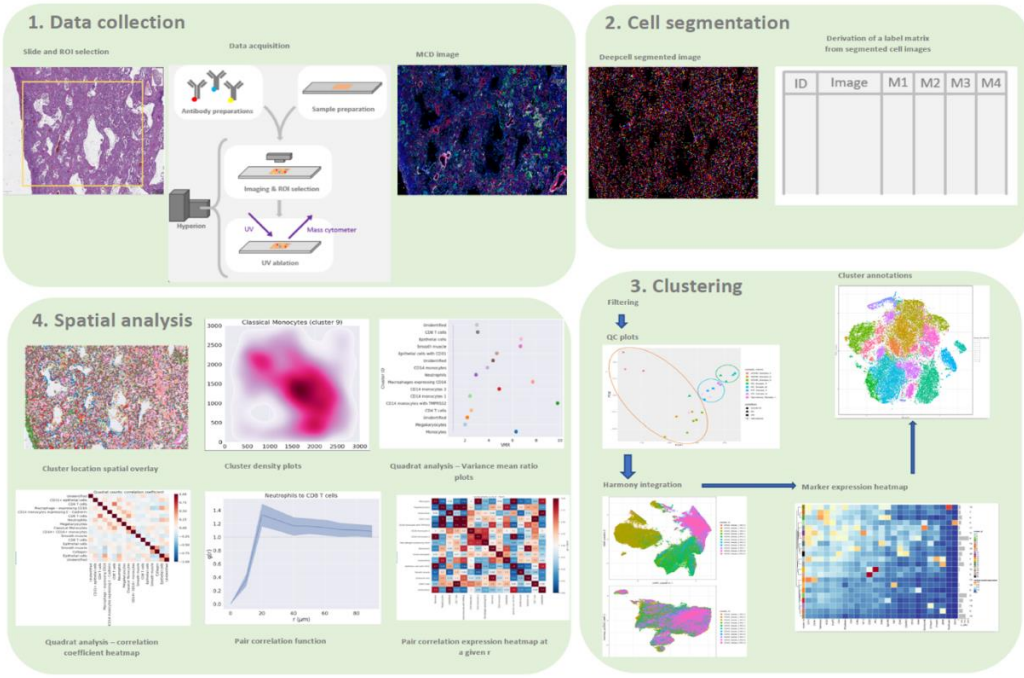


Figure 1. Analysis pipeline for spatial biology images. Dr Steve Taylor has developed a pipeline for end-to-end management of spatial biology images, including cell segmentation, clustering and the integration of ecological and statistical spatial analysis (Sergeant et al, 2021).

Translational potential

Spatial biology will allow us the opportunity to taxonomically reclassify disease based upon cell interactions and cellular neighbourhoods. Understanding shared and disease-specific cell interactions will allow us to triangulate the signalling processes that regulate cell intercommunication and then design or re-purpose drug interventions to manipulate these interactions. Spatial biology will also facilitate biomarker development with multi-marker discovery techniques driving generation of high throughput panel markers and machine learning led interrogation of H&E stained slides.

Training opportunities

The project specific training opportunities are listed above. This project would best suit a mathematician with an interest in applying statistical and topological data analysis techniques to biological image data or a pathologist with an interest in digital image analysis and mathematical modelling.

References:

JA Bull et al. (2020). Combining multiple spatial statistics enhances the description of immune cell localisation within tumours. Scientific Reports 10: 18624. DOI: 10.1038/s41598-020-75180-9

JA Bull and HM Byrne (2022). Macrophage sensitivity to microenvironmental cues influences spatial heterogeneity of tumours. bioRxiv preprint 2022.05.26.493564. DOI: 10.1101/2022.05.26.493564

O Vipond et al (2021). Multiparameter persistent homology landscapes identify immune cell spatial patterns in tumours. PNAS 118 (41): e2102166118. DOI: 10.1073/pnas.2102166118

Sergeant MJ, Hughes JR, Hentges L, Lunter G, Downes DJ, Taylor S. Multi Locus View: an extensible web-based tool for the analysis of genomic data. Commun Biol. 2021 May 25;4(1):623

26. Modulation of tumour immunogenicity by IGFs in prostate cancer [1] – Prof. Macaulay

Primary Supervisor: Prof. Valentine Macaulay

Additional Supervisors: Prof. Tim Elliot

Eligibility: Track 1 students are eligible to apply for this project.

Lay Summary

Prostate cancer is the commonest cancer in men in the UK and the second commonest cause of cancer deaths. The risk of prostate cancer is higher in men with high blood levels of a hormone called IGF-1. We think high IGF-1 can help cancers avoid detection by the immune system. We will test these ideas in 3 ways. First, we will see if high IGF-1 causes changes in the cancer cells themselves or in the main types of immune cell: killer cells that destroy cancer cells, and blocker cells that stop killer cells from working. Secondly, we will grow pieces of prostate cancer in the lab. We will see if adding IGF-1 affects the type of immune cells and how near they can get to the cancer cells. If the killer cells can't get very close, they can't destroy the cancer. Thirdly, we will test tissue samples from men in a clinical trial of a new anti-IGF drug given before prostate surgery. We will compare the number and type of immune cells in prostate tissue before and after the trial drug. This project may lead to better treatment, by helping men with prostate cancer benefit from immune boosting drugs. The results may also help us figure out how to reduce the risk of men developing prostate cancer in the first place.

Abstract

Most prostate cancers have a suppressive ('cold') tumour immune microenvironment (TIME) with exclusion of tumour infiltrating lymphocytes (TILs) especially CD8+ cytotoxic T cells, defective antigen processing machinery (APM), and many immunosuppressive TILs eg regulatory T cells (Tregs). Cancer risk is affected by serum IGF-1 (sIGF-1): subjects with very low sIGF-1 exhibit dwarfism and almost complete cancer protection, while high sIGF-1 increases prostate cancer risk. IGF-1 signals via IGF receptors (IGF-1Rs) to promote cell survival, invasion and androgen receptor (AR) activation. In other disease models IGF-1 enhances Treg function, while IGF blockade rescues APM components, inducing CD8 dependent anti-tumour immunity. We hypothesise that IGF-1 contributes to cancer risk via TIME immunosuppression. Using big data, patient derived explants (PDEs) and samples from men on a trial of IGF blockade pre-prostatectomy, we will ask whether IGF-1 affects: 1) tumour intrinsic or extrinsic TIME properties? 2) T cell phenotype/redistribution in prostate PDEs? 3) TIL localisation or exclusion? The findings may inform therapy and suggest novel approaches to prostate cancer risk reduction.

Research objectives

the aims are informed by evidence (Fig 1) that the prostate cancer TIME is affected by sIGF-1 and also by endogenous IGF-1 (eIGF-1) that is secreted by epithelial and stromal cells.

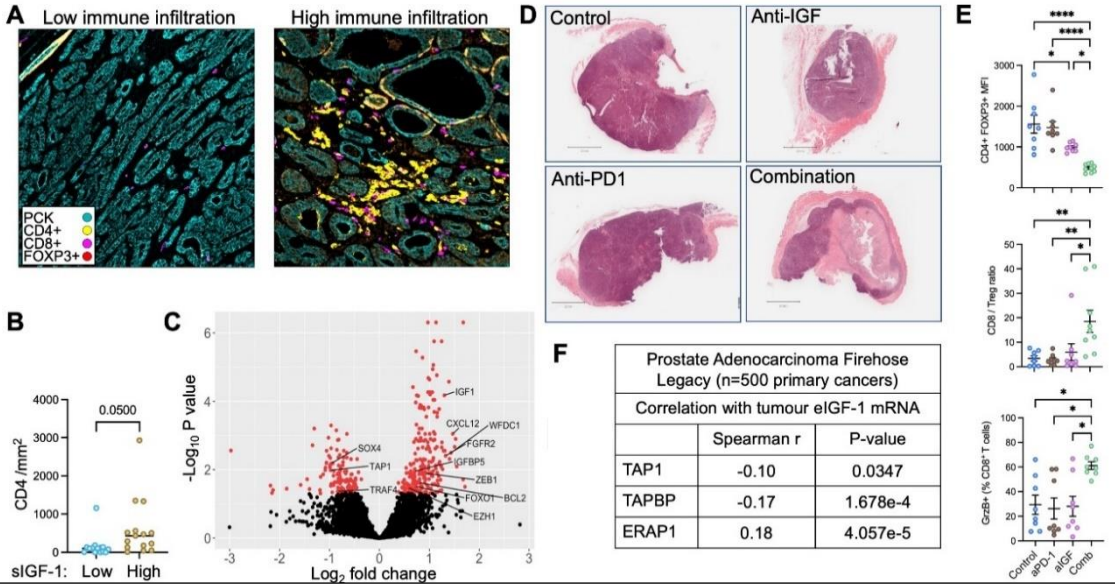


Figure 1. IGF-1 influences the TIME. A. Multiplex immunofluorescence (mIF) for pancytokeratin (PCK, epithelium), cytokeratin 5 (CK5, benign glands), immune markers. B. sIGF-1 associates with CD4+ TILs in prostatectomies of men with low (7.2-12.0 nmol/l) vs high sIGF-1 (21.7-31.5 nmol/l). **C.** RNAseq from same cases: differentially expressed genes (DEGs) in high eIGF-1 tumours include upregulated IGFBP5 (indicates IGF-1R activation) and downregulated TAP1 that transports antigenic peptides. **D-E**. Mice bearing Myc-CaP allografts treated with anti-PD-1, anti-IGF xentuzumab or combination. IGF:PD-1 co-inhibition increases tumour necrosis (D). FOXP3 TIL positivity is suppressed by anti-IGF-1 and PD-1:IGF co-inhibition (F, upper), and co-inhibition increases CD8+ cell/FOXP3+ Treg ratio and % granzyme B positive CD8+ TILs (E centre, lower). IGF-1 also upregulates PD-L1 in prostate cancer cells *in vitro* (not shown). **F.** Correlation of eIGF-1 and APM components.

Supporting ***tumour extrinsic effects,*** sIGF-1 associates with CD4+ TILs (Fig1A-B), IGF blockade reduces FOXP3+ Treg TILs, and Supporting ***tumour extrinsic effects,*** sIGF-1 associates with CD4+ TILs (Fig1A-B), IGF blockade reduces FOXP3+ Treg TILs, and IGF:PD-1 co-inhibition increases CD8+/Treg ratio and GrzB+ CD8+ cells (Fig 1E). ***Tumour intrinsic effects*** on the APM are suggested by the correlation of high eIGF-1 (Fig 1C, F) with low TAP1 and TAPBP and high ERAP1 that trims peptides for presentation by MHC-I, potentially contributing to immune evasion by altering the peptide repertoire.

A plausible mechanism through which high IGF-1 promotes prostate cancer risk is therefore:

•High sIGF-1 and/or eIGF-1 upregulate PD-L1 and immunosuppressive cytokines in healthy or premalignant prostate tissue to promote Treg proliferation/function, resulting in a high tissue Treg setpoint.

•Tregs condition tissue in favour of immune suppression/exclusion, favouring progression to invasive cancer.

•CD8+ T cells are primed by tumour antigens eg those from AR ligand-binding domain (LBD). But in tissues conditioned to be immunosuppressive, primed TILs are suppressed/excluded, less able to control new cancers.

•IGF-1 deregulates APM components in tumour cells, further contributing to immune escape.

This mechanism suggests a tractable hypothesis to explain how the TIME is shaped by IGF-1:

•Tumour extrinsic effects that maintain Treg function and infiltration, suppressing CD8+ TILs

•Tumour intrinsic effects by regulating the APM, influencing ability of CD8+ TILs to recognise AR derived epitopes

•Thus IGF-1 prevents CD8+ T cell infiltration, localisation to malignant glands and lysis of prostate cancer cells.

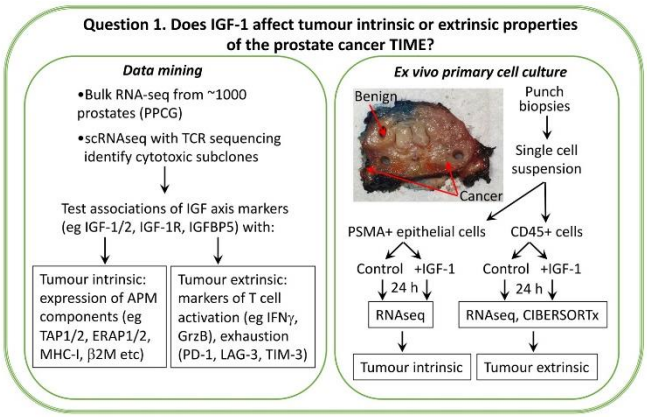
The project will establish a new collaboration between Professor Elliott and Dr Macaulay, who will co-supervise the Clinical Fellow to investigate these hypotheses using the following models, samples and collaborations:

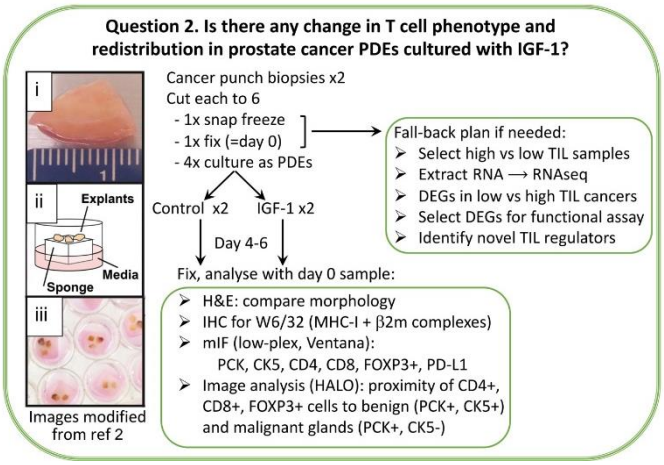
•*Highly curated RNAseq data*: ~1000 primary prostate cancers (collaborator: Dr Woodcock, Pan Prostate Cancer Group, PPCG).

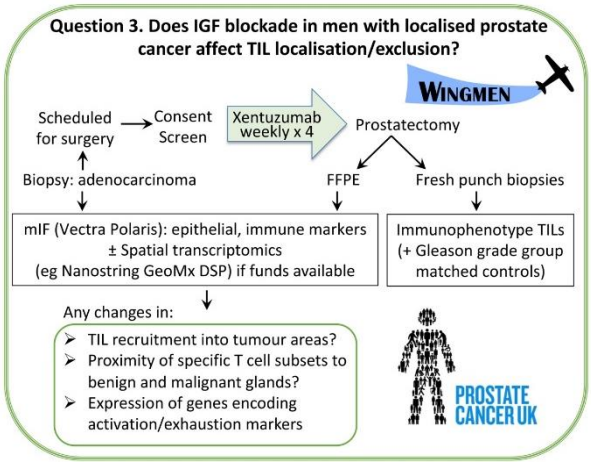
•*Prostate scRNAseq data* from the Macaulay lab and Dr Massie, Cambridge [1], with TCR sequencing to identify cytotoxic subclones and their expression of IGF axis genes.

•Patient derived explants (PDEs, ref 2) that maintain morphology, viability for ≤6 days (collaborators: Mr Lamb, Profs Verrill, Mills).

•WINGMEN samples (Mr Lamb, Prof Verrill).







Depending on the findings, further investigations could include:

•***Murine model*** *to assess TIL numbers, phenotype, intratumoral location.* 'HIT' mice (from Shoshana Yakar, NY) harbour a hepatic IGF-1 transgene with ~3-fold higher sIGF-1 [3]. Myc-CaP allografts in WT or HIT mice will be analysed by mIF, TIL phenotyping and RNAseq with digital cytometry (ImmuCC) to infer immune cell populations.

•***Functional assays*** to investigate effects of IGF-1 or IGF blockade on i) T cell killing and suppression in co-cultures of prostate cancer cells and T cells, ii) AR antigenic priming and relative ability to prime in immunosuppressive environment, iii) immunopeptidomics to identify IGF-induced changes in antigen processing and presentation.

Translational potential

identification of a novel MOA for IGF-1 in the TIME has clear relevance to:

•***Therapy***: Few prostate cancers respond to immune checkpoint inhibition (ICI). If IGF-1 enables Tregs to suppress CD8+ TILs, IGF blockade may cause Treg suppressed CD8+ cells to be less exhausted and thus rescuable by ICI.

•***Cancer risk***: immunosuppressive actions of IGF-1 may contribute to the ability of high IGF-1 to enhance the risk of prostate cancer. Understanding this effect may suggest new approaches to risk reduction.

Training opportunities

The student will be trained in analysis of big data, explant and cell culture, flow cytometry and mIF. If resources become available, it may be possible to provide training in high-cost techniques to address our hypotheses at the single cell (scRNAseq, CyTOF) and tissue levels (CellDive, spatial transcriptomics).

References:

1. Tuong et al. *Cell Rep* 2021. 37:110132. **2.** Centenera et al. *Mol Oncol* 2018. 12:1608-1622. **3.** Cannata et al. *Endocrinology* 2010. 151:5751-61.

27. Exploiting synthetic defects in metabolism and DNA repair to improve the treatment of glioma and AML [1,2,3] – Prof. McHugh

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

Lay Summary
The most common forms of brain tumour remain very hard to treat effectively, as do advanced blood cancers. We have discovered a change in the biochemistry of both these cancers that might mean they can be treated in a new, unexpected way. This new approach would involve targeting the cellular machinery that normally helps cell keep their chromosomes in pristine condition. In this project, we will comprehensively explore the potential of this new approach and work with chemists to generate agents that might enable the development of new drugs.

Abstract
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 a-ketoglutarate (α-KG) and CO_2 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 a-ketoglutarate as a co-substrate, including a family of metal dependent dioxygenases, the ALKB family (Rohle *et al.,* 2013).

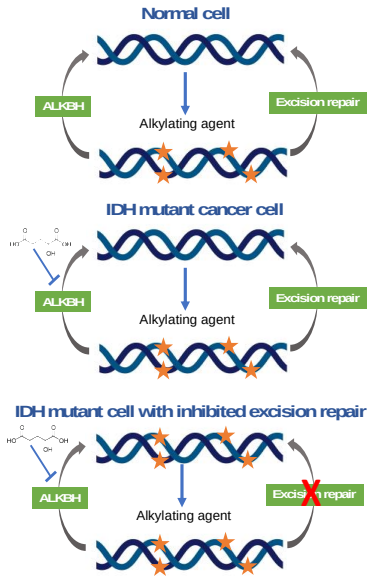


Figure 1. *Cells depend upon both the ALKBH enzymes and excision repair to remove DNA damage. Loss of both can be lethal in tumour cells.*

ALKBH2 and ALKBH3 are DNA repair enzymes that directly remove 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 excision repair factors. This, in turn, suggested that cancer cells harbouring IDH mutations could be sensitive to simultaneous loss of DNA exision repair genes by virtue of their reduced ALKB repair activity (**Fig. 1**). Follow-up studies suggest that this hypothesis is correct, and that inactivation of excision repair enzymes in IDH mutant tumour cells can be lethal.

Experimental plan
We will explore the detailed biology of the synthetic relationship of DNA repair defects with the key clinically-relevant IDH1 R132H patient mutation, as well as ALKBH2/3 disrupted cells. By performing CRISPR-Cas9-based screens that target all know DNA damage response (DDR) genes in IDH mutant cells, we will survey and define the full range of DDR genes required for survival of IDH mutant cells and therefore identify additional new therapeutic targets. 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.

We will characterise the nature of the DNA repair defects observed in repair defective IDH mutant (and ALKBH 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 mutated in IDH1 or lacking ALKBH2/3 that is synthetic with additional repair pathway loss. Furthermore, inhibitors of IDH and ALKBH2/3 are available, developed in the group of Prof. Chris Schofield (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. Moreover, we will work in collaboration with our chemistry collaborators to generate improved inhibitors of key excision repair factors that can be used to selectively target IDH deficient tumours, work which builds upon well-developed work in this area in our laboratories. These tools, both genetic and chemical, will ultimately be combined by the student to perform key proof-of-principle experiments to explore this novel approach to treating two cancers of unmet need.

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. The student will also benefit from interactions with clinical colleagues involved in treating glioma and AML, as part of their thesis committee.

References:

- Cohen AL, *et al.* (2013) IDH1 and IDH2 mutations in gliomas. Curr Neurol Neurosci Rep, 13: 345
- 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
- Fedeles 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
- Jalbert LE, *et al.* (2017) Metabolic Profiling of IDH Mutation and Malignant Progression in Infiltrating Glioma. Sci Rep, 7: 44792
- O'Connor MJ (2015) Targeting the DNA Damage response in cancer. Mol Cell, 60(4), 547-560
- Rohle D, *et al.* (2013) An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells. Science, 340: 626-30
- 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

28. Cellular and matrix interactions of F4/80, an adhesion GPCR which defines murine tissue macrophages, in the normal and tumour microenvironment [1,2,3] - Prof. Kim Midwood

Primary Supervisor: Prof. Kim Midwood
Additional Supervisors: Prof. Siamon Gordon
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Lay Summary

Our immune system detects tumors and activates 'killer' immune cells to destroy them. However, tumors can turn off these cells, switching the immune response instead to become 'tolerant', and to help the growth and spread of the cancer. Drugs that turn killer cells back on have revolutionized the treatment of people with cancer. However, this approach does not work for many patients, nor all types of tumor, and can be associated with severe side effects caused by the re-activated killer cells attacking healthy tissues. Our recent data show how a different type of immune cell, the macrophage, uses a protein called F4/80 to communicate with, and switch on, tolerant cells, and how this is protein essential for tumors to survive and thrive. However, nothing is known about how F4/80 works. We will investigate how F4/80 switches on tolerant cells in tumors. This will lead to a better understanding of how cancer highjacks the immune response for its own gain, and enable the design of new therapies that block this pathway, which are safer and effective in more patients that existing drugs.

Abstract

The Mononuclear Phagocyte System consists of embryonic and bone marrow-derived monocytes, macrophages and dendritic cells which are widely dispersed as sessile cells throughout all tissues in health and disease. They are terminally differentiated migratory leucocytes which sense physiologic and pathologic changes in their microenvironment through a variety of plasma membrane receptors that regulate their biosynthetic responses to maintain homeostasis (Fig.1A,B)[1]. In addition to specialised phagocytic receptors to recognize and clear senescent, apoptotic and necrotic cells and microbes, they are potent secretory cells able to nourish or destroy abnormal host cells and microbial targets, depending on their functional activation state. Tissue resident and recruited macrophages express adhesion molecules such as the F4/80 antigen, also known as EMR1 or ADGRE1, a widely used biomarker and the founder member of a family of 7 transmembrane G protein coupled receptors (GPCRs) that possess large extracellular epidermal growth factor like (EGF) repeats. Human myeloid cells express a closely related EMR2 receptor, which is absent in the mouse, as well as an EMR1 homologue with distinct properties. In spite of extensive studies of its specific expression in the mouse, the ligands of F4/80 in extracellular matrix (ECM) and cells in different tissues remain unknown. However, a major breakthrough in our understanding of its immunological function derived from studies using F4/80 knockout (KO) mice, which revealed this molecule to be dispensible for macrophage development, but responsible for peripheral tolerance. Required for Treg generation in vivo in response to a model antigen introduced into the anterior chamber of the eye [2], F4/80 expression is also essential for organ transplantation [3] and engraftment of Lewis lung cancer cells (Fig.1C-E). This project will explore the role of F4/80 in tumour-host interactions in vivo and identify cellular and extracellular binding partners of this adhesion GPCR.

Research objectives

Aim 1 will investigate the cellular and molecular basis of F4/80-mediated tolerance using a syngeneic, orthotopic breast cancer grafting model (well established at KIR)[4] in wild type or F4/80 null mice (maintained at the Dunn School). Immune profiling of tumor, spleen and lymph nodes during tumor growth using multicolour spectral flow cytometry (Aurora, KIR) will reveal F4/80 dependent changes in abundance, and activation/polarization status, across myeloid and lymphoid compartments. Multiplexed immunofluorescent imaging (GE CellDive, KIR) will reveal cell interaction partners of F4/80+ macrophages, and the tissue localization of these cellular networks. scRNA seq of sorted CD45+ cells from wild type and F4/80 knockout tumor bearing mice will reveal cell type-specific transcriptional changes associated with loss of F4/80, and pathway/interaction network analysis will highlight candidate effector molecules. Cellular interactions, and their contribution to F4/80-mediated tolerance, will be validated in vivo (e.g. by cell type or effector molecule depletion/blockade), and in vitro (e.g. using tumor-immune cell co-cultures)[4]. Aim 2 will identify extracellular binding partners of F4/80. More than 30 adhesion GPCRs exist, utilizing their sizable extracellular domains to form multimeric protein complexes of signalosome-like structures. Despite identification of extracellular ligands for other adhesion GPCRs, including the interaction of chondroitin sulphate (CS) and CD55 with close family members EMR2 [5] and CD97 [6] respectively, F4/80 remains an orphan receptor. F4/80 expression is elevated in specific murine breast tumor associated macrophage subpopulations with enriched APC capabilities. These cells localize to extracellular matrix tracks that support cell infiltration into the tumor, whilst F4/80 low cells are restricted to the periphery (Fig.1F). Multiplexed imaging using a panel of matrix markers will identify tumor components that interact with F4/80+ cells in these tracks. In parallel, the adhesion of fetal liver macrophage cell lines from wild type and F4/80 knockout mice [7](Dunn School), to purified matrix molecules (e.g. tenascin-C (TNC), fibronectin, collagen type I/IV, osteopontin, CS)(KIR) as well as more complex

matrices (e.g. matrigel, tumor derived cell free matrices) which better recapitulate the 3D environment of the tumor, will be assessed. Binding sites within F4/80 for ligands will be mapped as for CS-EMR2 [6] and downstream signalling examined.

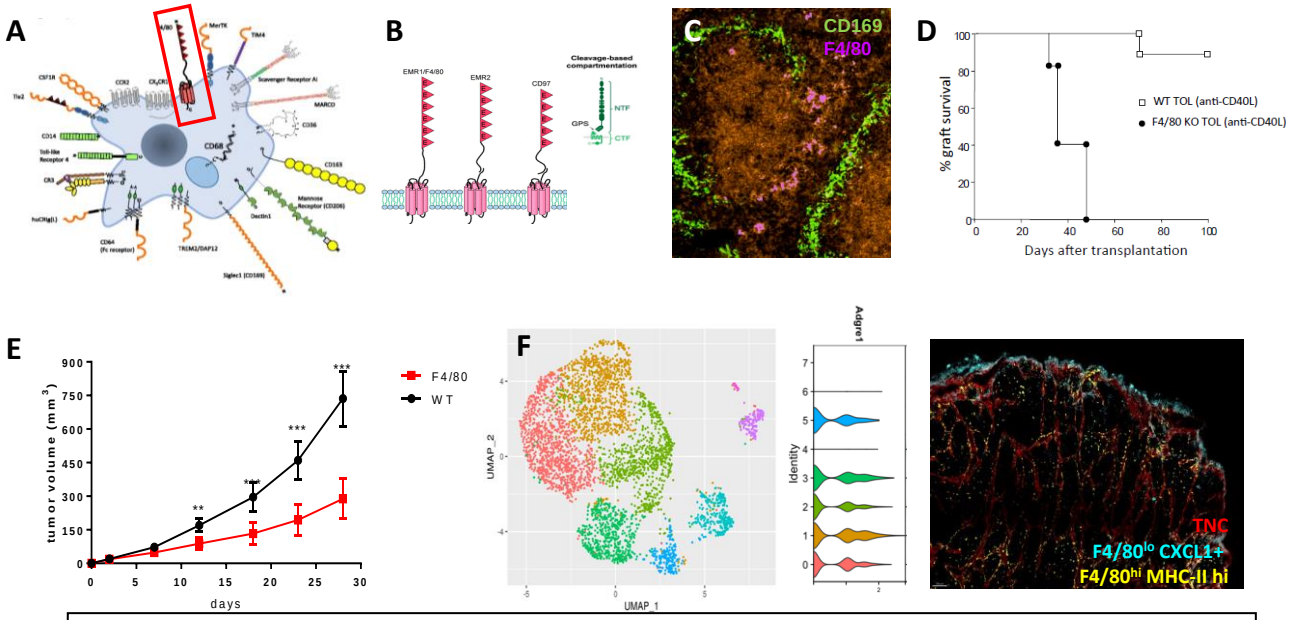


Figure 1. (A) Selected macrophage receptors. (B) Three of the EGF-TM7 family of adhesion GPCRs; EMR1, EMR2 and CD97. (C) F4/80+ APCs located in the spleen marginal zone 7d after antigen inoculation of the eye anterior chamber [2]. F4/80 deficiency abrogates tolerance during heart allograft [3](D) and Lewis cancer cell transplant (unpublished)(E). (F) F4/80 expression is elevated in MHC^{hi} macrophage subpopulations localized exclusively to matrix-rich tracks in murine breast tumors (our unpublished data).

Translational potential

Macrophages are an integral component of tumours, responding to, and in turn, influencing the malignant cells, as well as all stromal and other immune cellular and extracellular components of the tumour microenvironment. Their interactions with the extracellular matrix could affect their own growth, adhesion, migration and activation, as well as of the malignant cells and metastases. We need to learn more about their polarization, and the switch from trophic to cytotoxic potential, crucial in the tumour-host interaction. This project will establish some of the basic principles for further translation to human cancer. Among the EGF-TM7 family CD97 and EMR2 have been investigated in a range of human cancers, with, for example, changes in the cellular compartmentalization of EMR2 correlating with poor prognosis in breast cancer [8]. F4/80 has been neglected hitherto. Evidence that it mediates peripheral tolerance via APC, which can be abrogated by targeting F4/80, makes this a compelling model for further research in mouse models of cancer. Moreover, study of the autoproteolyic cleavage of these adhesion GPCRs (Fig1B), may also be relevant to shedding of soluble receptor in vivo, which could contribute to cancer pathogenesis and to diagnosis through its presence in plasma or other body fluids [9].

Training opportunities

the student will be trained in techniques including: in vivo tumor models, immune cell isolation/activation, 2D & 3D matrix adhesion assays, multiplexed tissue imaging, spectral flow cytometry, RNA seq dataset generation and bioinformatic analysis in mouse and human pathology.

References:

[1] Gordon, S. and A. Plüddemann, Tissue macrophages: heterogeneity and functions. BMC Biology, 2017. [2] Lin, H.H., et al., The macrophage F4/80 receptor is required for the induction of antigen-specific efferent regulatory T cells in peripheral tolerance. J Exp Med, 2005. [3] Conde, P., et al., DC-SIGN(+) Macrophages Control the Induction of Transplantation Tolerance. Immunity, 2015. [4] Deligne C et al, Matrix-Targeting Immunotherapy Controls Tumor Growth and Spread by Switching Macrophage Phenotype. Cancer Immunol Res. 2020. [5] Stacey, M., et al., The EGF–like domains of the human EMR2 receptor mediate cell attachment through chondroitin sulfate glycosaminoglycans. Blood, 2003. [6] Aust, G., L. Zheng, and M. Quaas, To Detach, Migrate, Adhere, and Metastasize: CD97/ADGRE5 in Cancer. Cells, 2022. [7] Fejer, G., et al., Nontransformed, GM-CSFdependent macrophage lines are a unique model to study tissue macrophage functions. PNAS, 2013. [8] Davies, J.Q., et al., Leukocyte adhesion-GPCR EMR2 is aberrantly expressed in human breast carcinomas and is associated with patient survival. Oncol Rep, 2011. [9] Boucard, A.A., Self-activated adhesion receptor proteins visualized. Nature, 2022.

29. Molecular and epigenetic mechanisms of Ikaros function in Multiple Myeloma [1,3] – Prof. Milne

Primary Supervisor: Prof. Thomas Milne
Additional Supervisors: Dr Sarah Gooding
Eligibility: Track 1 and 3 students are eligible to apply for this project.

Lay Summary
The goal of modern cancer medicine is to be able to precisely target cancer cells while leaving normal cells unharmed. In order to accomplish this, much work is required to better understand drug function on a molecular level. Treatment for multiple myeloma (MM), a form of blood cancer, has been revolutionised by a class of drugs called immunomodulatory drugs (IMiDs). IMiDs have been shown to have specific molecular targets, including a protein called Ikaros. Ikaros is important for cancer cell survival, where it helps turn genes on and off when needed. However, MM patients treated with IMiDs will eventually relapse, where in many cases these cancer cells have developed a way to survive without Ikaros. In this project, we hope to determine how Ikaros functions on a molecular level so we can better target altered Ikaros independent pathways in patients with relapsed myeloma, for which there is currently no cure.

Abstract
Multiple myeloma (MM) is a disease of mature lymphoid cells called plasma cells. The transcription factor Ikaros is essential in MM, and is a target of degradation by immunomodulatory imide drugs (IMiDs) such as lenalidomide. Interestingly, Ikaros has an opposite activity in less mature B cell leukaemias such as Acute Lymphoblastic Leukaemia (ALL), where loss of Ikaros is actually associated with a worse prognosis. Together, this suggests that the role of Ikaros in promoting disease is context specific. To better understand how MM develops resistance to drugs that target Ikaros such as lenalidomide, we are proposing to better understand the molecular mechanisms of Ikaros function in MM, with a particular focus on how Ikaros controls enhancer function and the epigenetics of gene expression.

Research Objectives

IKZF1 (Ikaros) is a widely expressed transcription factor that is generally important for normal haematopoietic development and is also more generally expressed in leukaemia [1,2,3]. As well being involved in ALL[1], Ikaros is a target of lenalidomide and pomalidomide, drugs that are part of the class of immunomodulatory drugs (IMiDs) used to treat multiple myeloma[4]. 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. The molecular function of Ikaros is not completely understood, but it can interact with both activating and repressing complexes, as well as potentially mediate interactions between enhancers and promoters through epigenetic mechanisms[2], although this has not been robustly established with high resolution 3C methods. 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 in MM using a high resolution 3C method
AIM 3) To determine if iMiD resistance in MM is due to acquisition of alternate enhancer activation pathways

AIM 1) Most work on enhancers (including our own [5,6]) 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 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[2]. This will be the first step in understanding its function in MM.

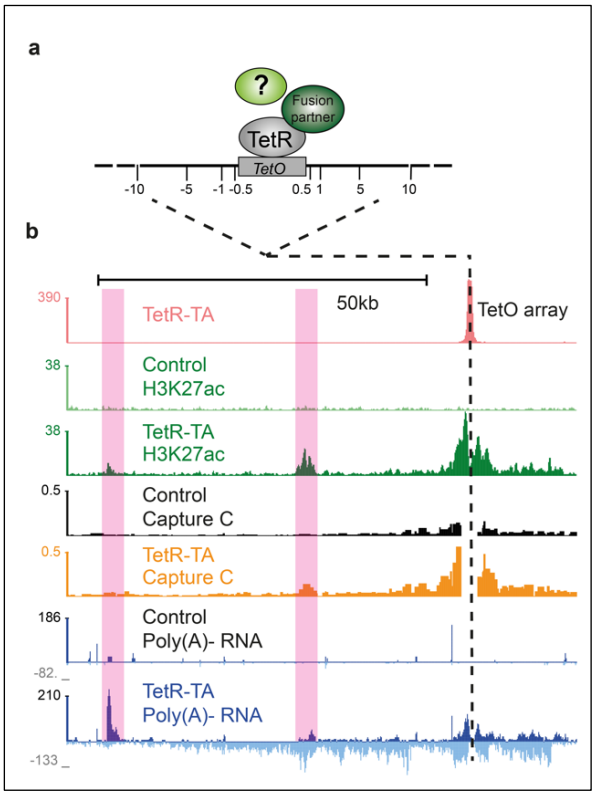


Figure 1: A transcription factor transactivation domain (TA) that is sufficient to create a* de novo *enhancer in a gene desert. (a) *An array of 14 TetR binding sites (TetO) in a gene desert region inserted into chromosome 8 in mouse ES cells. Expression of the TetR-TA in these cells anchors the TA 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 5 and 6) 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*

AIM 2) Once we've established the functional capabilities of Ikaros in our gene desert system, we will perform knockdowns of Ikaros, and IMiD agent treatments to degrade Ikaros in myeloma cell lines and primary samples. We will then perform RNA-seq, ChIP-seq and use 3C approaches such as NG Capture C to identify direct targets regulated by Ikaros and 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 MM. Dr Gooding, who has worked extensively on IMiD resistance in MM, and Prof Ramasamy, who leads the Oxford myeloma service, will be essential for guiding work with primary 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 in most patients. 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.

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) Read, K. A. *Immunol Rev* **300**, 82-99, doi:10.1111/imr.12936 (2021).
3) Harman, J. R. *et al.* **Milne T.A.** *Gen Res*, doi:10.1101/gr.268490.120 (2021).
4) Martinez-Hoyer, *Exp Hematol* **91**, 22-31, doi:10.1016/j.exphem.2020.09.196 (2020).
5) Crump, N. T. *et al.* **Milne T.A.** *Nat commun* **12**, 223, doi:10.1038/s41467-020-20400-z (2021).
6) Godfrey, L. *et al.* **Milne T.A.** *Nat commun* **10**, 2803, doi:10.1038/s41467-019-10844-3 (2019).

30. Clonal structure and therapeutic targeting of aggressive forms of mastocytosis [1,2,3] – Prof. Nerlov

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

Lay Summary
Systemic mastocytosis (SM) is a group of blood cancers where the production of mast cells is increased. About one third of patients also develop a second type of myeloid blood cancer, a phenomenon called 'SM with additional haematological neoplasm (SM-ANH)'. Mast cells are sentinel immune cells produced by the bone marrow that reside in the gut, skin, lung and liver where they detect invading infectious organisms and initiate the immune response. When overproduced, mast cells accumulate in body organs (liver, spleen, gut) and bone marrow, and cause inflammation, severe symptoms, tissue damage, and ultimately organ failure. The median survival of patients is around 3 years for advanced SM and around 2 years for SM-AHN. SM is almost always driven by a mutation in the gene encoding the 'KIT' receptor, which stimulates mast cell production, and this is the key therapeutic target. Very recently, a specific inhibitor of the common D816V mutation has been developed, which is revolutionizing care of patients with SM, with >80% response rates, generating a lot of excitement in the field of targeted cancer therapeutics. However, treatment with a KIT inhibitor does not specifically target the second AHN cancer, which can sometimes become even more aggressive once the SM is eliminated. A key obstacle to progress towards curative treatment is the lack of understanding about why SM predisposes a patient to develop a second malignancy, and then why the second cancer expands once the SM is treated. In particular, it is not understood if the SM and AHN derive from the same cancer stem cell or if they evolve concurrently. This project will utilize advanced mouse models and unique access to samples from patients to study the gene activity and cellular architecture of the cancer(s) present, with an overarching goal of identifying improved methods for treating SM and related blood cancers.

Research objectives
The aim of this project is to use state-of-the-art genomics (combined single cell RNAseq/ATACseq (multiome) analysis, single cell genotyping) profiling of normal and patient samples to identify the hierarchical structure of SM and SN-AHN, and in particular to pinpoint the self-renewing cells at the top of the respective hierarchies. Based on these analyses we will both identify putative therapeutic targets (oncogenic mutations as well as other molecular vulnerabilities), and design accurate preclinical mouse models of SM/SM-AHN, using multiple oncogenes and cell type-specific recombinase drivers. The preclinical mouse models will be used to test single and combination therapies, complemented by analysis of human disease samples in xenografts and organoids.

Aim 1: In which stem- or progenitor cells are SM-causing mutations present? Using combined single cell RNAseq and ATACseq we have already generated a map of the normal human hematopoietic stem- and progenitor cell (HSPC) compartment. We will use this map to project similar analysis of disease samples with added single cell genotyping to identify where mutant cells reside in the malignant clone. The genotyping will be informed by exome sequencing of the entire patient cell population. This will allow the progressive accumulation of genetic hits within HSPCs to be visualized, and the point at which all key mutations have been accumulated identified, which is where the malignant stem cells are predicted to reside. This will also allow differences in mutational load between the SM and AHN components to be identified. In addition, using single cell RNAseq the gene expression profiles of the same patient samples can be projected onto the normal hierarchy, and malignant and normal populations compared to identify aberrantly expressed genes, form which putative therapeutic targets can be extracted.

Aim 2: Generation of informed genetic models of SM and SM-AHN. We have previously identified the progenitor populations that generate mast cells in both mice and humans, and shown that these cellular pathways are highly homologous(1,2). Based on the findings from Aim 1 we can therefore design mouse models that accurately recapitulate the mutational landscape, by designing recombinase drivers that can activate the relevant mutations (KIT, TET2 etc.) at the correct point of the mast cell differentiation process. Multiple recombinases will be used to allow mutations to be introduced sequentially, and in the cell types in which they are found in the human disease. We anticipate that mutations may occur in earlier multi-lineage progenitors in SM-AHN, in order to generate a multi-lineage disease. In that scenario, we will compare the effect of introducing the same mutations at different points of the hematopoietic hierarchy. The disease phenotypes of the models will be validated, including the functional validation of the identity of self-renewing

malignant cells by disease re-initiation after transplant, and the resulting transplantation models used to develop therapeutic strategies, including the use of in vivo CRISPR-based screening for novel therapeutic targets (Aim 3).

Aim 3: Therapeutic targeting. The mouse models will be used to identify therapeutic targets using targeted in vivo dropout screens based on the aberrant gene expression seen in malignant cells. These will be combined with known pharmacological agents (e.g. KIT inhibitors) to test combination therapies. In particular, any combinations that have the potential to target both the SM and AHN components of SN-AHN will be prioritised. Where relevant, humanized disease models (e.g. xenografting of primary patient samples into immune-deficient mice) will be used to further validate the findings.

Translational potential

There is a clear unmet need to improve SM and SM-AHN therapies. Both supervisors are engaged in the clinical development and commercialization of preclinical data, using the extensive translational framework and excellent clinical environment available in Oxford. There will therefore be ample opportunity to engage with these processes.

Training Opportunities

The PIs (Claus Nerlov, Bethan Psaila) together have both clinical expertise and extensive experience with generation of mouse models of myeloid malignancies(3,4), and the use of these to test pharmacological strategies(5). Both laboratories use single cell genomics extensively, and are experienced in analysis of single cell data(2,6), as well as CRISPR-based screening for therapeutic targets. This studentship will therefore offer training in genetic modelling, molecular biology, single cell technologies, bioinformatics and target discovery. There will be excellent opportunities for grant writing and industry collaboration if of interest, and to attend national/international conferences to develop an independent research network.

References:

1. Drissen, R., Buza-Vidas, N., Woll, P., Thongjuea, S., Gambardella, A., Giustacchini, A., Mancini, E., Zriwil, A., Lutteropp, M., Grover, A., Mead, A., Sitnicka, E., Jacobsen, S. E. W., and Nerlov, C. (2016). Distinct myeloid progenitor-differentiation pathways identified through single-cell RNA sequencing. Nat Immunol *17*, 666-676.
2. Drissen, R., Thongjuea, S., Theilgaard-Monch, K., and Nerlov, C. (2019). Identification of two distinct pathways of human myelopoiesis. Sci Immunol *4*.
3. Bereshchenko, O., Mancini, E., Moore, S., Bilbao, D., Mansson, R., Luc, S., Grover, A., Jacobsen, S. E., Bryder, D., and Nerlov, C. (2009). Hematopoietic stem cell expansion precedes the generation of committed myeloid leukemia-initiating cells in C/EBPalpha mutant AML. Cancer Cell *16*, 390-400.
4. Di Genua, C., Valletta, S., Buono, M., Stoilova, B., Sweeney, C., Rodriguez-Meira, A., Grover, A., Drissen, R., Meng, Y., Beveridge, R., Aboukhalil, Z., Karamitros, D., Belderbos, M. E., Bystrykh, L., Thongjuea, S., Vyas, P., and Nerlov, C. (2020). C/EBPalpha and GATA-2 Mutations Induce Bilineage Acute Erythroid Leukemia through Transformation of a Neomorphic Neutrophil-Erythroid Progenitor. Cancer Cell *37*, 690-704 e698.
5. Valletta, S., Thomas, A., Meng, Y., Ren, X., Drissen, R., Sengul, H., Di Genua, C., and Nerlov, C. (2020). Micro-environmental sensing by bone marrow stroma identifies IL-6 and TGFbeta1 as regulators of hematopoietic ageing. Nat Commun *11*, 4075.
6. Psaila, B., Wang, G., Rodriguez-Meira, A., Li, R., Heuston, E. F., Murphy, L., Yee, D., Hitchcock, I. S., Sousos, N., O'Sullivan, J., Anderson, S., Senis, Y. A., Weinberg, O. K., Calicchio, M. L., Center, N. I. H. I. S., Iskander, D., Royston, D., Milojkovic, D., Roberts, I., Bodine, D. M., Thongjuea, S., and Mead, A. J. (2020). Single-Cell Analyses Reveal Megakaryocyte-Biased Hematopoiesis in Myelofibrosis and Identify Mutant Clone-Specific Targets. Mol Cell *78*, 477-492 e478.

31. Multi-cancer detection testing in clinical practice [1,2,3,4] – Dr Nicholson

Primary Supervisor: Dr Brian Nicholson
Additional Supervisors: Prof. Eva Morris
Eligibility: Track 1, 2, 3 and 4 students are eligible to apply for this project.

Lay Summary

A core strategy within the NHS Long Term Plan is to diagnose three quarters of cancers at an early stage by 2028. Cancer screening is only routinely available for three cancers in England and Wales, identifying one in ten of adult cancers. Nine out of ten people diagnosed with cancer visit their GP with symptoms in the year prior to being diagnosed. NHS GPs urgently refer patients with cancer symptoms according to a set of nationally agreed guideline criteria via urgent pathways that investigate different cancer types in 2 weeks. Half of all cancers are diagnosed this way in the NHS. As cancer shares symptoms with many other conditions patients can require a number of urgent referrals before a cancer diagnosis is made. To remedy this Rapid Diagnostic Centres (RDC) have been set-up at pace across the NHS to investigate cancer across multiple different cancer sites in patients with non-specific cancer symptoms. However, even with these pathways in place, many more patients are referred for cancer investigation than are diagnosed with cancer. This causes unnecessary anxiety for patients and uses NHS time and resource.

Attention is turning to whether non-invasive multi-cancer early detection (MCED) testing might improve cancer screening and assist doctors in identifying the most appropriate symptomatic patients for cancer investigation. A number of companies are developing MCED blood tests that analyse patterns of abnormal DNA in the blood released from cancers. MCED tests commonly provide a result that indicates whether a cancer has been detected, and in some cases they suggest what sort of cancer it is. They look for signatures of many types of cancer from a single blood test. These exciting and novel technologies offer great promise in detecting cancer early. However, there is limited research to support their adoption in screening or the clinic. For example, it remains unclear whether MCED testing should take place in GP surgeries before a referral to hospital, in the hospital after referral, or in asymptomatic people as part of a national screening programme. The successful candidate will be supported to develop and lead research into MCED testing using methods that suit their intended career path.

Abstract

Non-invasive MCED tests presents a new opportunity to improve early cancer detection by optimising patient selection for targeted cancer testing. Whilst MCED technologies are designed to detect a cancer signal across multiple cancer sites, their performance varies by cancer site and cancer stage. These technologies will not be used in isolation by clinicians in primary or secondary care: they will be used in people with a prior risk of cancer based on their risk factors (most importantly age), symptoms, signs, and test results, who are referred into clinical pathways for definitive testing. Care will be needed to select at-risk populations that complement the performance characteristics of the test to balance the likelihood of missed cancers and unnecessary referrals for invasive or expensive investigation. With a rapidly increasing number of MCED technologies in development, their performance characteristics are likely to improve. However, understanding the performance characteristics of MCED technologies alone will not be sufficient to guarantee the success of their implementation. Many promising innovations fail to reach clinical adoption as little attention has been given to the drivers of uptake in clinical practice. Successful clinical implementation of MCEDs in clinical practice is critically dependent upon intimate understanding of the patient, clinician and system-level factors that influence uptake. The successful candidate would join an exciting multidisciplinary programme of work investigating the accuracy, utility, and implementation of MCED testing in NHS clinical practice.

Research objectives

There is scope for the successful candidate to develop research objectives within the broad framework of the MCED focussed CRUK Oxford Cancer Centre's Early Detection theme. The Early Detection theme focusses on patient selection for MCED testing, MCED test development, and MCED test evaluation in clinical practice. The successful candidate will be supported to develop and lead research into MCED testing using methods that suit their intended career path. Examples of areas for development could be to:

- compare the performance of existing risk algorithms and clinical guidance to identify populations most at risk of cancers (combined and individually) who could be offered MCED testing by using existing health records data or by developing studies to collect new cohort data. These multi-parametric algorithms could take patterns of a patient's symptoms, signs,

test results, consultation patterns, medical history and risk factors to calculate their individual risk of cancer diagnosis to be updated as MCED tests are completed.

- utilise the Rapid Diagnostic Centre Digital Research Platform (RDC-DRP) curated to include clinical, research, and biobank data derived from the expanded Suspected CANcer (SCAN) pathway and biobank. The RDC-DRP could support fundamental and basic science researchers seeking to study early-stage disease and enhance risk factor and symptom data capture, clinical epidemiologists interested in the MCED signatures in patients with non-specific symptoms, and health services researchers hoping to use an online secure patient survey portal to collect patient data prior to and following their appointment.

- develop community-based prospective MCED cohorts and trials engaging patients across to promote diversity and inclusivity with the team who delivered the SYMPLIFY study. Together with a focus on assessing the accuracy and placement of MCED technologies within NHS clinical workflow key implementation questions could be asked using qualitative methodologies to understand the public, patient, clinician and system-level factors that influence MCED uptake and impact.

Translational potential
In order for the NHS to maximise the benefit of MCEDs for patients in clinical practice research is required to understand how MCEDs complement existing diagnostic pathways, if they replace commonly used diagnostic tests, and how patients and practitioners will use them. As MCEDs develop, with improved or different analytical performance, the candidate's research findings will be required to understand where to best place MCED in the diagnostic pathway. Oxford is uniquely placed to investigate MCED technologies as the supervisory team are involved in the development of MCED technologies and NHS evaluations of MCEDs in clinical practice.

Training Opportunities
In addition to the training provided by the Cancer Science DPhil programme, NDPCHS offers broad methodological expertise in applied health services research and evidence-based health care with training available to support the approach chosen by the candidate under guidance from their supervisory team. For example, the Medical Statistics group specialises in quantitative diagnostic, prognostic, monitoring, and prediction methodologies, the Medical Sociology and Health Experiences Research Group specialises in social science informed, qualitative and mixed methods implementation studies of health and illness, and the Primary Care Clinical Trials Unit delivers world class clinical trials in the community. In addition, the Oxford-led NIHR Community Healthcare MedTech and In vitro Diagnostics Co-operative (CH-MIC) works upstream and downstream of the CE-marking process to both influence the development of novel technologies and the evaluation of clinic-ready products.

32. Investigating the role of C5aR1 as a regulator of macrophage biology and recovery from intestinal injury [1,2,3] – Dr Olcina

Primary Supervisor: Dr Monica Olcina
Additional Supervisors: Dr Simon Buczacki
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Lay Summary

Radiotherapy is commonly used for the treatment of tumours and for some patients this treatment is very effective. Unfortunately, following radiotherapy for the treatment of tumours in the abdomen, patients can experience side effects such as increased bowel movement frequency, urgency and soiling. Furthermore, high-risk tumours are less susceptible to the killing effects of radiotherapy which results in treatment failure1–3. The identification of safer and more effective treatments could therefore significantly improve radiotherapy. We have found that blocking the action of a protein called C5aR1 may increase the effectiveness of radiotherapy while helping mice live with fewer debilitating side effects4. Mice where C5aR1 has been blocked have increased numbers of immune cells called macrophages and these macrophages appear to be key to the protective effects observed. In this project we want to investigate exactly how these macrophages control reduced toxicity in the intestine. We are very interested in understanding exactly what kinds of macrophages are present in the guts of the mice where C5aR1 has been blocked, and how C5aR1 may regulate the recruitment of these macrophages to the sites of injury. In collaboration with Simon Buczacki, we will also investigate whether these macrophages can promote the regeneration of the intestine following injury and ultimately how this regeneration contributes to reduced side effects following treatment. Understanding how particular macrophage populations regulate intestinal regeneration will help us identify future treatments to reduce treatment-related side effects. Importantly, since recruitment of these protective macrophage populations might be controlled by C5aR1, these studies will also be essential for our understanding of how to use drugs blocking C5aR1 in the most effective way in order to reduce treatment side effects.

Abstract

Little is known about how to most effectively modulate the immune response to improve tumor control while simultaneously reducing normal tissue toxicity. We have been working to identify innate immunity targets that could simultaneously improve tumour response and reduce normal tissue toxicity following radiotherapy and have identified innate immune receptor, C5aR1 as a key modulator of both tumour response and radiation-induced bowel toxicity (Figure 1)4. Our data suggest that increased numbers of a particular intestinal macrophage population in C5aR1-/- mice mediate normal tissue protection including significantly increased long-term survival of mice following abdominal irradiation. Consequently, mice treated with the potent, selective and orally active C5aR1 inhibitor, PMX205, display reduced signs of bowel toxicity. Importantly, treatment with PMX205 also results in improved tumour radiation response4. The aim of this project is to investigate the mechanisms underlying reduced bowel toxicity following C5aR1 blockade, including characterisation of protective macrophage populations in the intestines of C5aR1-/- mice and the potential effects of these macrophages on intestinal stem cell functions.

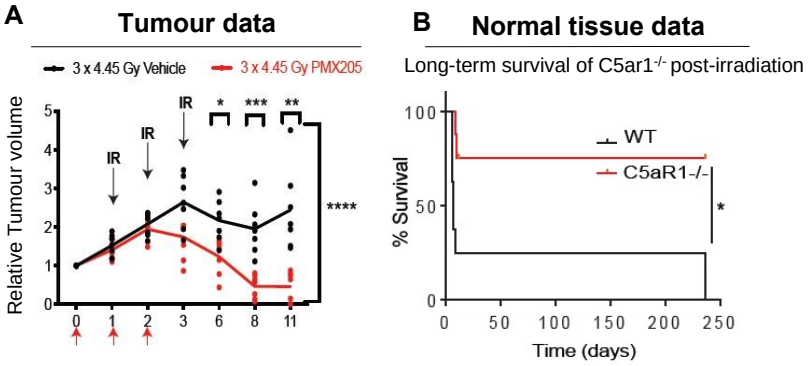


Figure 1. A. C5aR1 inhibition with inhibitor PMX205 improves tumour radiation response in an MC38 colorectal subcutaneous model**.** PMX205 treatment is indicated with red arrows. **B.** C5aR1-/- mice display increased survival following whole abdominal irradiation.

Research objectives

The overall objective of this project is to investigate the role of macrophages in reducing bowel toxicity following treatments such as radiotherapy. In particular, the student will investigate whether macrophages enriched following C5aR1 depletion provide an anti-inflammatory milieu and/or regenerative factors which facilitate stem cell

regeneration/differentiation. The mechanisms by which C5aR1 recruits particular macrophage populations to the site of injury will also be investigated. Understanding how particular macrophage populations regulate intestinal regeneration following injury will help us understand how to identify future therapies to reduce treatment-related side effects. How to most effectively modulate macrophage biology to reduce treatment-associated toxicity is currently unclear so this project will address this knowledge gap.

We will explore these hypotheses using a variety of tools including, co-cultures of macrophages and intestinal organoids (expertise in organoid culture available in the Buczacki lab) and macrophages (expertise in macrophage isolation and co-culture available in the Olcina lab). The effects of C5aR1 on macrophage recruitment will be analysed in murine models. The student will also analyse spatial transcriptomics datasets from intestinal crypts and macrophages of WT and C5aR1$^{-/-}$ mice (generated by the Olcina lab). These analyses will include assessment of the effects of C5aR1 loss on stem cell regeneration and differentiation gene signatures. Any changes observed will be validated using techniques such as qPCR and immunohistochemistry. The collaboration with the Buczacki lab will be greatly facilitated by having a joint student pursuing this project. Having a co-supervisor with expertise in intestinal stem cell biology will be key to the success of this project.

Translational potential

Diarrhea induced following intestinal irradiation is a dose-limiting toxicity for some abdominal tumours. Increasing the therapeutic index of radiotherapy through normal tissue radioprotection would be a critical step in radiotherapy dose escalation. This study opens the possibility of using C5aR1 inhibitors to increase the therapeutic index of radiotherapy.

Training Opportunities

By undertaking this project, the student will benefit from working with two laboratories with complementary expertise in innate immunity and radiobiology (Olcina) and intestinal stem cell biology (Buczacki). The student will therefore have the opportunity to learn a range of techniques including how to work with organoid-macrophage co-culture systems, isolation of intestinal macrophages, flow cytometry, immunohistochemistry and standard cell and molecular biology techniques. The student will also have access to spatial transcriptomics data and will receive training in how to analyse these data. The student will likely also acquire experience in using colorectal cancer models with clinically relevant molecular phenotypes while working both independently as well as in a team. Career development opportunities will be offered, including attendance of national and international conferences and transferable skills training courses.

References

1. Hauer-Jensen, M., Denham, J. W. & Andreyev, H. J. N. Radiation enteropathy--pathogenesis, treatment and prevention. *Nat. Rev. Gastroenterol. Hepatol.* **11**, 470–9 (2014).
2. Moding, E. J., Kastan, M. B. & Kirsch, D. G. Strategies for optimizing the response of cancer and normal tissues to radiation. *Nat. Rev. Drug Discov.* **12**, 526–42 (2013).
3. Olcina, M. M. & Giaccia, A. J. Reducing radiation-induced gastrointestinal toxicity -The role of the PHD/HIF axis. *J. Clin. Invest.* **126**, (2016).
4. Olcina MM, Melemenidis S, Nambiar DK, Kim RK, Casey KM, von Eyben R, Woodruff TM, Graves EG, Le QT, Stucki M, G. A. Targeting C5aR1 Increases the Therapeutic Window of Radiotherapy. *bioRxiv* (2020) doi:https://doi.org/10.1101/2020.10.27.358036.

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.

Lay Summary

Oesophageal and gastro-oesophageal junction (GOJ) cancers are the fastest growing cancer in the Western population. Improving the accuracy and efficiency of diagnosis, treatment and surveillance is the rationale behind the developments of computer-aided systems in medical imaging. However, obtaining reliable imaging readouts from Computed Tomography (CT) or Positron emission tomography (PET)-CT, which can be used for surveillance purposes, is time consuming and suffers from observer biases.

Currently, in the normal clinical pathway, CT is the best initial modality for surveillance of the distant spread i.e. cancer cells spreading to other parts of the body. Different types of image analysis i.e. integrating PET and CT has been shown to be superior to CT alone for detecting changes in lymph nodes as PET/CT can depict spread in normal-sized lymph nodes through the detecting of its metabolic activity using a chemical compound (Fluorodeoxyglucose, FDG) injected into patient. However, PET/CT still plays a little role in the current surveillance of GOJ tumors.

The proposed approach envisages to develop computer-aided tools that could integrate the metabolic information provided by PET and anatomical information available in CT. The integration of multimodal data such and PET and CT is expected to support obtaining reliable readouts from these data, and therefore improve detection of GOJ tumours. The obtained multimodal readouts could be also utilised in developing models for patient survival or cancer recurrence rate prediction, which could support treatment and patient-care decision-making processes. Furthermore, in the absence of robust scientific evidence and no consensus on surveillance strategy after treatment oesophageal, the proposed project could also support analysis of data collected in clinical trials related for GOJ cancers.

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

Work Package 1 (WP1). 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 recurrent tumor. We envisage that the project will start investigation on supervised (or weakly-supervised) detection of the recurrent 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 e.g. YOLO (Bochkovsky2020), 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.

Work Package 2 (WP2). 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 recurrence using multimodal imaging and non-imaging data 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).

Work Package 3 (WP3). Deployment of machine-learning model trained using retrospective data (ENSURE) to the on-going randomised clinical trial (SARONG).

ML is effectively used to learn risk predictions from the retrospective datasets that can subsequently be prospectively tested in SARONG RCT. This national UK RCT (funded by NIHR-HTA) will randomise 952 patients to intensive radiological surveillance (CT or PET-CT every 6 months for 3 years) or symptomatic clinical follow-up. Testing machine-learning model trained on retrospective data on prospective studies has been only reported in few papers, it offers promise to better capture complex relations between extracted features, and for example in (Kalscheur2018) researchers were able to improve discrimination between patients who would do better or worse following cardiac resynchronization therapy. This project, and the already approved SARONG trial (to start in April 2023) creates a unique and highly sought-after opportunity to explore this area of clinical machine learning.

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:

(Bochkovsky2020) Bochkovskiy, Alexey, Chien-Yao Wang, and Hong-Yuan Mark Liao. "Yolov4: Optimal speed and accuracy of object detection." arXiv preprint arXiv:2004.10934 (2020).
Chen, Xiaoyang, et al. "Fast and Accurate Craniomaxillofacial Landmark Detection via 3D Faster R-CNN." IEEE Transactions on Medical Imaging (2021).
(Katzman2018) Katzman, Jared L., et al. "DeepSurv: personalized treatment recommender system using a Cox proportional hazards deep neural network." BMC medical research methodology 18.1 (2018): 1-12.
(Lee2018) Lee, Changhee, et al. "DeepHit: A deep learning approach to survival analysis with competing risks." Thirty-second AAAI conference on artificial intelligence. 2018.
(Lee2019) Lee, Changhee, Jinsung Yoon, and Mihaela Van Der Schaar. "Dynamic-DeepHit: A deep learning approach for dynamic survival analysis with competing risks based on longitudinal data." IEEE Transactions on Biomedical Engineering 67.1 (2019): 122-133.
(Ting2017) Ting, Daniel Shu Wei, et al. "Development and validation of a deep learning system for diabetic retinopathy and related eye diseases using retinal images from multiethnic populations with diabetes." Jama 318.22 (2017): 2211-2223.
(Rajpurkar2017) Rajpurkar, Pranav, et al. "Chexnet: Radiologist-level pneumonia detection on chest x-rays with deep learning." arXiv preprint arXiv:1711.05225 (2017).
(Esteva2017) Esteva, Andre, et al. "Dermatologist-level classification of skin cancer with deep neural networks." Nature 542.7639 (2017): 115-118.
(Arcadu2019) Arcadu, Filippo, et al. "Deep learning algorithm predicts diabetic retinopathy progression in individual patients." NPJ digital medicine 2.1 (2019): 1-9. (Rahman2020) Rahman, Saqib A., et al. "Machine learning to predict early recurrence after oesophageal cancer surgery." Journal of British Surgery 107.8 (2020): 1042-1052.
(Niu2020) Niu, Peng-Hui, et al. "Artificial intelligence in gastric cancer: Application and future perspectives." World Journal of Gastroenterology 26.36 (2020): 5408.
(Jin2021) Jin, Cheng, et al. "Predicting treatment response from longitudinal images using multi-task deep learning." Nature communications 12.1 (2021): 1-11.
(Kalscheur2018) Kalscheur MM, Kipp RT, Tattersall MC, Mei C, Buhr KA, DeMets DL, et al. Machine learning algorithm predicts cardiac resynchronization therapy outcomes: lessons from the companion trial. Circ Arrhythm Electrophysiol. 2018;11(1):e005499.

34. Targeting cancer associated fibroblasts in chromosomally unstable oesophageal cancer [1,2,3] – Dr Parkes

Primary Supervisor: Dr Eileen Parkes
Additional Supervisors: Dr Sheraz Markar and Prof. Simon Buczacki
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Lay Summary

Cancer of the oesophagus is increasing in the UK and affecting people at a younger age. Together with a lack of successful therapeutic options, oesophageal cancer is on a course to impact increasing numbers of people in the coming years. A key feature of oesophageal cancer is tangled or knotted DNA in the cancer cells. Usually, this tangled DNA should help immune cells recognise the cancer cells and destroy them – but in oesophageal cancer, this doesn't happen. We have discovered one potential strategy the cancer uses is to cause the fibroblasts (supporting cells) to change their behaviour. We think that fibroblasts near cancer cells with tangled DNA become "stiff" and harder for the immune cells to move around. We want to investigate fibroblasts in the context of oesophageal cancers that receive chemotherapy and immunotherapy, to understand differences between fibroblast behaviour before and after treatment. We also want to see if fibroblast differences are associated with changes in immune cell behaviour inside and around the cancer. By doing this, we want to find new ways to treat oesophageal cancer in the future.

Abstract

With the advent of immune checkpoint blockade as standard of care in oesophageal adenocarcinoma (OAC), there is now a pressing need to identify new combination strategies for immune checkpoint blockade-resistant disease (the majority of OAC). OAC is characterised by chromosomal instability - we previously identified that highly chromosomally unstable (CIN-high) OAC carries a particularly poor prognosis, associated with treatment resistance. We also observe that CIN-high OAC has high expression of genes involved in organisation and remodelling of the extracellular matrix, which determines the shape, structure and stiffness of the tumour. This is consistent with a distinct fibroblast phenotype in CIN-high cancers, contributing to immunotherapy resistance.

Research objective

Here we will interrogate the fibroblast and extracellular matrix profile of OAC using a recently constructed tissue microarray and a prospective collection of fresh tissue from patients with OAC treated with immune checkpoint blockade combinations. In concert with this, blood samples from patients receiving treatment for OAC will be collected and profiled. The aims are:

(1) Identify the fibroblast phenotype in CIN-high OAC. Using multiplex IHC and digital spatial profiling, fibroblasts and extracellular matrix (Figure 1) adjacent to areas of high chromosomal instability will be profiled. Complementing this, fresh OAC samples will be disaggregated and sorted (pre-treatment) with flow cytometry and RNA-seq analysis of fibroblasts identifying potential targets on fibroblasts in CIN-high cancers. We have previously optimised a flow cytometry panel for profiling fibroblasts which will be employed in this aim. This will be the first time fibroblasts in OAC have been profiled and phenotyped. Collaborative partners in this aim include OUH surgeons and gastroenterologists, as well as the tissue banking team and tissue histopathology laboratory. Outcome: Characterisation of fibroblasts in OAC and identification of distinctive phenotypes of fibroblasts in CIN-high vs. CIN-low disease in the context of therapeutic response.

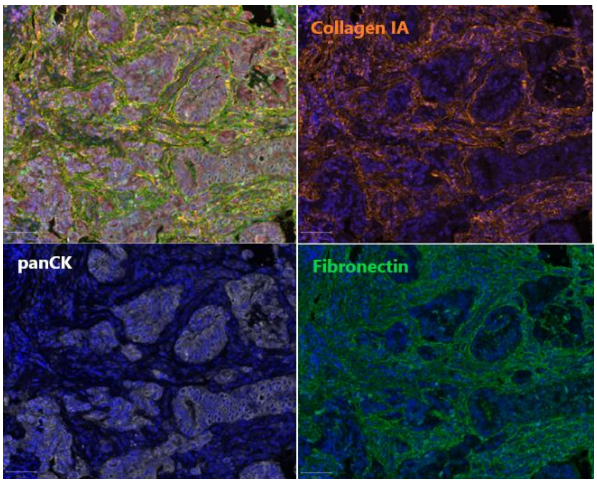


Figure 1: Multiplex immunohistochemistry showing extracellular matrix in oesophageal adenocarcinoma. Top left panel – merged image. Orange: Collagen IA. Green: Fibronectin. White: panCK. DAPI is shown in dark blue.

(2) *Interrogate fibroblast phenotype in patients treated with immune checkpoint blockade.* Organoids +/- matched fibroblasts (in collaboration with Professor Simon Buczacki) will be established from patient samples. The ability of T cells to infiltrate organoids will be assessed using imaging. In parallel a novel tissue microarray from immune checkpoint blockade-treated disease will be built, identifying fibroblast and ECM phenotype and correlates of response. This will include pre-treatment biopsy samples as well as matched resections – focusing on the characterisation of fibroblasts in immune checkpoint blockade-resistant cancers. **Outcome**: Novel organoids generated and characterised as well as identifying tumour microenvironment features associated with resistance to immune checkpoint blockade treatment in OAC

(3) *Correlate immune phenotypes using peripheral samples.* Under the leadership of Mr Sheraz Markar, peripheral samples from patients with OAC will be available. We will use existing expertise to remove peripheral blood mononuclear cells (using magnet selection) and sort CD8+ cells from these for further RNAseq profiling. These peripheral samples will be investigated for correlates of immune phenotype and clonality with the fibroblast features in the tumour microenvironment from the same patients, to determine if T-cell clonal expansion (a marker of response to immune checkpoint blockade) is affected by fibroblast phenotype in the local tumour environment. **Outcome:** First study to profile T cell clonality in relation to tumour microenvironment features in oesophageal adenocarcinoma.

The overall goal is the identification of novel tumour microenvironment targets in immune checkpoint blockade-resistant CIN-high OAC for subsequent investigation in a combination early phase study.

Translational Potential

This proposal will identify and characterise new therapeutic opportunities in the immuno-oncology space by combining targeting of cancer-associated fibroblasts in CIN-high cancers with immune checkpoint blockade. Targeting the tumour microenvironment addresses an urgent barrier to successful immune recognition of cancer cells, and as such supports the future development of combination immuno-oncology strategies. Moreover, this project supports a personalised immuno-oncology approach by specifically targeting CIN-high cancers.

Training opportunities

The successful applicant will receive training in flow cytometry, 3D culturing of organoid models, cell sorting, digital pathology analysis as well as bioinformatic training for RNAseq analysis. The Parkes and Buczacki labs are established teams including postdoctoral fellows, DPhil students as well as technical support. Hands on training and support will be provided for all the techniques outlined. Students will also have the opportunity to attend bioinformatics training to learn or advance their bioinformatic skills.

References

Li…Parkes, Izar, Bakhoum. Metastasis and immune evasion from extracellular cGAMP hydrolysis. Cancer Discovery; 2021.

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35. Important differential roles for two-pore channels TPC1 and TPC2 in melanoma tumourigenesis and metastasis[1,2,3,4] – Prof. Parrington

Primary Supervisor: Prof. John Parrington
Additional Supervisors: Prof. Angela Russell
Eligibility: Track 1, 2, 3 and 4 students are eligible to apply for this project.

Lay Summary

Melanoma, a type of skin cancer, is an aggressive cancer whose UK incidence increased 45% over the last decade. Understanding how cancer develops and spreads through the body through the process of metastasis, is critical for developing new ways to diagnose and treat cancer. In this project we will investigate the role of the two-pore channel proteins (TPC1 and TPC2), which generate cellular 'signals', in melanoma tumour development and metastasis. This investigation is stimulated by our recent finding that removing TPC2 from cells derived from an immature melanoma, inhibited their migratory and invasive characteristics. In contrast loss of TPC2 in cells derived from a metastatic melanoma, enhanced their migratory and invasive characteristics. This suggests that inhibition of TPC2 may have very different consequences depending on tumour stage and other differences between melanoma cells, which could be very important in terms of treatments that seek to target TPC2. We have also shown that cells in which TPC2 is deleted form slow-growing tumours when injected into mice, yet cause more organ damage and faster death compared to control cells. This suggests some toxic effect of such cells in the body, possibly through metastasis. Our deletion of TPC1 from melanoma cells had very different effects to TPC2 deletion, suggesting that TPC1 and TPC2 play differential roles in cancer development and metastasis. What we still barely understand is what such differential roles are, and how they relate to cellular and bodily mechanisms. Here we will identify such mechanisms that are key to the role of TPCs in melanoma, and test drugs that interact with TPCs. Although focused on melanoma, we expect our findings to help us better understand tumour development and metastasis of other cancer types; indeed our other recent studies are showing interesting effects of TPC2 deletion in breast and colon cancer cells.

Abstract

Intra-tumour microenvironment stresses, combined with cell plasticity, drive changes in cell phenotype that underpin metastatic dissemination and contribute to resistance to targeted and immunotherapies (1,2), the major cause of cancer-related deaths. In cutaneous melanoma, an aggressive cancer whose UK incidence increased 45% in the past 10 years, the role of phenotype-switching in disease progression is well-established (3). Notably, phenotypic identity is controlled by the microphthalmia-associated transcription factor MITF, which coordinates many aspects of melanoma biology (4). However, many melanoma cells within individual tumours, particularly invasive or therapy-resistant cells, do not express MITF. Ca^{2+} signalling likely plays a critical role in controlling melanoma progression, at least in part via regulation of the MITF-family of transcription factors. This is consistent with observations that Ca^{2+} signalling plays a vital role in tumour metastasis and progression (5). Yet whether Ca^{2+} signalling has different effects in different phenotypic states is unknown. Recently we showed that the endolysosomal two-pore Ca^{2+} channels (TPC1 and TPC2) play important roles in melanoma progression. Thus, in $MITF^{High}$ B16 cells TPC2 KO inhibited ability to migrate and invade a 3D matrix; in contrast TPC2 KO in MITF-negative CHL1 cells enhanced migratory and invasive characteristics (6). Importantly, recently we showed that while TPC2 KO cells form slow growing tumours they cause more organ damage and faster death compared to WT cells. Significantly, we have identified an important link between TPC2 and the Hippo signalling pathway that also regulates melanoma phenotypic identity (6). Our other recent unpublished studies have shown that while TPC2 KO has little effect on melanoma cell proliferation, TPC1 KO significantly reduced proliferation rate. This suggests that TPC1 and TPC2 may play different roles in melanoma tumourigenesis. Here we will identify critical molecular and cellular events that are key to TPC role in melanoma.

Research objectives

We and others have shown that the endolysosomal calcium channels, TPC1 and TPC2, play key roles in a broad range of physiological processes (7). By generating TPC2 KO melanoma cells we have shown that TPC2 plays an important role in cancer *in vitro* by regulating invasion (6), and *in vivo* where TPC2 KO slows tumourigenesis but increases organ damage and lethality; that TPC2 impacts on Hippo signalling, a key pathway that controls melanoma phenotype in response to exposure to extracellular matrix (6); and that *TPC2* gene polymorphisms in UK individuals correlates with susceptibility to skin cancer (8). Most importantly, the effect of TPC2 KO differs in melanoma cells exhibiting different phenotypes marked by differential MITF expression. However, whether other factors, such as different genetic driver mutations, also affect the outcome of TPC2 mutation is unclear.

Objectives: In this project our objectives will be to:
(1) Identify molecular basis of TPC2's apparently differing roles in MiTF^{High} vs MITF^{Low}melanoma cell. We will use cell lines generated by the Goding lab in which endogenous MITF/TFEB and TFE3 have been knocked out alone or in combination and study the effect of TPC2 KO in such cell lines. Assays to be used include proliferation, invasion/migration/differentiation, activation of Hippo signalling, secretion of extracellular matrix, and assays for the integrated stress response that plays a critical role in melanoma invasion and adoption of a de-differentiated phenotype.
(2) Identify TPC2's role in tumourigenesis in an *in vivo* mouse model. Tumours derived from TPC2 KO melanoma cells injected into mice grow to a smaller size than tumours from injected WT cells yet are far more detrimental to the animal (data not shown). We will investigate whether TPC2 KO cells *in vivo* are less proliferative, but more metastatic, using luciferase-tagged cells that enable tumour growth and metastatic dissemination to be imaged over time *in vivo*.
(3) Study role of TPC1 in melanoma. We recently used CRISPR/Cas to create TPC1 KO melanoma cells. Unlike TPC2 KO that has no impact on proliferation, TPC1 KO greatly inhibits proliferation (data not shown). We will perform general analysis of the effect of TPC1 and TPC2 KO on melanoma cell tumourigenesis and metastasis *in vitro* and *in vivo*.
(4) Assess effect of novel chemicals that interact with TPCs on melanoma progression/metastasis. We will screen for novel chemicals that interact with TPC1 or TPC2 and then assess the effect of such novel chemicals on melanoma progression. This will be the first step towards identifying their therapeutic potential as anti-cancer drugs.

Plan of Work and Predicted Outcomes.
1. The role of TPC2 in melanoma. TPC2 KO in B16 cells inhibited their migration and invasion of a 3D matrix, but in CHL1 cells had the opposite effect. TPC2 KO may have different effects depending on phenotype related to MITF status. This will be explored using melanoma cell lines knocked out for MITF-or the related TFEB or TFE3 alone or in combination. Cell growth, migratory characteristics, and other parameters will then be compared. We will determine MITF protein and mRNA expression after glutamine starvation, known to induce a Ca^{2+}-dependent increase in MITF expression. In addition, given calcineurin's effect in dephosphorylating the MITF-related factors TFEB/3 to promote their cytoplasmic nuclear localization, we will examine the regulation of TFEB/3 in WT and TPC2 KO cells by examining their phosphorylation using antibodies generated by the Goding lab. Immunofluorescence will be used to examine TFEB/3 subcellular localization in response to amino acid or glucose starvation that would normally promote their nuclear accumulation. To distinguish whether other factors affect the differential response between MITF^{High} B16 cells vs MITF^{Low} CHL1 cells to TPC2 KO, we will create TPC2 KOs in other melanoma cell lines with different driver mutations, or in which MITF or related proteins are knocked out. We have shown that TPC2 KO or knockdown activates the Hippo pathway effectors YAP/TAZ, drivers of tumourigenesis and metastasis (8). TPC2 KO CHL melanoma cells have reduced expression of ORAI1, a key component of store-operated calcium entry (SOCE), previously shown to inhibit YAP/TAZ. We will measure SOCE directly in WT vs TPC2 KO cell lines and examine the relationship between SOCE and TPC2-mediated endolysosomal Ca^{2+} release in CHL1 cells using fluorescent tagged versions of TPC2 and ORAI1 and imaging Ca^{2+} to investigate the physical and functional interactions between these two signalling proteins. Finally, we will use CRISPR/Cas to create melanoma cell lines with polymorphisms in the *TPC2* gene modelled on those that we recently identified in the UK Biobank (8), to see how such changes affect the properties of melanoma cells.
2) Role of TPC2 in tumourigenesis in an *in vivo* mouse model. Injecting WT or TPC2 KO melanoma cells into mice revealed decreased tumour growth, but increased lethality. In this project we will use luciferase-tagged melanoma cells to follow in real time tumour growth and metastatic dissemination of WT vs TPC KO cells. We will examine tissues and organs from mice injected with WT or TPC2 KO cells, following tumour development, to identify molecular/cellular changes, including formation of micrometastases, that may explain why the TPC2 KO tumours are so lethal. We will analyse the blood of mice harbouring tumours derived from WT vs TPC2 KO melanoma cells, to see if circulating cytokines differ in these two situations. We will study the transcriptomes of the WT vs TPC2 KO tumours using 3'RNA-seq, at different stages of tumour growth, to see how RNA expression changes in the two types of tumour during their growth *in vivo*..
(3) Role of TPC1 in melanoma. TPC1 and TPC2 have different intracellular locations and biophysical properties. Currently little is known about TPC1's role in melanoma. We recently showed that unlike TPC2 KO that has no impact on proliferation of melanoma cells, TPC1 KO greatly inhibits rate of proliferation (data not shown). We will therefore use a similar approach to that taken with TPC2 and make various TPC1 KO cell lines and examine their differentiation, and proliferative and migratory properties *in vitro*. We will also investigate potential links with other signalling proteins linked to melanoma like MITF and YAP/TAZ. Finally, we will study how TPC1 KO affects the tumour formation and metastatic dissemination of cells injected into mice. We will be particularly interested to identify similarities but also differences between TPC1 and TPC2's roles in melanoma, as this could have important therapeutic implications.
(4) Effect of pharmacological modulators of TPCs in melanoma progression. A number of chemicals that interact pharmacologically with TPC2 have been identified but they are far from specific in their action (9). In this project we will draw on the expertise of Prof. Angela Russell and Dr Margarida Ruas, in screening and testing novel pharmacological

agents, to identify novel, specific agonists and antagonists of TPC1 and TPC2, and test their effects on melanoma cell biology and particularly their capacity for proliferative and invasive and migratory properties *in vitro* and *in vivo*.

Translational potential

One of the major contributors to disease progression and therapy resistance is the ability of plastic cancer cells to adopt distinct phenotypic states in response to the microenvironment. Our preliminary data strongly implicate TPC2 in disease progression in vitro and in vivo and suggest that TPC2 may play a phenotype-specific role in the disease. Because TPC2 is potentially druggable (9), these observations have significant implications for treatment. The project will decipher the role of TPC2, and for the first time the related protein TPC1, in vitro and in vivo in regulating key melanoma associated transcription factors and signalling pathways, reveal the role of TPCs in melanoma lethality and explain the impact of TPC2 polymorphisms on melanoma incidence. As TPC1 and TPC2 are widely expressed in other cancers, the results obtained here will have implications beyond melanoma.

Training opportunities

The student will be trained in a wide variety of cell biology, molecular biology, and biochemistry techniques including cell culture, RTqPCR, CRISPR/Cas gene editing, enzyme assays, immunoblot and immunofluorescence analysis, Ca2+ imaging, in vivo animal work, histology, data analysis, and statistics.

References

1. Rambow, Marine & Goding (2019) *Genes Dev* 33:1295; 2. García-Jimenéz & Goding (2019) *Cell Metab* 29:258; 3. Falletta *et al.* (2017) *Genes Dev* 31:18; 4. Goding & Arnheiter (2019) *Genes Dev* 33:983; 5. Alharbi, Zhang & Parrington (2021) *Cancers* 13:179; 6. D'Amore *et al.* (2020) *Cancers* 12:2391; 7. Jin *et al.* (2020) *Trends Pharm Sci*. 41:582; 8. Alharbi & Parrington (2021) *Genom Med* 6:58; 9. Alharbi & Parrington (2021) *Cell Chem Biol* 28:1103.

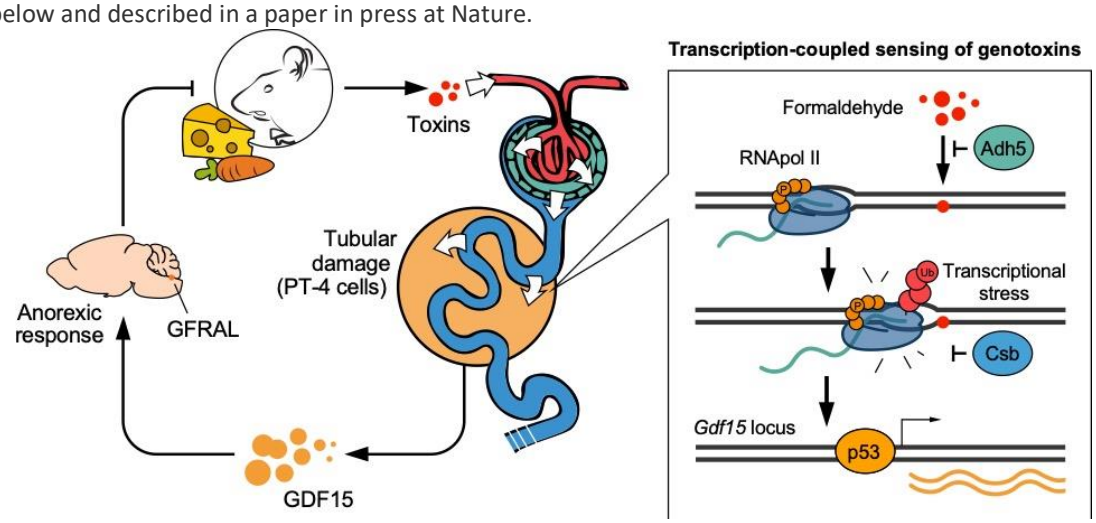
36. DNA damage induced cachexia in cancer and other pathological states [1,2,3] –Prof. Patel

Primary Supervisor: Prof. KJ Patel
Additional Supervisors: Dr Ross Chapman
Eligibility: Track 1 and 4 students are eligible to apply for this project.

Lay Summary
Cachexia is a common clinical state that is seen in many patients with cancer and it manifests itself by progressive decline in body weight and muscle volume. Ultimately this condition is progressive and it significantly impacts on such cancer patients to withstand the disease and the treatments required to cure or control it. Cachexia ultimately contributes to cancer mortality and declining quality of life, however why this process happens is not understood and without understanding the underlying the biology we will not be able to devise treatments to prevent or reverse this state. Our project builds on our basic discovery of an anorexic signalling pathway triggered by damage to the DNA of certain cells in the kidney, this new response very likely explains why certain anticancer drugs cause anorexia and weight loss. The reason why we believe that the kidney may play a central role in driving certain forms of cachexia in cancer patients is because many drugs to treat cancer damage this organ and lead to activation of the anorexia cascade. The second reason to consider the kidney is that kidney cancer is often associated with severe cachexia and it is plausible that inappropriate activation in these cancer cells of the anorexia pathway may contribute to this. We want to better understand this pathway and define how it might be engaged in cancers that are commonly associated with cachexia.

Abstract
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 Cockayne 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 CRISPER 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 objectives
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

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.

References

Mulderrig L, Garaycoechea JI, Tuong ZK, Millington CL, Dingler FA, Ferdinand JR, Gaul L, Tadross JA, Arends MJ, O'Rahilly S, Crossan GP, Clatworthy MR, Patel KJ (2021) 'Formaldehyde transcriptional stress triggers an anorexic DNA damage response' (in press). *Nature*

Marteijn, J.A., Lans, H., Vermeulen, W., and Hoeijmakers, J.H. (2014). Understanding nucleotide excision repair and its roles in cancer and ageing. *Nat Rev Mol Cell Biol* **15**, 465-481.

Dingler, F.A., Wang, M., Mu, A., Millington, C.L., Oberbeck, N., Watcham, S., Pontel, L.B., Kamimae-Lanning, A.N., Langevin, F., Nadler, C., Cordell, R.L., Monks, P.S., Yu, R., Wilson, N.K., Hira, A., Yoshida, K., Mori, M., Okamoto, Y., Okuno, Y., Muramatsu, H., Shiraishi, Y., Kobayashi, M., Moriguchi, T., Osumi, T., Kato, M., Miyano, S., Ito, E., Kojima, S., Yabe, H., Yabe, M., Matsuo, K., Ogawa, S., Gottgens, B., Hodskinson, M.R.G., Takata, M.*, and Patel, K.J.* (2020). Two Aldehyde Clearance Systems Are Essential to Prevent Lethal Formaldehyde Accumulation in Mice and Humans. *Mol Cell*. 80: 996-1012

Garaycoechea, J.I., Crossan, G.P., Langevin, F., Mulderrig, L., Louzada, S., Yang, F., Guilbaud, G., Park, N., Roerink, S., Nik-Zainal, S., Stratton, M.R., and Patel, K.J. (2018). Alcohol and endogenous aldehydes damage chromosomes and mutate stem cells. *Nature*. 553: 171-177.

Burgos-Barragan, G., Wit, N., Meiser, J., Dingler, F.A., Pietzke, M., Mulderrig, L., Pontel, L.B., Rosado, I.V., Brewer, T.F., Cordell, R.L., Monks, P.S., Chang, C.J., Vazquez, A., and Patel, K.J. (2017). Mammals divert endogenous genotoxic formaldehyde into one-carbon metabolism. *Nature*. 548: 549-554.

37. Development of the next generation linear accelerator for FLASH radiotherapy with megavoltage x-rays [1,2,3,4] – Dr Petersson

Primary Supervisor: Dr Kristoffer Petersson
Additional Supervisors: Prof. Amato Giaccia
Eligibility: Track 1, 2, 3 and 4 students are eligible to apply for this project.

Lay Summary
FLASH Radiotherapy is a new type of radiation treatment of cancer. The treatment is delivered with a 1,000 times higher radiation intensity compared to standard treatments. The result is very quick treatments, similar to the time it takes to take an X-ray image at the dentist. This new treatment technique shows great promise in our biology experiments, killing cancer tissue while preserving healthy tissue better than normal radiation treatments. We would like to use this new technique to treat our cancer patients. However, generating the high intensity radiation is difficult, requiring large and expensive machinery. Our research programme aims to find a smaller and cheaper technical solution. Together with experts from the company Teledyne e2v, we will push the available power generating components to and above their current limits, to find their true potential for FLASH radiotherapy. These should be much higher for FLASH because of the short treatment times. We will also try to improve on the efficiency of the radiation production. Radiation is produced by electrons being accelerated onto a piece of heavy metal, a so-called "target". By changing the design of this target, our calculations indicate that the efficiency of our radiation production will increase severalfold. Our best target design and FLASH-optimised power generating solution will be coupled with other components optimised for FLASH delivery to form a complete linear accelerator optimised for FLASH Radiotherapy.

Abstract
FLASH radiotherapy is a novel radiotherapy delivery methodology using ultra-high dose rates. Recent global research has indicated that FLASH can reduce radiation-induced damage in healthy tissues with similar anti-tumour effects as conventional radiotherapy, the so-called "FLASH effect" 1. In addition, FLASH works with hypofractionated regimens, and treatment times are in the order of 100 ms 2. Such short treatment times may increase patient throughput, and most importantly will effectively negate the problem of patient motion during radiation delivery.
The Oxford Institute for Radiation Oncology (OIRO) is world leading in Radiation Oncology research, and Kristoffer Petersson, Group Leader for FLASH Radiation, is recognised as a leading figure in FLASH radiotherapy research and in the investigation of the FLASH effect. Teledyne e2v is the world leader in RF solutions for radiotherapy and its magnetrons, thyratrons, modulators and accessories are present in >98% of linear accelerator driven radiotherapy systems worldwide. Teledyne e2v has long-term relationships with all of the major radiotherapy systems manufacturers, including Elekta, Varian and Accuray. We will work with them in the development of a FLASH beam generation system.
To date, most FLASH research is performed using proton or electron beams. Aside from the many technical challenges in delivering clinical FLASH, the use of these particles requires large and expensive facilities if they treat anything but the most superficial of tumours. The FLASH Programme in OIRO is seeking to develop a solution using Megavoltage X-Rays. This will be at a lower cost, with a smaller system footprint and reduced infrastructure requirements – particularly with regards to radiation shielding.

Research Objectives

1. **Prove the potential for ultra-high dose rates from conventional hardware**
   Teledyne would provide commercially available hardware to drive a conventional X-Ray linac system and assist OIRO in pushing the operating parameters as close to FLASH levels as is possible. The extremely short timescale of a FLASH treatment fraction may unlock interesting opportunities for the technical advancement of hardware for radiotherapy systems.
2. **Evaluate upgrades to the base system to multiply the dose rate**
   One of the major challenges in providing FLASH X-Rays is that conventional X-Ray targets are only on the order of 1% efficient. OIRO is developing novel targets to provide a substantial increase in target gain. By using an accelerator with a removable target, the team will be able to evaluate its designs.
3. **Identify and implement a new, higher dose rate linear accelerator**

Having established the capability of the RF system and the X-Ray target, attention will turn to the design of an appropriate electron gun and accelerator to complete the demonstrator system.

4. **Route to commercialisation**
   The shorter-term technology advancements will enable us to engage with the major radiotherapy systems manufacturers to discuss opportunities for these improvements to be designed into their future platforms.

Translational Potential
If successful, this project will provide a future pathway to:

1. A larger number of local FLASH treatment centres as opposed to a small number of large regional or national treatment centres.
2. Treatment centres at a price point which is affordable in lower income countries

This is essential if FLASH is to become widely adopted and accessible.

It is recognised that such a commercially available FLASH system will not be achieved for some years. However, it is intended that this programme of research will provide shorter term incremental technology improvements which will be applicable to further advancement in the delivery of conventional radiotherapy. This may include radically reduced time spent in breath holds, electron FLASH for superficial tumours, improved system reliability, etc. We foresee that we will produce world leading FLASH research that will be published in high-impact journals and that our technical solutions will be implemented in the next generation of medical linear accelerators. This will serve as a first step towards a clinical FLASH X-Ray treatment device.

Training opportunities

The student will have the opportunity to train at Teledyne e2v, learning about RF solutions and their implementation in radiotherapy. This general knowledge of RF sources will be essential for the work carried out during the DPhil project. At OIRO, the student will earn about FLASH radiation biology and dosimetry from the members of the FLASH Radiation research group. Additionally, the electron FLASH linear accelerator available at OIRO will serve as a reference for all the work carried out throughout the project [3].

References:

1. Wilson JD, Hammond EM, Higgins GS, Petersson K. Ultra-High Dose Rate (FLASH) Radiotherapy: Silver Bullet or Fool's Gold? *Frontiers in Oncology.* 2020;9.
2. Montay-Gruel P, Acharya MM, Goncalves Jorge P, et al. Hypofractionated FLASH-RT as an Effective Treatment against Glioblastoma that Reduces Neurocognitive Side Effects in Mice [published online ahead of print 2020/10/17]. *Clin Cancer Res.* 2021;27(3):775-784.
3. Berne A, Petersson K, Tullis IDC, Newman RG, Vojnovic B. Monitoring electron energies during FLASH irradiations [published online ahead of print 2020/12/29]. *Phys Med Biol.* 2021;66(4):045015.

38. Comprehensive study of oncogenic JAK2 proteomes as a basis for improved MPNs therapies [1,2,3] – Dr. Pinto-Fernández

Primary Supervisor: Dr. Adán Pinto-Fernández
Additional Supervisors: Dr Benedikt Kessler
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Lay Summary

- Myeloproliferative neoplasms (MPNs) are progressive blood cancers where the bone marrow overproduces mature white blood cells. Of interest, an activating mutation in a cellular protein called JAK2 is found in most MPNs cases.
- Ruxolitinib (Jakafi) is a highly potent and selective JAK2 inhibitor approved for MPNs in 2011 and has demonstrated major benefits to patients. However, most patients with mutated JAK2 do not respond to this drug and, therefore, the identification of more efficient therapies remains critical. (More info in references 1 and 2, at the end of the proposal).
- This PhD project aims to understand why current therapies targeting JAK2 for the treatment of myeloproliferative neoplasms do not work in patients carrying the above-mentioned mutation. Understanding the functional consequences of this mutation will provide novel insights for overcoming resistance to therapy and eventually improve the survival and life quality of MPNs patients.
- To investigate this, we have performed comprehensive, large-scale, studies of cellular proteins in a number of blood cancer cell lines harbouring either, the "normal" unaltered or the mutated protein.
- Initial analysis of the data suggested new roles for JAK2 in mitochondrial (a cellular component) functions. Interestingly, JAK2 inhibitors interfered with these functions. However, this only happens in cells without the mutation unveiling a potential resistance mechanism in the cells with the mutation.
- In addition to these observations, previously published data described that interfering with mitochondrial functions induced cell death in MPNs cells containing the activating mutation in JAK2.
- In summary, the appointed PhD student, with the guidance of experts in cancer research and applying advanced biomedicine and bioinformatics tools, will aim to validate and expand the above-described findings in order to better understand how mutated JAK2 works and to identify novel, more efficient, treatments for MPNs.

Abstract
We performed a comprehensive mass spectrometry - based interactomic, proteomic, and phospho-proteomic analysis to identify proteins associated with activated JAK2 in five different blood cancer cell lines harbouring wildtype JAK2 (TF1 and F36P) and the mutation associated with MPNs, JAK2 V617F (HEL, SET2, and UKE1). The cells were treated with Erythropoietin (EPO) at different time points (0, 5 min., 15 min., and 30 min.) in the presence/absence of two JAK2 inhibitors, 1824 (Ruxolitinib) or 532. Interactome data has been fully analysed and proteome and phospho-proteome data have been acquired and preliminarily analysed. The proposed research involves the validation of the interactome data, the analysis of the proteome and phosphor-proteome data y the study of JAK2 roles in MPNs cancer cell antigenicity. Project overview in Figure 1.

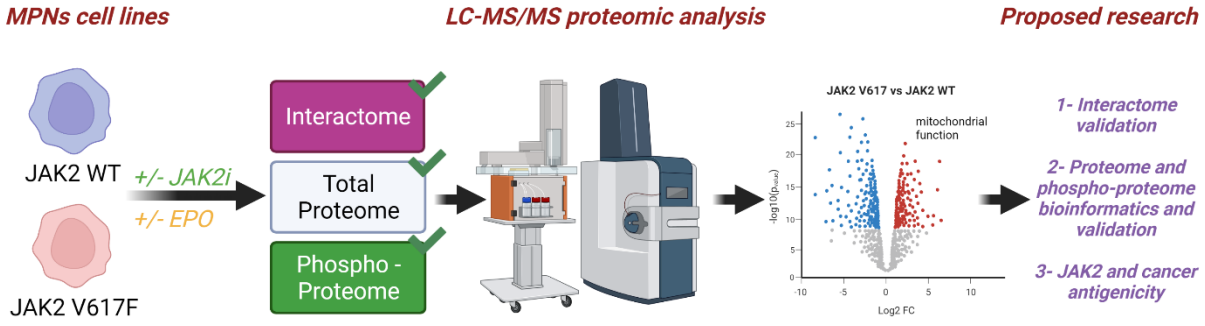


Figure 1: Discovery of JAK2 wildtype versus V617F mutant dependent molecular pathways. *Proteomic workflow including interactome, phospho-proteome, and total proteome analysis of five different blood cancer cell lines. Proposed research includes the validation of the JAK2 interactome, the comprehensive bioinformatic analysis of the total proteome and phosphor-proteome data and the study of JAK2 in cancer immunogenicity.*

Analysis of the JAK2 interactome unveiled the **association of wildtype JAK2 with mitochondrial components** upon activation with EPO. Notably, JAK2 inhibitors did prevent EPO-dependent binding of JAK2 WT to such mitochondrial components. Most importantly, this **regulation seemed to be lost in cells harbouring the activating V617F mutation (Figure 2)**. Our results represent novel biology associated with JAK2 signalling and EPO activation cascades and could possibly provide a potential link to oxygen sensing and energy metabolism, affecting cellular proliferation and differentiation.

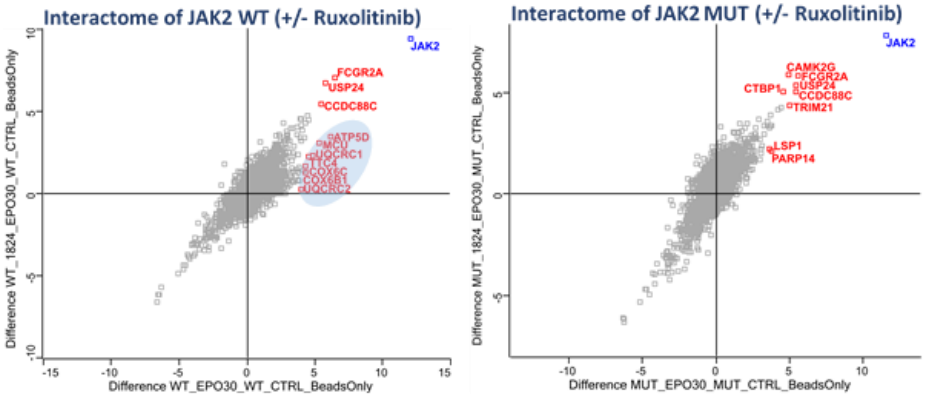


Figure 2: JAK2 inhibition prevents association of mitochondrial components to wildtype (circled in blue), but not to V617F JAK2. *Comparative scatter plots showing the JAK2 interactomes in WT cells (left) and JAK2V617F cell lines (right). Conditions EPO 30 minutes treatment in the presence/absence of JAK inhibitor 1824 (Ruxolitinib) were plotted. The bait is labelled in blue and selected interactors in red.*

Research objectives

- To study the co-localisation/interaction between JAK2 (mutant and WT), upon EPO stimulation (+/- JAK inhibitor and +/- JAK2 genetic inhibition), and the identified mitochondrial components by co-immunoprecipitation and confocal microscopy. These experiments aim to validate the proteomics data.
- To measure mitochondrial activity reflected by oxygen consumption rate (OCR) and extracellular acidification rates (ECAR) in the same conditions using a Seahorse Real-Time Cell Metabolic Analyser and ROS production using molecular probes in a live cell imaging system.
- Evaluate whether combined use of JAK2 inhibitors and ROS inducers such as LAAO, CCCP, Rotenone, Cisplatin, enhances MPN anti-tumour therapy.
- Comprehensive bioinformatic analysis of the remaining data (phospho-proteome and total proteome) and further validation.
- Study of JAK2-dependent tumour antigenicity in T cell activation/T cell killing assays (in collaboration with Prof. Tao Dong).

Translational Potential

With this research plan, we expect to uncover novel JAK2 biology that could explain resistance mechanisms to current therapy and allow the characterisation of additional targets for the treatment of MPNs. With the first three aims of the proposal, we want to validate our interactomics data suggesting that JAK2, upon activation, has important roles in mitochondrial function and cellular metabolism. For instance, it has been shown that mutated JAK2 induced the accumulation of reactive oxygen species (ROS) in patients with MPNs, highlighting key roles for the JAK2 pathway in mitochondrial activity and intracellular ROS generation (reference 3). The authors of this study suggested that targeting ROS accumulation may prevent the development of JAK2V617F MPNs. Further supporting this hypothesis, another study demonstrated that a ROS inducer exhibited cytotoxicity and induced apoptosis in JAK2V617F MPNs cell lines in a ROS production-dependent manner (reference 4). Based on this, we will determine whether ROS inducers enhance the anti-cancer effects of JAK2 inhibition. Importantly, additional JAK2 biology will be explored using the remaining proteomic analysis, potentially providing additional insights into resistance to JAK inhibition. Finally, the interplay between JAK2 status/inhibition and its implications to resistance to current immunotherapy will be also investigated.

Training Opportunities

The student will have opportunities to gain expertise in advanced bioinformatics, proteomics, metabolic studies (Seahorse), cellular biology (immunofluorescence, tissue culture, live cell imaging, gene knockdown/knockout etc), immunology (T cell activation and T cell killing) and biochemistry (immunoprecipitation and immunoblotting).

References

1. Schieber M, Crispino JD, Stein B. Myelofibrosis in 2019: moving beyond JAK2 inhibition. Blood Cancer J. 2019;9(9):74.
2. Staerk J, Constantinescu SN. The JAK-STAT pathway and hematopoietic stem cells from the JAK2 V617F perspective. JAKSTAT. 2012;1(3):184-90.
3. Marty C, Lacout C, Droin N, Le Couedic JP, Ribrag V, Solary E, et al. A role for reactive oxygen species in JAK2 V617F myeloproliferative neoplasm progression. Leukemia. 2013;27(11):2187-95.
4. Machado-Neto JA, Traina F. Reactive oxygen species overload promotes apoptosis in JAK2V617F-positive cell lines. Rev Bras Hematol Hemoter. 2016;38(3):179-81.

39. Neutrophils as novel targets in colorectal cancer [3] – Prof. Powrie

Primary Supervisor: Prof. Fiona Powrie
Additional Supervisors: Prof. Irina Udalova
Eligibility: Track 3 students are eligible to apply for this project.

Project Summary

Cancer is a disease in which cells of the body divide uncontrollably and, in some instances, spread to other parts of the body. In the intestine, cancer is often located in the large bowel (colon) or back passage (rectum) and termed 'colorectal cancer' (CRC). CRC is the second most common cancer in the UK and difficult to treat as a large proportion of disease fails to respond to new checkpoint therapies that harness the immune system. One of the reasons for that is that in CRC the immune system becomes harmful producing inflammation that fuels the cancer process. In our earlier work we developed mouse models of inflammation driven cancer and identified immune cells and their products that promote disease. Our unpublished work has identified particular immune cells, neutrophils as associated with inflammation driven cancer and high amounts of neutrophils in human CRC tumour tissue is associated with a poor outcome. We have also identified an enzyme peptidylargine deiminase 4 (PAD4) expression in pre cancer cells as a hallmark of the developing inflammation driven cancer lesion. PAD4 is also expressed in neutrophils. In this project we will build on this preliminary data to assess the role of neutrophils and the PAD4 pathway in CRC development, progression and response to therapy. Utilising cutting edge tools we will work at the intersection of model systems (mouse models and human cancer cell cultures) and assessment of human disease to newly identify how neutrophils and their products fuel CRC. We expect knowledge gained from this project will lead to new understanding of how inflammation fuels CRC that will drive new diagnostic and therapeutic approaches.

Abstract

Colorectal cancer (CRC) is a heterogeneous disease of the intestinal epithelium that is characterised by the accumulation of DNA mutations, tumour development and often fatal metastasis. Many forms of CRC are refractory to immune checkpoint therapies thought to be attributable to low neo-antigen generation and protumorigenic inflammation driving the cancer niche1 Consistent with that, patients diagnosed with inflammatory bowel disease (IBD) have an increased risk of developing CRC[2]. Over the last decade we have developed a number of models of microbe driven colitis associated cancer (CAC) and demonstrated the role of novel innate lymphoid cells and IL-22 in tumour development and progression[3]. We also identified amounts of the IL22RA gene as prognostic in human KRAS mutant CRC4 Longitudinal analysis in microbe driven CAC identified accumulation of neutrophils and epithelial expression of the citrullination enzyme peptidylarginine deiminase 4 (PAD4) as early features of the cancer niche (Figure 1A&B). Pharmacological inhibition of PAD4 reduced tumour burden, indicating a functional role for this enzyme in sustaining the tumour response. In other spontaneous models of CRC, neutrophils play a crucial role in disease[5,6] with preliminary data on a range of models showing EC PAD4 expression (unpublished).

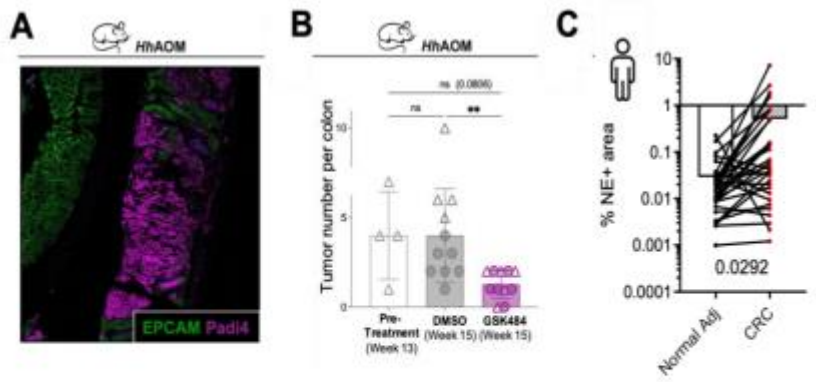


Figure 1: PADI4 expression in tumor epithelial cells and neutrophils are present in a subset of human CRC. A) Immunofluorescence staining of epithelial EpCAM and PAD4 in tumours of *Hh*OM mice. B) Tumour numbers of HhOM mice treated with the PAD4 inhibitor GSK484 (daily intraperitoneal injections for the last 14 days of the model. C)Gene expression of neutrophil-derived elastase (NE) in normal adjacent and tumours derived from CRC patients.

Neutrophils are cells that play a crucial role in the innate immune response and are often associated with inflammatory diseases. In colitis, the infiltration of neutrophils is a major feature of disease severity scores and progression[7], and neutrophils have been recently linked to therapeutic nonresponse in IBD[8]. Furthermore, neutrophils have the highest correlation with adverse outcomes in colon, lung and pancreatic cancer[9]. However, the precise mechanistic role(s) of neutrophils in cancer remain ambiguous10 with longitudinal and functional data still lacking in CRC. Initial data from our lab has shown a subset of CRC patients with neutrophil infiltration (Figure 1C). Based on a substantial body of preliminary data we hypothesise that neutrophils shape a specific inflammatory niche in CRC which promotes mutation-driven PAD4-dependent tumorigenesis. This DPhil project will test this hypothesis using disease positioned CRC models (Inflammation Hh AOM; spontaneous BRAFMutALK5-/-mouse models) and proteomics to identify neutrophil pathways associated Figure 1: PADI4 expression in tumor epithelial cells and neutrophils are present in a subset of human CRC. A) Immunofluorescence staining of epithelial EpCAM and PAD4 in tumours of HhOM mice. B) Tumour numbers of HhOM mice treated with the PAD4 inhibitor GSK484 (daily intraperitoneal injections for the last 14 days of the

model. C)Gene expression of neutrophil-derived elastase (NE) in normal adjacent and tumours derived from CRC patients. with CRC. The functional role of PAD4 in neutrophils and EC will be assessed. Pathways identified will be tested in tumour organoid cultures, mouse CRC models and validated in human CRC subsets.

Research objectives

A. Identification of neutrophil mediated pro-tumorigenic processes: 1) Neutrophil proteomics. We will identify protein candidates acting on cancer cells using bulk proteomics of sorted neutrophils from human CRC samples and murine CRC models. A proteomic approach is preferred to transcriptomics as neutrophils store many proteins and RNA yield is low[11]. We will use our biobank of sorted neutrophils from CRC patients (n= 20 patients) and compare their protein profile to neutrophils sorted from mouse CRC models over disease course (HhAOM and BRAFMutALK5-/-mouse models). Candidate proteins prioritised by shared expression in human will be tested in tumour organoid cultures established in the Powrie lab (below) and probed by immunofluorescence on CRC tissue. This aim will be supported by an established collaboration with Prof. Doreen Cantrell an expert in leukocyte proteomics 2) Neutrophil epithelial cell cross talk. Our lab has established intestinal organoids models from both normal and CRC epithelial cells which we will use to test the activity of proteins identified in Aim 1. Key measurements will involve assessment of epithelial cell transformation and cancer cell proliferation and migration. Subsequently, we will co-culture neutrophils and organoids derived from human patients to test the therapeutic potential of candidate proteins identified in Aim 1. This aim will be supported by our ongoing collaboration with Prof Irina Udalova who is an expert in neutrophil biology and has access to a cell line of neutrophils that can be genetically manipulated[12]. 3) Therapeutic potential of blocking neutrophil pathways in mouse pre-clinical models. We will use two mouse models of CRC, an inflammation-driven cancer model (HhAOM) and a spontaneous CRC model (BRAFMutALK5-/-) to evaluate the contribution of neutrophils to tumour development and progression. We will employ small drug inhibitors to target proteins identified in Aim1 and Aim2 and neutrophil-specific chemokine receptors (such as CXCR1/2 blocking antibody) to study the role of neutrophils. Read-outs will include tumour size, gene profiling, FACS analysis and multiplex imaging to profile the TME.

B. Mechanism of PAD4 mediated pro-tumour effects: 1) Cellular origin of PAD4 function in tumor development. To determine whether PAD4 mediates tumour maintenance through effects in EC or neutrophils we will cross PAD4flox/flox mice to VillinCRE mice (EC-specific) or LysMCRE mice (neutrophil-specific) and assess their impact on tumour development in inflammatory and spontaneous CRC models. 2) Role of PAD4 on epithelial cell transformation. We will screen cancer organoids derived from mice and patients to select PAD4 positive cancer organoids and use them in downstream assays +/- PAD4 inhibitor (GSK484) to assess the role of PAD4 in controlling cancer-associated pathways and explore its citrullinated targets using proteomics. 3) PADI4 expression in human CRC and its association with prognosis. We will screen our CRC human cohort for PAD4 expression using immunohistostaining and assess correlation of expression in tumour cells and/or neutrophils with CRC prognosis

Translational potential

While the relation between neutrophils infiltrates and poor prognosis in CRC is well documented, the role of this understudied cell type in tumorigenesis has been largely ignored. A ‘two-pronged approach’ analysing human and murine proteomic data will pinpoint relevant therapeutic targets on a protein level and simultaneously unravel innate mechanisms that can be experimentally targeted at different timepoints in pre-clinical models. These mouse models will also allow us to probe if mechanisms discovered also play a role in early on-set disease rather than late-stage tumour establishment.

Training opportunities

The student will be based at the Kennedy Institute of Rheumatology which is a world-renowned institute and is housed in a state-of-the-art research facility with close ties to the Churchill Hospital and the Translational Gastroenterology Unit at the John Radcliffe Hospital to access human biological samples. This project provides a broad training in cancer biology and immunology covering a range of cellular, molecular computational techniques. Students have access to cutting edge technologies such as disease positioned mouse models, ex vivo organoid models derived from human patients – neutrophil co cultures systems, multiplex imaging of tumour tissue and in silico methods of proteomic analysis.

References

[1] Janney, A., Powrie, F. & Mann, E. H. Nature 585, 509-517 (2020). [2] Jess, T., Rungoe, C. & Peyrin-Biroulet, L. Clin Gastroenterol Hepatol 10, 639-645 (2012). [3] Kirchberger, S. et al. J Exp Med 210, 917-931 (2013). [4] McCuaig, S. et al.. Clin Cancer Res, 6 (2020). [5] Leach, J. D. G. et al. Nat Commun 12, 3464 (2021). [6] Jackstadt, R. et al. Cancer Cell 36, 319- 336 e317 (2019). [7] Wera, O., Lancellotti, P. & Oury, C. J Clin Med 5(2016). [8] Friedrich, M. et al. Nat Med (2021). [9] Gentles, A. J. et al. Nat Med 21, 938-945(2015). [10] Jaillon, S. et al. Nat Rev Cancer 20, 485-503 (2020). [11] Hoogendijk, A. J. et al.. Cell Rep 29, 2505-2519 e2504 (2019). [12] Khoyratty, T. E. et al. Nat Immunol 22, 1093-1106 (2021).

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40. Targeting intrinsic and extrinsic mechanisms of blood cancer progression using genetic models and human bone marrow organoids [1,2,3] – Associate Prof. Psaila

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

Lay Summary

'Myeloproliferative Neoplasms (MPNs)' are a group of chronic blood cancers in which mutations, or 'typing errors' in the genetic code of blood stem cells lead to the over-production of blood cells. There are around 60,000 people living with an MPN in the UK. For most patients, the main consequence of an MPN is a significantly increased risk of blood clots, heart attacks and strokes. However, for around 1 in 20 patients, the cancer progresses to a much more severe stage called 'myelofibrosis', where destructive scar tissue ('fibrosis') builds up in the bone marrow, leading to low blood counts and poor survival. Currently, there are no treatments that reduce the risk of progression to myelofibrosis for patients with earlier stage MPNs.

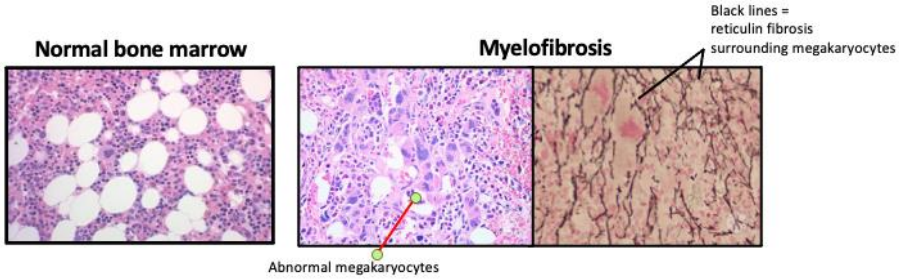
This project aims to understand why certain patients with MPNs progress to fibrosis, and to try to identify methods of preventing this. To do this, we are developing state-of-the-art mouse models that will help us model the disease more accurately and pinpoint what triggers fibrosis development. We will also implement a novel 'bone marrow organoids' platform – which enable us to replicate the complexity of human bone marrow in a dish using human cells in a 3D, multi-lineage context. Using these experimental systems, we will study the interactions between the MPN cancer clone and their bone marrow environment, to identify and validate ways of preventing fibrosis development. This studentship will provide ample opportunities for development of skills in cell culture, stem cell assays, molecular biology including single cell methods, advanced imaging and organoid cultures as well as training in bioinformatics. Our goal is to achieve novel insights into disease biology that have the potential to improve outcomes for patients with MPNs. These findings will also be relevant to patients with other blood cancers or disorders of tissue fibrosis affecting other body organs.

Research objectives

The majority of patients with MPNs do not progress to bone marrow fibrosis. Our hypothesis is that fibrotic progression is due to both 'cell intrinsic' factors – i.e. drivers within the MPN cancer cells themselves – and also 'extrinsic' influences, e.g. cells in the bone marrow niche that in some patients are more likely to respond, or indeed trigger, fibrosis-promoting signals from megakaryocytes and other cells from the MPN clone. This project will use state-of-the-art experimental systems to tease out the relative importance of these two contributors, to identify the key cellular/molecular pathways that may be targeted to prevent cancer progression in patients with less advanced disease.

Aim 1: What causes the generation of fibrosis-driving cells in MPNs?

Recently, our lab and others have used single cell profiling to identify functionally-distinct subsets of megakaryocytes and their progenitors[1-6]. We will ask whether megakaryocytes and platelets generated by certain subsets of haematopoietic stem cells (HSCs) are functionally distinct, and whether acquiring a MPN cancer driver mutation in a specific subset of stem cells[7,8] causes the emergence of pro-fibrotic megakaryocytes, and other related cell types. To do this, we will use a newly generated mouse model designed by the Nerlov lab that more faithfully recapitulates the emergence of MPNs than existing systems. This is based on a unique genetic system that enables introduction of a cancer driver mutation into a subset of HSCs with the ability to 'switch on' the mutation in a reproducible subset of stem cells, rather than in all of the cells at the same time. In parallel, we will study HSCs, megakaryocytes and platelets from MPN patients with and without fibrosis, to identify the genetic and epigenetic signatures that are unique to patients with fibrosis.

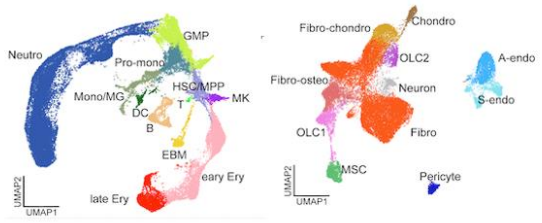


Aim 2: How does the bone marrow stroma accelerate progression to fibrosis?

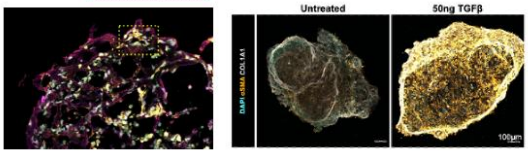
MPN mutations are often acquired early in life, but myelofibrosis typically presents in later decades of life. Haematopoiesis becomes progressively myeloid biased with age, and the pattern of haematopoiesis seen in patients with MPNs is, in many ways, a severe exacerbation of the normal ageing phenotype. This suggests that age-related changes to the bone marrow may be conducive for myelofibrosis development. To discover whether the bone marrow niche in older mice is more conducive to fibrosis in MPN, we will induce MPN driver mutations in young and older mice and ask how this alters the cellular and molecular architecture of the bone marrow stroma, and test whether the aged bone marrow microenvironment is more susceptible to MPN progression and fibrosis.

Studies will involve highly-multiplexed spatial proteomics, as well as a novel iPSC-derived bone marrow organoid system to validate targets in a human, 3-dimensional, multi-lineage setting[9]. The organoids support engraftment of patient cells and provide an exciting tool for high-throughput screens of fibrosis inhibitors modelling of normal and malignant haematopoietic cell-niche interactions**.** This will involve collaboration Kellie Machlus (Boston Children's Hospital)

Single cell profiling of myelofibrotic bone marrow, capturing haematopoietic cells and bone marrow stroma



Bone marrow organoids, containing stroma, lumen forming vasculature and myelopoietic niche to study haematopoiesis and model bone marrow fibrosis



Translational potential

The PI (Beth Psaila) is a clinician scientist, and the focus of her group is highly translational, prioritising research focussed on discovery of disease mechanisms and treatment targets that may improve outcomes for patients. We have ample access to primary patient samples. This year we have submitted 2 patents and have several commercial collaborations including with a WIMM spin-out company *Alethiomics* co-founded by Beth Psaila and Adam Mead last year, creating opportunities for lab members to interact with biotech, if of interest.

Training opportunities

There will be ample opportunity for training in advanced molecular biology techniques, in vivo models, bioinformatics, target discovery and iPSC work. I very much enjoy mentoring, and alumni from the group have an excellent track record in publishing, grant writing and securing personal fellowships/funding as well as positions in industry. We are a well-funded, highly collaborative lab and all team members are encouraged to attend relevant national/international conferences and supported in developing a personal research network.

References

1 Psaila, B. *et al.* Single-Cell Analyses Reveal Megakaryocyte-Biased Hematopoiesis in Myelofibrosis and Identify Mutant Clone-Specific Targets. *Mol Cell* **78**, 477-492 e478, doi:10.1016/j.molcel.2020.04.008 (2020).

2 Wang, H. *et al.* Decoding Human Megakaryocyte Development. *Cell Stem Cell* **28**, 535-549 e538, doi:10.1016/j.stem.2020.11.006 (2021).

3 Sun, S. *et al.* Single-Cell Analysis of Ploidy and Transcriptome Reveals Functional and Spatial Divergency in Murine Megakaryopoiesis. *Blood*, doi:10.1182/blood.2021010697 (2021).

4 Liu, C. *et al.* Characterization of Cellular Heterogeneity and an Immune Subpopulation of Human Megakaryocytes. *Adv Sci (Weinh)*, e2100921, doi:10.1002/advs.202100921 (2021).

5 Yeung, A. K., Villacorta-Martin, C., Hon, S., Rock, J. R. & Murphy, G. J. Lung megakaryocytes display distinct transcriptional and phenotypic properties. *Blood Adv* **4**, 6204-6217, doi:10.1182/bloodadvances.2020002843 (2020).

6 Pariser, D. N. *et al.* Lung megakaryocytes are immune modulatory cells. *J Clin Invest* **131**, doi:10.1172/JCI137377 (2021).

7 Carrelha, J. *et al.* Hierarchically related lineage-restricted fates of multipotent haematopoietic stem cells. *Nature* **554**, 106-111, doi:10.1038/nature25455 (2018).

8 Sanjuan-Pla, A. *et al.* Platelet-biased stem cells reside at the apex of the haematopoietic stem-cell hierarchy. *Nature* **502**, 232-236, doi:10.1038/nature12495 (2013).

9 Khan, A. O. *et al.* Human bone marrow organoids for disease modelling, discovery and validation of therapeutic targets in hematological malignancies. *bioRxiv* https://doi.org/10.1101/2022.03.14.483815 (2022).

41. Targeting Colorectal Cancer Cells with the p97 inhibitor CB-5339 [1,3] – Prof. Ramadan

Primary Supervisor: Prof. Kristijan Ramadan
Second Supervisor: Prof. Tim Maughan
Eligibility: Track 1 and 3 students are eligible to apply for this project.

Lay Summary

The standard systemic treatment of colorectal cancer (CRC) uses a combination of 5FU (a 60-year-old drug) with one of two newer agents: irinotecan (shortened to FOLFIRI) or oxaliplatin (FOLFOX). The choice between the two possible therapies has been challenging since we are currently missing clinically validated biomarkers to predict the efficacy of FOLFIRI and FOLFOX in each patient. Eventually all CRC patients develop resistance to these chemotherapy combinations. These drugs work in different ways to affect the function of DNA. Irinotecan damage involves the attachment of its target (called topoisomerase-1) to DNA in complexes which lead to cell killing. We have shown that these complexes can be repaired by a process called DNA-protein crosslink repair which includes three proteins called p97, SPRTN and TEX264. These three proteins cooperate together to break up the topoisomerase-1 DNA complex. When this works efficiently, it repairs the topoisomerase-1 DNA complex caused by irinotecan and this results in resistance to FOLFIRI treatment. Two of these proteins (p97 and SPRTN) are potential biomarkers which could predict patient response to irinotecan in CRC and we have filed a patent on this discovery. In this study we want to test if an orally available p97 inhibitor (CB-5339) may stop this repair process from working in some lab investigations to work out the mechanism and to see if this reverses resistance to FOLFIRI in some patient derived samples. The aim is to confirm that this pathway is driving resistance to FOLFIRI and then to improve responses to FOLFIRI chemotherapy in patient derived material by testing the combination of irinotecan and p97 inhibitor. Ultimately this line of work could define a biomarker to improve selection between FOLFOX and FOLFIRI, define a new mechanism of resistance and point the way to a new more effective combination therapy for CRC patients.

Abstract

Colorectal carcinoma (CRC) currently represents the third most common type of cancer worldwide, representing around 10% of all diagnosed cancers and with 1.85 million new cases diagnosed each year [1]. The systemic treatment of microsatellite stable (MSS) colorectal cancers, which currently represents around 80% of all cases, has not been significantly modified since the early 2000s. In fact, two main therapies with similar efficacy (FOLFIRI and FOLFOX) still represent the most common first line treatment[2, 3]. FOLFIRI is an irinotecan-based chemotherapy regimen which induces the formation of Topoisomerase 1 cleavage complex (**TOP1ccs**), a specific type of enzymatic DNA-protein crosslink (DPC) where TOP1 is covalently bound to the DNA[4]. In recent years, our laboratories (Ramadan, Maughan) have been able to demonstrate the importance of p97 ATPase/unfoldase, SPRTN protease and TEX264 for TOP1ccs repair and consequent cell survival [5, 6]. TEX264 is a p97-cofactor that serves as TOP1ccs receptor. TEX264 binds to TOP1ccs and recruits the p97-SPRTN complex to the site of DNA damage (Fig. 1), which allows the proteolytical degradation of TOP1ccs by p97 unfoldease activity and, consequently, the SPRTN protease activity.

The importance of p97 and SPRTN in determining the efficacy of TOP1 inhibitors like camptothecin and irinotecan has been validated in our laboratory in different human cancer cell lines (Fig. 2, and data not shown) and in CRC patients (Fig. 3). Further, we stablished SPRTN as the first biomarker for prediction of FOLFIRI response in CRC patients (patent filed in the UK and US). Despite these exciting advances, our attempts to develop SPRTN inhibitors, in collaboration with EVOTEC A.G., proves to be challenging.

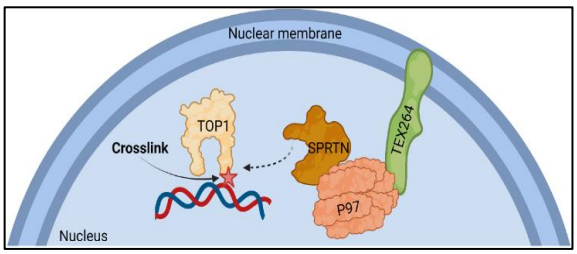


Figure 4. The role of p97-SPRTN- TEX264 machinery in repair/processing of cytotoxic TOP1ccs.

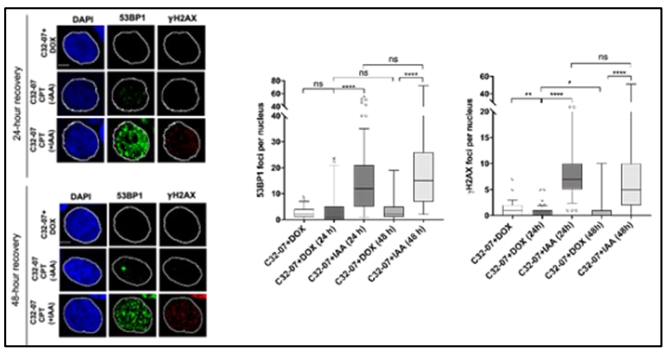


Figure 2. SPRTN depletion by auxin (IAA) inducible degradation increases the amount of DNA damage (γH2AX; 53BP1) in HCT116 cells treated with camptothecin (CPT) and delays its repair.

However, the p97 inhibitors are in clinical trials for various blood and solid malignancies, but not for colorectal cancer. CB-5083, a first-generation p97 inhibitor, demonstrated broad antitumour activity in various models but was limited by mild vision toxicity [7, 8]. However, our industry partner Cleave Therapeutics has developed the second generation of orally available p97 inhibitors, like CB-5339, which is in clinical trials for treatment of various malignancies [9]. CB-5339 successfully passed phase I clinical toxicity study and is currently entering phase II clinical trials. With the support of Cleave Therapeutics, we aim to investigate the molecular mechanisms of CB-5339 *in vitro* and in orthotopic CRC mouse model. Our work will offer the perfect opportunity to determine if p97 contributes to the survival of CRC cells treated with irinotecan, and if p97 inhibition can significantly increase the efficacy of FOLFIRI.

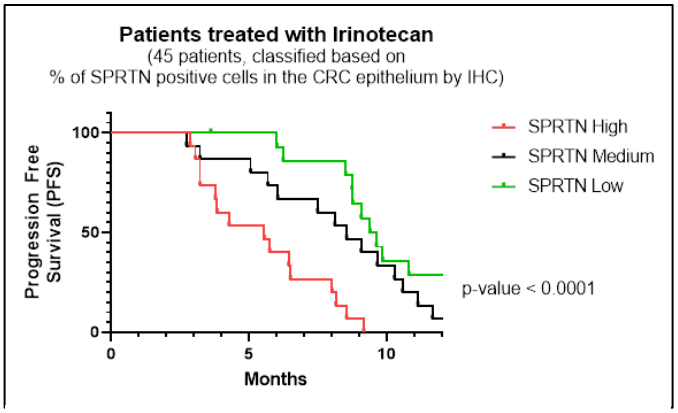


Figure 3. The overexpression of SPRTN correlates with a worse prognosis in patients treated with irinotecan.

Research objective

- Characterise the sensitivity of different CRC cell lines to the p97 inhibitor CB-5339
- Investigate the role of p97 in the repairment of TOP1ccs induced by topoisomerase inhibitors in CRC with the focus on DNA replication and S-phase cell proliferation
- Validate our findings using CRC orthotopic mouse models treated with FOLFIRI and CB-5339, in collaboration with Simon Leedham.

Translational potential

A deeper understanding of the role of specialised machinery (97-SPRTN-TEX264 complex) for repair of cytotoxic TOP1-ccs in mediating the response to topoisomerase inhibitors will allow us to better predict the response to FOLFIRI in CRC patients. Moreover, p97 inhibition is likely to synergize with irinotecan-based chemotherapy to further improve the outcomes of patients treated with TOP1ccs-inducing drugs in many cancer types. This is a collaborative work between the clinic (T. Maughan), basic science (K. Ramadan), pre-clinical validation (S. Leedham) and industry partner (Cleave Therapeutics; a support letter can be provided).

Training opportunities

The PhD student will have the opportunity to get trained in basic science (molecular, cell biology and rodent work) and understand how to translate a clinical problem to the bench and return this knowledge back to the clinics.

References

1. Mattiuzzi, C., F. Sanchis-Gomar, and G. Lippi, *Concise update on colorectal cancer epidemiology.* Ann Transl Med, 2019. **7**(21): p. 609.
2. Tournigand, C., et al., *FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study.* J Clin Oncol, 2004. **22**(2): p. 229-37.
3. Aggarwal, N., et al., *Systematic review and meta-analysis of tumour microsatellite-instability status as a predictor of response to fluorouracil-based adjuvant chemotherapy in colorectal cancer.* Int J Colorectal Dis, 2022. **37**(1): p. 35-46.
4. Thomas, A. and Y. Pommier, *Targeting Topoisomerase I in the Era of Precision Medicine.* Clin Cancer Res, 2019. **25**(22): p. 6581-6589.
5. Fielden, J., M. Popovic, and K. Ramadan, *TEX264 at the intersection of autophagy and DNA repair.* Autophagy, 2022. **18**(1): p. 40-49.
6. Fielden, J., et al., *TEX264 coordinates p97- and SPRTN-mediated resolution of topoisomerase 1-DNA adducts.* Nat Commun, 2020. **11**(1): p. 1274.
7. Tang, W.K., et al., *Structural Basis of p97 Inhibition by the Site-Selective Anticancer Compound CB-5083.* Mol Pharmacol, 2019. **95**(3): p. 286-293.
8. Leinonen, H., et al., *A p97/Valosin-Containing Protein Inhibitor Drug CB-5083 Has a Potent but Reversible Off-Target Effect on Phosphodiesterase-6.* J Pharmacol Exp Ther, 2021. **378**(1): p. 31-41.
9. Lina Benajiba, et al., *2870 Trials in Progress: A Phase I Study to Evaluate the Safety and Pharmacokinetic Profiles of CB-5339 in Participants with Relapsed/Refractory Acute Myeloid Leukemia or Relapsed/Refractory Intermediate or High-Risk Myelodysplastic Syndrome.* 62nd ASH Annual Meetig and Exposition, 2022.

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

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

Lay Summary
The most common childhood cancer is acute lymphoblastic leukaemia (ALL), a disease which leads to the accumulation of an immature type of white blood cell (lymphoid cells) in the bone marrow. This is thought to be caused in part by a block in normal differentiation of these lymphoid cells. There has been amazing progress in treating childhood ALL, but unfortunately a subset of childhood ALL continues to be unresponsive to treatment, especially in those patients that carry a mutation in the Mixed Lineage Leukaemia (MLL) gene. The most common mutation in the MLL gene results in an abnormal protein, MLL-AF4, that is responsible for the poor prognosis of many 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 immature ALL lymphoid cells. This treatment should be much milder than conventional approaches and be potentially applicable to incurable ALL types.

Abstract
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.1 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 model2 (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 objective
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 collaborator Dr Anindita Roy:
1. Analyze differentiation of ALL cells using a novel MLL-AF4 ALL model,[2] with a combination of tools as well as novel compounds.[1] 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 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 and proteomic analysis.
The overall workflow is depicted in **Fig. 1**.
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.[2] 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.

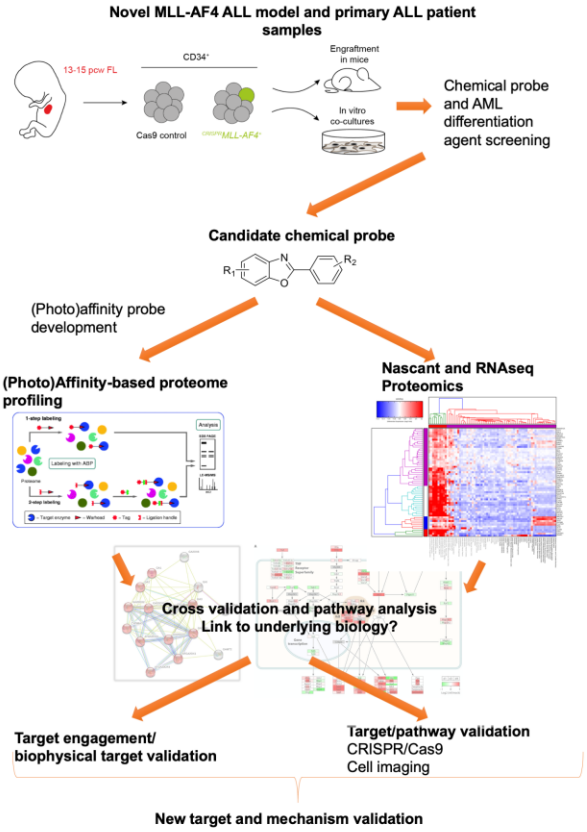


Fig. 1: Proposed project workflow.

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

[1] Josa-Culleré L; …..**Milne TA*** & **Russell AJ*** A Phenotypic Screen Identifies a Compound Series That Induces Differentiation of Acute Myeloid Leukemia Cells In Vitro and Shows Antitumor Effects In Vivo. *J. Med. Chem.* **2021**, *64*, 15608–15628. DOI: 10.1021/acs.jmedchem.1c00574.

[2] Rice S; …..**Milne TA*** & **Roy A*** A human fetal liver-derived infant MLL-AF4 acute lymphoblastic leukemia model reveals a distinct fetal gene expression program. *Nat. Commun.* **2021;** 12, 6905. DOI: 10.1038/s41467-021-27270-z.

[3]Wilkinson IVL; …..**Russell AJ*** Chemical Proteomics and Phenotypic Profiling Identifies the Aryl Hydrocarbon Receptor as a Molecular Target of the Utrophin Modulator Ezutromid. *Angew. Chem. Int. Ed.* **2020**; DOI: 10.1002/anie.201912392.

43. Bioinformatics and statistical approaches for the identification of genomic markers predictive of progression to B-cell malignancy using whole genome sequencing data from Genomics England [2,3,4] – Prof. Schuh

Primary Supervisor: Prof. Anna Schuh
Second Supervisor: Dr Dimitrios Vavoulis
Eligibility: Track 2, 3 and 4 students are eligible to apply for this project.

Lay Summary
Conditions that might lead to cancer in future and that involve the immune system occur about 15% of people over 65 years old. Most people will not progress to develop cancers of the immune system, but because their doctor cannot predict whether or not they will get cancer, all these people require regular "watch and wait" follow-up by cancer specialists. Unfortunately, the small number of people who do progress to cancer (~5%), cannot be cured by the time they need treatment. We think that if these people at risk of getting cancer could be reliably identified early, we might be able to cure their cancer.
OXPLORED (Oxford Pre-cancerous Lymphoproliferative Disorders: Analysis and Interception Study) is a clinical study that aims to identify new blood-based markers that might help doctors in future to reliably predict cancer development in people at risk. OXPLORED was developed by patients currently undergoing "watch and wait (worry)" follow-up. In collaboration with Genomics England, we will examine the entire genetic make-up of the pre-cancer cells of up to 800 participants using a technology called whole genome sequencing and state-of-the-art methods to analyse this type of big data. Our aim is to reliably classify the participants into low- and high-risk groups for developing cancer.
We think that if the small number of people at high-risk of developing cancer (~5%) is identified early (i.e., before they develop cancer) and reliably, we could potentially prevent cancer through earlier treatment. In addition, some of the markers that predict cancer development might help us to develop new "targeted bullet" treatment. We would also be able to re-assure the remaining 95% of people who will never get cancer, thus reducing their anxiety and improving their psychological well-being.

Abstract
B-cell pre-cancer clinical states occur in up to 15% of people over the age of 65[1,2,3]. On average, 1% of patients per year with Monoclonal Gammopathy of Undetermined Significance (MGUS) progress to multiple myeloma (MM), and 1-2% of patients per year with high-count Monoclonal B cell lymphocytosis (MBL) progress to Chronic Lymphocytic Leukaemia (CLL). Current guidelines do not recommend universal screening for MGUS or MBL. This is because the positive predictive value of current screening methods (serum electrophoresis and free-light chain (FLC) assays for myeloma and flow cytometry for CLL) is not high enough to confidently predict progression to malignancy in most individuals. Moreover, these assays are not readily available in multiple settings.
For MBL, risk of progression correlates with the absolute clonal CD19+/CD5+ B-cell count[4]. For MGUS, the risk of progression to a malignant blood cancer can be stratified by monoclonal antibody (MAB) type, MAB level and serum FLC ratio. Using a MAB threshold of 10g/L and a five-fold extended range of serum FLC ratios, excludes 93% of MGUS, but importantly still identified 98% of MM patients. This strategy identifies the decile of patients at highest risk of progression. The same strategy identifies two fifths of patients in whom risk of progression is reassuringly very low.
However, for the remainder of patients, risk stratification is inaccurate, and there is an urgent need for improvement to facilitate earlier diagnosis in the few who will progress and to reduce unnecessary anxiety in those who will not progress[5,6].
The aim of the OXPLORED study is to identify markers for the early prediction of the development of B-cell cancer in people at risk. The target recruitment population is 1650 patients on surveillance for CLL/multiple myeloma disease progression, and it includes two groups of early lymphoproliferative disorders: a) Monoclonal B-cell lymphocytosis and asymptomatic Chronic Lymphocytic Leukaemia, and b) IgA or IgG Monoclonal gammopathy and smouldering myeloma. Participants in the OXPLORED study provide samples for germline DNA, plasma for circulating tumour DNA analysis and tumour samples, either as sorted circulating B-cells or sorted bone marrow-based plasma cells, depending on the specific early lymphoproliferative disorder.
Through a collaboration between the OXPLORED Study and Genomics England, we have been allocated in-kind funding by Genomics England to sequence up to four hundred whole genome tumour-normal pairs (800 genomes; tumours: 100X; saliva germline: 50X) from participants in OXPLORED. Analysis of this dataset is the primary focus of the proposed research.

Research objective

The **primary objectives** of this proposal are: 1) to develop a predictive machine learning model for the binary classification of patients at risk of developing B-cell cancers into progressors and non-progressors, 2) to identify the combination of clinical and genomic predictors that underlie this classification, and 3) to further subdivide progressors and non-progressors into groups with distinct clinical and genomic characteristics.

The **major outcome** of this study will be an analytical pipeline, which takes as input multiple genomic data modalities (including coding and non-coding mutations, mutational signatures and hotspots, global measures of genomic complexity, and copy number aberrations) from a single patient, as well as the patient's clinical data (including sex, age, serum FLC ratio, MBL levels, and B-cell count), and generates at its output the probability that the patient will develop a B-cell malignancy, CLL or MM.

Our group together with an international group of collaborators have analysed whole genome sequences of 485 patients with CLL in need of treatment recruited into the Genomics England CLL Pilot (manuscript under revision in *Nat Gen*). Our analysis pipeline is housed in the GEL CLL Pilot embassy and was developed as part of the haematological malignancies GECIP. Our WGS analysis identified 5 different biological risk groups of patients defined by genomic characteristics. Therefore, a **secondary objective** of this study is to establish whether these genomic subgroups retain their prognostic power in the pre-malignant stages of the disease. Towards this aim, we will also apply the same analysis of mutations, global lesions, mutation signatures and hotspots, and copy number aberrations in the pre-malignant genomic dataset of the proposed study.

Translational potential

Given the rapid advances in analytical capability and reductions in cost, healthcare systems around the world including in the UK are beginning to replace multimodal testing for conventional biomarkers with WGS as a single comprehensive genomics platform for clinical diagnostics. We expect that machine learning techniques that combine non-coding changes and global measures derived from germline and tumour WGS with conventional biomarkers and clinical data, as we propose to do here, will enhance the diagnostic value of clinical WGS significantly. We also expect that our tools will be directly translated into the NHS whole genome diagnostics service and be used as part of clinical trials in the UK and within our international consortium to redefine existing risk groups for treatment stratification and early detection. Ultimately, we hope that improved risk stratification and personalisation of therapy through a comprehensive and global insight into the pre-malignant genome will lead to improved patient outcome.

Training opportunities

The DPhil candidate for this project will be working in a multidisciplinary environment of bioinformaticians, statisticians, cancer geneticists and clinicians. This will provide ideal conditions for developing technical skills in state-of-the-art methods for data analysis and software engineering, which are transferable to domains other than genomics, as well as making substantial scientific contributions to a cutting-edge research field. In particular, the student is expected to acquire expertise in the following domains: a) cancer genomics, particularly as applied in early detection, b) bioinformatics approaches for WGS data analysis, c) Bayesian approaches to data modelling and analysis, and d) supervised and unsupervised machine learning techniques, including deep learning, for the integration of heterogeneous data modalities, feature selection, prediction, classification, and clustering. The student is also expected to hone essential soft skills, including time management, presentations skills and task planning, which will allow them to communicate and operate effectively in a vibrant and diverse team of clinical and computational scientists.

References:

[1] Kyle et al. *N Engl J Med* **346**(8) 2002 -- [2] Kyle et al. *N Engl J Med* **354**(13) 2006 -- [3] Landau et al. *Nature* **526**(7574) 2015 -- [4] Landgren et al. *N Engl J Med* **360**(7) 2009 -- [5] Ojha et al. *Leukemia* **28**(12) 2014 --[6] Rawstron et al. *N Engl J Med* **359**(6) 2008

44. Epigenetic control of cancer cell phenotypes via nuclear F-actin based chromosome motility [1,2,3,4] –Prof. Shi

Primary Supervisor: Prof. Yang Shi
Second Supervisor: Prof. Eric O'Neill
Eligibility: Track 1, 2, 3 and 4 students are eligible to apply for this project.

Lay Summary
Pancreatic ductal adenocarcinoma (PDAC) is one of the leading causes of cancer death with a 5-year survival below 5%. Poor survival is due to the fact that early stages of disease that are treatable with surgery do not cause obvious symptoms which results in diagnosis when the cancer is more aggressive and less treatable. Therefore, it is important to improve the ability to diagnose PDAC earlier in disease progression and identify new ways to treat the disease. A major difficulty to identifying individuals that are at-risk of PDAC earlier is that symptoms are non-specific. Therefore, identifying how a normal pancreatic cells changes into a cancer cell is key to providing signals we can use to diagnose earlier. It is known that one of the first steps in cancer is the loss of cell identity, where a cell forgets its normal function and starts to return to their original embryonic or stem cell-like state. These transitions are controlled by modifications on DNA and proteins that control gene expression called 'Epigenetics'. We aim to investigate how cell identity is normally controlled in order to understand both how cancer cell develop and to find novel ways to reverse cancer by promoting them to return to normal. To do this we will take advantage of recent discoveries in both the O'Neill and Shi labs which have highlighted key mechanisms that control the emerging concept of a 'nuceloskeleton' (a structural frame in the cell nucleus) and epigenetic marks that control gene expression[1-3].

Abstract
The hippo tumour suppressor pathway regulates tissue size in development and although the contribution of this pathway to cancer is evident from tumour models and pan-cancer transcriptomics, somatic mutations are rare[4]. Our research has demonstrated how epigenetic silencing of RASSF1A is responsible for YAP activation in human tumours and correlates with poor survival across all major solid malignancies. Such 'epigenetic plasticity' allows dynamic switching between phenotypes and supports progression of lesions and the appearance of cancer stem-like cells (CSCs) in solid tumours[42]. During development, increasing evidence implicates the co-factor YAP1 as a key determinant of phenotype by supporting pluripotency or differentiation through activation of distinct transcription programmes in response to RASSF1-hippo signalling[5]. Moreover, the hippo pathway transduces mechanical forces from the microenvironment to guide proliferation, stem cell behaviour and differentiation6. Our recent work has identified RASSF1A and MST2 reside at the nuclear envelope to sense mechanical force and influence both chromatin and nuclear actin. This project aims to consolidate these discoveries to understand how genomic motility between repressive heterochromatin and phase separated transcription factories are controlled by nuclear actin to influence cell phenotype. We will also explore how nuclear actin influences the stabilisation of cell phenotypes through re-wiring DNA methylation, specifically 5'hmc by Tet2, to influence differentiation state and clinical outcome in pancreatic cancer.

Research objective
(i) Defining the molecular mechanisms that influence cell fate will allow us to target the epigenetic plasticity behind tumour heterogeneity, progression and therapeutic resistance.
(ii) EON is an expert in hippo pathway signalling and epigenetics in pancreatic cancer and YS is an expert in epigenetic control of cell-phenotype in cancer.

This project asks three questions;

- How does RASSF1A regulation of nuclear actin affect chromatin at specific loci associated with pluripotency or differentiation?

Outcome: an understanding of how nuclear actin guides the movement of specific genes into regions of repressive chromatin or active transcription.

- Does mechanical force impact ATR-RASSF1A signalling to influence plasticity?

Outcome: defining specific extracellular or cytoplasmic cues that can trigger gene positioning and influence cell phenotype.

- Can targeting phenotypic plasticity improve therapy in PDAC?

Outcome: Can we promote differentiation in PDAC to improve therapeutic responses and survival?

Translational potential

The potential of precision cancer medicine is limited by therapeutic resistance arising from tumour heterogeneity. Heterogeneity underpins cancer progression and results from a combination of genomic instability and epigenetic plasticity; the dynamic alterations of the epigenome responsible for establishing cell phenotype. The tumour microenvironment governs epigenetic plasticity but exactly how multiple states are generated and maintained unknown[7]. Personalised therapies targeting driver mutations are largely circumvented by the presence of genetically diverse resistant subclones. In contrast, epigenetic plasticity is reversible and an attractive target to prevent resistant phenotypes appearing or to revert phenotypes of recalcitrant populations (e.g. cancer stem-like cells) to improve overall therapeutic efficacy. Moreover, as plasticity in tumours can result in genome instability[8], the underlying alterations may highlight specific vulnerabilities not apparent from genetics alone. To understand how plasticity occurs in tumours, we need to understand how the mechanisms governing cell phenotype are influenced by epigenetics and microenvironmental cues.

The genome kinases ATM and ATR phosphorylate RASSF1A-Ser131 to influence chromatin, transcription, and DNA replication. We now know that this influences plasticity and have shown how a SNP in *RASSF1* (rs2073498) encodes a mutation, RASSF1A^{A133S}, that disrupts phosphorylation at Ser131[9], blocks the formation of nuclear actin (preliminary data) and hinders differentiation. RASSF1A^{A133S} is prevalent in Caucasian populations with a minor allele frequency (MAF) of ≤0.17 in European cohorts and associates with early onset tumorigenesis in multiple cancers. We generated *Rassf1*^{A133S} mice that accelerate pancreatic and colorectal tumour models, supporting the hypothesis that RASSF1A maintains differentiation and prevents phenotypic plasticity in human tumours. This model gives us the opportunity to direct model an emerging pathological SNP in humans, while also provide a platform for strategies to intervene in hyperplastic phenotypic model.

Training opportunities

In addition to standard cell culture assays the candidate will receive training in high content and real-time microscopy, epigenetics (inc ChIPseq, bioinformatics), phase separation and transcription factories, nuclear F-actin filaments etc. In addition, there are opportunities to explore the in vivo relevance in mouse models of pancreatic cancer. The student will contribute to understanding how F-actin impacts on the regulation of specific genes involved in pluripotency and differentiation. Research outputs will be the identification of epigenetic complex subunit interactions with actin and how these are altered in cell undergoing mechanical stimulation.

References:

1. Wu, D. *et al.* Glucose-regulated phosphorylation of TET2 by AMPK reveals a pathway linking diabetes to cancer. *Nature* **559**, 637-641 (2018).
2. Eyres, M. *et al.* TET2 Drives 5hmc Marking of GATA6 and Epigenetically Defines Pancreatic Ductal Adenocarcinoma Transcriptional Subtypes. *Gastroenterology* **161**, 653-668 e616 (2021).
3. Chatzifrangkeskou, M. *et al.* RASSF1A is required for the maintenance of nuclear actin levels. *EMBO J* **38**, e101168 (2019).
4. Harvey, K.F., Zhang, X. & Thomas, D.M. The Hippo pathway and human cancer. *Nat Rev Cancer* **13**, 246-257 (2013).
5. Papaspyropoulos, A. *et al.* RASSF1A uncouples Wnt from Hippo signalling and promotes YAP mediated differentiation via p73. *Nat Commun* **9**, 424 (2018).
6. Dupont, S. Role of YAP/TAZ in cell-matrix adhesion-mediated signalling and mechanotransduction. *Exp Cell Res* **343**, 42-53 (2016).
7. Easwaran, H., Tsai, H.C. & Baylin, S.B. Cancer epigenetics: tumor heterogeneity, plasticity of stem-like states, and drug resistance. *Mol Cell* **54**, 716-727 (2014).
8. Pefani, D.E. *et al.* RASSF1A-LATS1 signalling stabilizes replication forks by restricting CDK2-mediated phosphorylation of BRCA2. *Nat Cell Biol* **16**, 962-971, 961-968 (2014).
9. Yee, K.S. *et al.* A RASSF1A polymorphism restricts p53/p73 activation and associates with poor survival and accelerated age of onset of soft tissue sarcoma. *Cancer Res* **72**, 2206-2217 (2012).

45. Understand the developmental origin of Clear Cell Sarcoma using long-read single-cell sequencing[1,2,3] – Associate Prof. Snelling

Primary Supervisor: Associate Prof. Sarah Snelling
Second Supervisor: Dr Adam Cribbs
Eligibility: Track 1, 2 and 3 students only are eligible to apply for this project.

Lay Summary
Clear Cell Sarcoma (CSS) is a rare form of cancer called soft tissue sarcoma that is most common in younger people. It affects the hands, feet, legs and arms. Unfortunately, the symptoms of CSS, which include lumps under the skin, fatigue and night sweats, often only arise when the cancer has progressed. Diagnosis can also take a long time as there are other cancer types must be excluded first and often involves painful and invasive biopsies. This means that the outlook for patients with CSS is poor as the original tumour is often large by the time the patient is diagnosed with CSS and is likely to have spread to other parts of the body. Additionally, treatments are limited and CSS can occur again after initial treatment.

To improve the outlook for patients with CSS it is very important to be able to accurately diagnose the disease and identify new and effective treatments. The cells that cause CSS are important to identify, as are the changes within these cells that cause the cancer to grow and spread. This work aims to address these critical needs by analysing which cells are within CSS tumours and comparing this to the cells within soft tissues normally present within the hands, feet, legs and arms (for example tendons). This will help to identify 1. Which tissues CSS arises from 2. Which cells proliferate and spread to cause CSS and 3. Diagnostic markers (molecules that are specifically only seen in CSS tumours and not other sarcoma tumours) that allow quicker and less invasive detection of CSS 4. Potential targets within the cells that can be used for drug-based treatment of CSS.

Our team has been developing methods to accurately and quickly identify the different cells within tissues of our bodies and to assess how DNA within these cells changes when cancer is present. We will now apply these methods to CSS to address this important, unanswered question in cancer treatment.

Abstract
Clear Cell Sarcoma (CCS) of tendons, is a rare malignant soft tissue sarcoma, typically derived from neural crest cells. It usually presents in the distal lower extremities of young adults, frequently attached to tendons. It behaves like a high-grade soft tissue sarcoma and is associated with poor overall survival due to spreading to other parts of the body with recurrence after treatment also being very common. CCS neoplastic cells express the EWSR1-ATF1 fusion gene in most cases, with EWSR1-CREB1, EWSR1-CREM or EWSR1-DDIT3 fusion genes comprising a smaller subset of cases.
Prof Snelling leads the Tendon Seed Network chapter of the Human Cell Atlas, whose aim is to investigate the cell architecture of healthy human tendons. We have performed single-cell sequencing on several anatomically different tendon tissues across several healthy donors. Tendons have historically been thought of as acellular, however we have shown that tendons are composed of a diverse and rich cellular microenvironment. We are now applying these technologies to generate healthy tissue atlases of other joint-resident soft tissues including synovium and ligament

Considering that very little is known about the developmental origin of CSS, we hypothesise that CSS may develop from cells residing within the tendon. As such, we will leverage the reference maps of healthy musculoskeletal tissues with single-cell sequencing data of CSS that we will generate as part of this proposed project. We will cross reference these datasets of CSS and healthy soft tissues to identify whether the cellular origin of CSS is tendons or another tissue type. We will then determine the oncogenic drivers of CSS with the long-term goal of utilising this data to enable identification of novel therapeutics to treat this currently incurable cancer.

In collaboration with the Snelling 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-seq[1], a method to overcome low basecalling accuracy making long-read single-cell transcriptomic sequencing highly accurate. This new technology enables us to measure translocations, alternative splicing, and allows variant calling. We have begun to apply this technology to understand the development of drug resistance in Sarcomas.

Research objective
Our aim is to apply long-read single-cell sequencing technology to primary CCS patient samples and then generate computational models that will help us to identify the developmental origin and oncogenic drivers of this cancer.

Work package 1: Apply scCOLOR-seq to Clear Cell Sarcoma tumours.

The student will apply scCOLOR-seq to investigate the gene expression, isoform expression, translocations, and copy number variation within 10 CSS primary tumours. Specifically, the aim of this work package will be to identify genomic signatures that can provide us with a better understanding of the developmental origin on CCS.

Work package 2: Development of a computational analysis strategy to improve long-read single-cell sequencing.

Working simultaneously alongside work package 1, bespoke computational analysis pipelines will be written to help process the long-read sequencing data. The student will work alongside Dr Cribbs, who will provide extensive computational training, to develop skills in python and R programming, as well as software development. We have already developed generic long-read single-cell sequencing workflows (ref). The student will expand the development of this code with an emphasis on cross comparison analysis of our healthy tendon, ligament, synovium and soft tissue datasets and the long-read single-cell data generated during work package 1.

Work package 3: Develop and apply machine learning models to understand the genomic features that are important for developing Clear Cell Sarcoma.

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 features that are important for CCS pathogenesis. Specifically, we will develop a classifier model using data (structural variation, isoform expression, gene expression) generated from the long-read sequencing experiments. Knowledge from this model will be used to identify features that drive the development of CSS and then generate a priority list of potentially druggable targets for functional validation.

Translational potential
The stated aim of this project is to study the developmental origin of Clear Cell Sarcoma and identify drug targets for therapy. By its very definition, this work is likely to identify novel therapeutic intervention points within the development of Clear Cell Sarcoma. 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 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. As part of this project, you will collaborate and be co-supervised by Prof Pillay, a clinical pathologist at UCL who will provide samples for this study.

References:
1. Philpott, M. et al. Nanopore sequencing of single-cell transcriptomes with scCOLOR-seq. *Nat Biotechnol* (2021).
2. Baldwin, M.J., Cribbs, A.P., Guilak, F. *et al.* Mapping the musculoskeletal system one cell at a time. *Nat Rev Rheumatol* **17,** 247–248 (2021).

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

Primary Supervisor: Associate Prof. Chunxiao Song
Second Supervisor: Dr Shivan Sivakumar
Eligibility: Track 1, 2, 3 and 4 students only are eligible to apply for this project.

Lay Summary

Pancreatic cancer has the worst survival of any human cancer, mostly due to detection at an advanced stage. Furthermore, there are no blood tests to detect or diagnose pancreatic cancer at a stage for curative treatment intent. Therefore, new approaches for pancreatic cancer detection are urgently needed.

Circulating cell-free DNA (cfDNA) - the free-floating DNA in blood originating from cell death in healthy and diseased tissues - holds great potential for early cancer detection. From cfDNA, one can obtain a wealth of information, including genetic changes (for example, mutations, which are changes in the DNA sequence) and epigenetic changes, which refers to genetic modifications that impact gene activity without changing the DNA sequence. Epigenetic changes are known to occur early in tumour development in a tissue-specific manner. Recent studies have shown that epigenetic alterations in cfDNA are to be one of the most promising molecular indications for early cancer detection. This is because DNA epigenetic alterations not only reveals the tumour itself, but also provides the crucial tissue-specific information to determine the location of the tumour in early detection. However, it is technologically challenging to detect epigenetic changes efficiently from cfDNA.

Recently, we developed a new method to detect epigenetic changes that is substantially better than previous methods in providing higher quality data and reducing cost. It is ideal for epigenetic analysis in cell-free DNA and our pilot study showed it holds great promise for early detection of pancreatic cancer. This project will build upon the early result to be a comprehensive study on pancreatic cancer early detection. We will combine epigenetic information with genetic information such as mutations to further improve the sensitivity of our method and apply it to multiple large cohorts of pancreatic cancer for independent validation. This is an important step to realize the full potential of liquid biopsy (blood test) for early cancer detection.

Abstract

Multimodal, genome-wide characterization of epigenetic and genetic information in circulating cell-free DNA (cfDNA) could enable more sensitive early cancer detection, but it is technologically challenging. 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) (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.

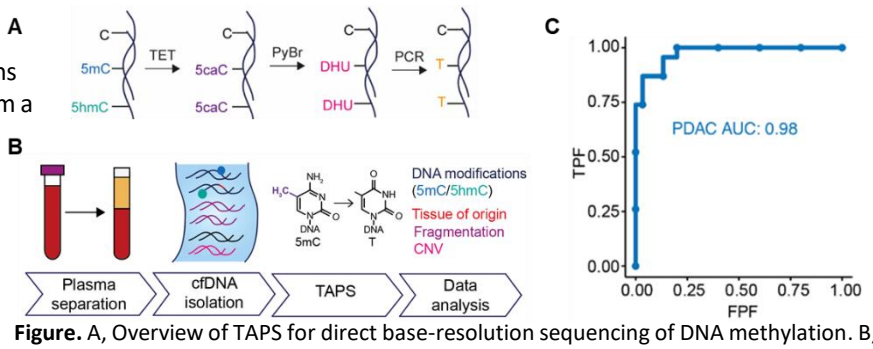


Figure. A, Overview of TAPS for direct base-resolution sequencing of DNA methylation. B, Schematic representation of the cfTAPS approach for cfDNA analysis. C, ROC curve of cfTAPS classification for PDAC and non-cancer controls (n = 53, PDAC = 23, non-cancer controls = 30).

Research objective

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 stage. Furthermore, there are no blood tests to detect or diagnose PDAC at a stage for curative treatment intent. 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.

References:

1. Liu Y, *et al.*, Bisulfite-free direct detection of 5-methylcytosine and 5-hydroxymethylcytosine at base resolution. *Nat. Biotechnol.* **37**, 424-429 (2019).
2. Siejka-Zielińska P, *et al.*, Cell-free DNA TAPS provides multimodal information for early cancer detection. *Sci. Adv.* **7**, eabh0534 (2021).

47. Exposing and exploiting metabolic vulnerabilities in cancer using connexin channel uncouplers: Do electrically-coupled networks of cancer cells mitigate the consequences of mutations in essential metabolic genes? [2,3] –Prof. Swietach

Primary Supervisor: Prof. Pawel Swietach
Second Supervisor: Prof. James McCullagh and Prof. Eamon Gaffney
Eligibility: Track 2 and 3 students only are eligible to apply for this project.

Lay Summary
Gene mutations occur randomly, and some may confer a growth advantage onto cells. Growth that evades normal checks and controls can lead to cancer, which explains why human tumours are enriched in 'pro-growth' mutations. Many mutations will, however, produce inactive proteins and may be damaging to cells, particularly if their function is deemed essential for survival. Studies of cultured cancer cells have determined that certain genes involved in metabolism are essential, that is, their inactivation causes cells to die. A prediction borne from this finding is that invasive cancers should not carry inactivating mutations in essential genes. Surprisingly, this prediction has not been verified in human cancers, which puts into question the concept of 'essential genes'. Our preliminary findings may provide an explanation for this paradox. We speculate that cancer cells can compensate for mutations in essential genes by accessing functional proteins in neighbouring cells that do not carry the 'lethal' mutation. This takes place by exchanging small metabolites between cells across channels called gap junctions. For example, a mutated cell that is unable to process a specific metabolite can exploit its immediate neighbour for the missing enzyme activity. In this scenario, both cells survive, despite one of them bearing a mutation in an 'essential' gene. If this rescue mechanism is important for cancer, then blocking gap junctions should expose major metabolic vulnerabilities. Blocking gap junctions therapeutically would render cancer cells more susceptible to treatments that interfere with essential functions. This project will study the postulated rescue mechanism and evaluate its therapeutic potential. To study the role of gap junctions in networks of cancer cells, we will use genetic engineering, culture-dish preparation, mouse models, and mathematical modelling.

Abstract
Gene mutations occur spontaneously, subject to the genetic instability of cells and the microenvironment they live in. Mutations that allow cells to overcome normal checks and controls can result in cancer. Such mutations will become enriched in tumours through a process called positive selection. In contrast, loss-of-function mutations in essential genes will have the opposite effect on cells. Cells bearing such mutations are predicted to undergo negative selection, i.e. appear less abundant than predicted from the rate of random mutations. Multiple genes, notably those involved in metabolism, have been described as essential in vitro because their inactivation is lethal in cultured cancer cells. However, negative selection is exceedingly rare in human cancers, which argues against the notion of 'essential genes'. In other words, mutations in so-called essential genes are as frequent as mutations in most other genes. We hypothesise that this paradox arises as a consequence of diffusive connectivity between cells in vivo, established by means of gap junctional channels coded by connexin genes. Our recent findings indicate that an isolated mutation in an essential metabolic process does not result in a disadvantageous phenotype because cell-to-cell diffusive connectivity allows mutated cells to access resources (e.g. wild-type enzymes) in neighbouring cells. Such 'recipient' cells will not undergo negative selection. Blocking connectivity by pharmacological or genetic means would introduce a vulnerability in tumours, making these more likely to succumb to the lethality of mutations in essential genes. Strikingly, connexin genes such as Cx26 are almost never mutated in human cancers, and we hypothesise that this protects individual cancers from becoming vulnerable under metabolic stress. This project will study the role of inter-cellular metabolic coupling using colorectal cancer (CRC) cells expressing various combinations of connexin genes. To test the therapeutic potential of targeting connexins, CRC cells grown in vitro and in vivo will be genetically engineered to lack a specific isoform. The project will evaluate evidence for negative selection under conditions where cells are unable to compensate for each other's metabolic defects. Our objective is to test connexins as a novel target for exploiting tumour metabolic vulnerabilities. If connexins are shown to play a key role in reducing the lethality of mutations in essential genes, our deliverables will challenge a central paradigm in carcinogenesis models that the individual cell is the unit under selection. The alternative model would postulate that coupled cellular networks represent a unit under selection, a dramatic shift in our understanding of tumourigenesis.

Research objective

- To develop cell lines carrying mutations in essential genes coding for proteins involved in metabolism, and expressing various combinations of connexins. This will establish the biological resources for *in vitro* and *in vivo* experiments. We currently have CRISPR clones with inactivated glycolytic metabolism, oxidative metabolism, and pentose phosphate shunt.
- To optimise co-culture protocols that test for functional rescue of cells carrying mutations in essential genes. This will involve assays including metabolic profiling, proliferation assays, etc. This deliverable will test the hypothesis that cell-to-cell connectivity can result in a sharing of resources between cells. This part of the project will be supported by the metabolic expertise and state-of-the-art metabolomics facilities in Oxford (James McCullagh).
- To verify *in vitro* observations in tumour xenografts. Selected experiments will be performed in mice to confirm evidence for metabolic coupling and rescue. Animals will be used for testing gap junctional inactivation, specifically Cx26 and Cx43 which our pilot data have implicated in CRC. We will use the latest technology to target cargo to acidic tumour regions (pHLIP).
- To model the process of selection with and without connectivity and investigate its impact on carcinogenesis using the mathematics of game theory. This will explore the significance of cellular networks versus individual cell responses to metabolic stress. The predictions in silico will inform the next line of experiments. This part of the project will be supported by expertise in mathematical biology (Eamonn Gaffney).

Translational potential

- A large volume of evidence has demonstrated the existence of essential genes in cultured cells. Inactivating mutations in these genes can stunt growth *in vitro*, yet the translation of this striking effect to cancer treatment has had limited success. This is primarily because evidence for negative selection is not convincing. This project will re-evaluate the potential for targeting essential genes under conditions that eliminate their safe-guard mechanisms (i.e. cell-to-cell connectivity). This may result in successful re-purposing of many drugs.
- Gap junctions are not routinely considered as targets for cancer therapy partly due to their ubiquitous role in other cells. However, modern targeting strategies such as those that exploit tumour hypoxia or acidosis offer a means of reducing unwarranted effects.

Training opportunities

- Cell physiology and biology: use of gene editing techniques, implementation of high-throughput assays, and development of methods to study cell-cell coupling;
- *In vivo* techniques including surgery, imaging, and histology;
- Metabolomics and single-cell techniques
- Mathematical modelling (game theory, selection).

References:

1. Monterisi, Michl, Hill, Hulikova, Abdullayeva, Bodmer, Swietach. Solute exchange through gap junctions lessens the adverse effects of inactivating mutations in metabolite-handling genes. https://elifesciences.org/articles/78425
2. Martincorena, Raine, Gerstung, Dawson, Haase, Van Loo, et al. Universal Patterns of Selection in Cancer and Somatic Tissues. *Cell* 2017;171:1029-577.
3. Zapata, Pich, Serrano, Kondrashov, Ossowski, Schaefer. Negative selection in tumor genome evolution acts on essential cellular functions and the immunopeptidome. *Genome Biol* 2018;19:67.
4. Dovmark, Hulikova, Niederer, Vaughan-Jones, Swietach. Normoxic cells remotely regulate the acid-base balance of cells at the hypoxic core of connexin coupled tumor growths. *FASEB J* 2018;32:83-96.
5. Dovmark, Saccomano, Hulikova, Alves, Swietach. Connexin-43 channels are a pathway for discharging lactate from glycolytic pancreatic ductal adenocarcinoma cells. *Oncogene* 2017;36:4538-50.
6. Michl, Wang, Monterisi, Blaszczak, Beveridge, Bridges, Koth, Bodmer, Swietach. CRISPR-Cas9 screen identifies oxidative phosphorylation as essential for cancer cell survival at low extracellular pH. *Cell Rep* 2022 38(10):110493.

48. 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: Dr Christiana Kartsonaki
Eligibility: Track 1, 2, 3 and 4 students only are eligible to apply for this project.

Lay Summary
Cancers with alterations in the BRCA1 or BRCA2 genes, related to risk of breast, ovarian, and other cancers, continue to grow in spite of persistent damage to their chromosomes. A class of drugs termed PARP inhibitors (including the drug olaparib), takes advantage of this damage to stop the tumour growth. These drugs have clear benefits for patients, but often stop working after some time, as the tumour becomes resistant to the drug. It is not clear how this happens, although several possibilities have been proposed. With this project, we aim to identify how the process of protein production from DNA differs between cancer cells with BRCA1/2 alterations which have never been exposed to olaparib and cancer cells that stopped responding to olaparib. We will then try to assess whether any such differences occur at specific locations in the genome which affect this process. It is hoped that this work will eventually lead to identifying genes/proteins which can be measured to help predict how well olaparib may work for each patient and therefore to inform their treatment decisions.

Abstract
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
(i) 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 subpopulations 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 Oct. 2023 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 ability through univariable 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.

(ii) 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.

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 protocols 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 blotting techniques necessary to validate any candidate genes and pathways.

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49. Imaging metabolism in cancer and the heart to assess efficacy and safety of mitocans[1,3] – Dr Timm

Primary Supervisor: Dr Kerstin Timm
Second Supervisor: Prof. Adrian Harris and Prof. Lisa Heather
Eligibility: Track 1 and 3 students only are eligible to apply for this project.

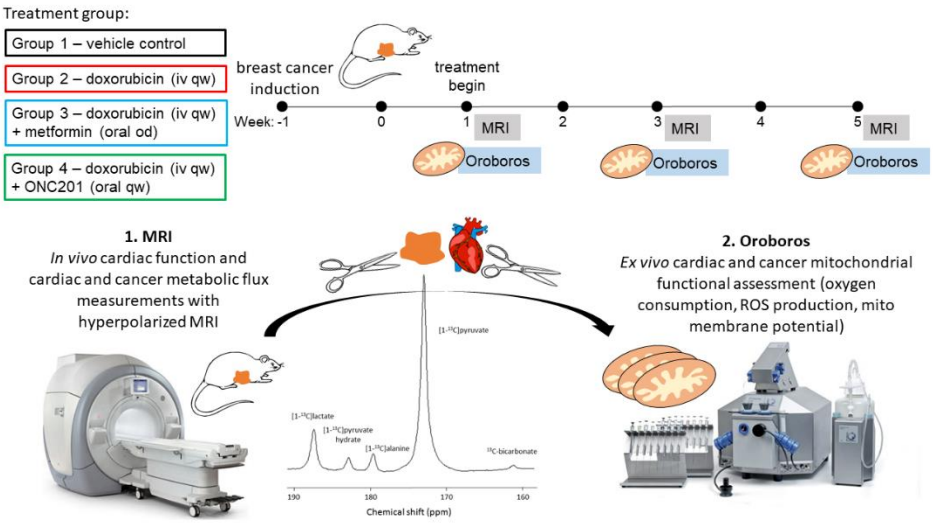
Lay Summary
Cancer cells rely on energy generation to sustain their growth, and a lot of this energy is made in the cell's mitochondria, the so-called "powerhouse" of the cell. New cancer treatments aim to target those mitochondria and thus impair the cancer's ability to generate energy. However, other organs in the human body are very reliant on energy generation in the mitochondria too, namely the heart. This could lead to devastating side effects of mitochondria-targeted cancer treatments, culminating in heart failure. This project aims to use an imaging technique that is also available for patients, which can determine how the cancer and heart generate energy in the mitochondria, and can thus assess whether a mitochondrially-targeted treatment is a) killing cancer cells and b) negatively impacting the heart. We will combine this with measurements of mitochondrial function in a laboratory setting to fully understand imaging results. The same imaging could be offered to cancer patients and could improve cancer care in the future, maximising tumour treatment response and minimizing long-term side-effects on the heart.

Abstract
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 ^{13}C 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
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-^{13}C]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 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].

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.

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.

References:

[1] Weinberg, F. & Chandel, N.S. *Ann. N. Y. Acad. Sci.* (2009) [2] Roth, K.G. et. al. *Trends Mol. Med.* (2020).[3] K. Kobayashi & J. R. Neely, *Circ. Res*. (1979) [4] Day, S.E. et al. *Nat. Med.* (2007) [5] Timm, K.N. et al. *J. Biol. Chem.* (2017) [6] Nelson, S.J. et al. *Sci. Transl. Med.* (2013) [7] Gallagher, F.A. et al. *Proc. Natl. Acad. Sci. U. S. A.* (2020) [8] Timm, K.N. et al. *Commun. Biol* (2020) [9] Park, J.M. et al. *Circ. Res.* (2020 [10] Moslehi, J.J. N. Engl. J. Med. (2016) [11] Mattern, J. et. al. *Zeitschrift für Krebsforsch. und Klin. Onkol.* (1975) [12] El-Mir, M.Y. et al. *J. Biol. Chem.* (2000) [13] Marinello, P.C. et al. *Sci. Rep.* (2019) [14] Bowker, S.L. et. al. *Diabetologia* (2010) [15] Saif, M.W. et al. *Cancer Chemother. Pharmacol.* (2019) [16] Lord, S.R. et al. *Cell Metab.* (2018) [17] Dilman, V.M. & Anisimov, V.N. *Cancer Lett.* (1979) [18] Greer, Y.E. et al. *Oncotarget* (2018) [19] Baccelli, I. et al. *Cancer Cell* (2019) [20] Tarpey, M.D. et. al. *J. Biol. Chem.* (2019) [21] Perry, J.B. et al. *J. Mol. Cell. Cardiol*. (2019)

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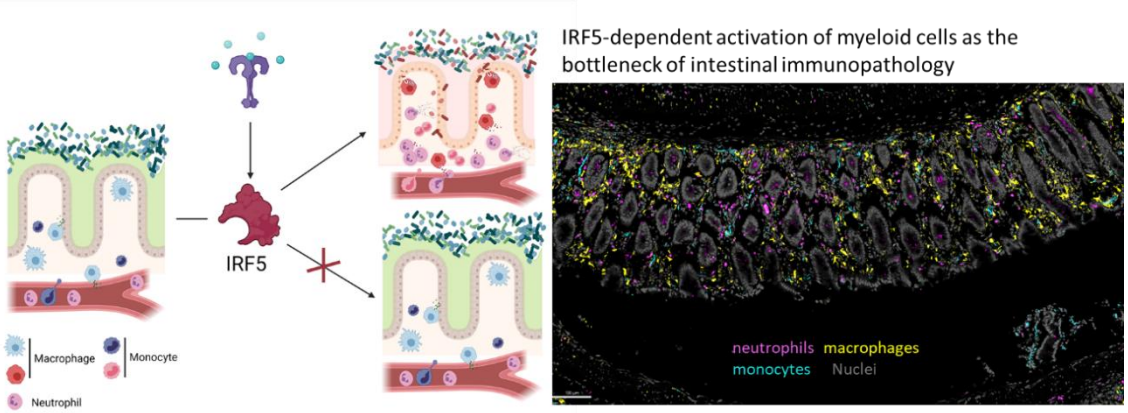
50. Heterogeneity of myeloid cells in colorectal cancer: the role of IRF5 [1,2,3] –Prof. Udalova

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

Lay Summary
The intestinal immune system is a delicately balanced system between peaceful coexistence with good bacteria and food particles and raising an immune response upon bad infection. Dysregulation of the intestinal immune system can lead to inflammation, which may progress to colorectal cancer (**CRC**). Myeloid cells (macrophages, monocytes, neutrophils) are innate immune sensors, which play a central role in maintenance of normal organ function, initiation of inflammation, restoration of tissue upon injury and response to chemotherapy in cancer. By integrating cues of their immediate tissue microenvironment, they can adapt their functions according to tissue-specific needs. The goal of the project is to dissect the contribution of specific myeloid cells in the resolution of intestinal inflammation and changes that may occur that would lead to the inflammatory response going awry, which would ultimately progress to CRC. It focuses on a key intrinsic regulator of myeloid cell phenotype called IRF5. Specific aims of this project are to (1) profile the spatial multitude of myeloid cells in resolution of inflammation and compare to CRC; (2) understand the myeloid cell-specific role for IRF5 in resolution of inflammation and (3) examine the role of IRF5 inhibition or enhanced expression in CRC. Understanding the molecular mechanisms orchestrating specific function of these cells is crucial for identification of treatment options for both inflammatory bowel diseases (**IBD**) and CRC.

Abstract
Dysregulation of the intestinal immune system can lead to inflammation, which may progress to colorectal cancer (CRC) [1]. Myeloid cells 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. Interferon Regulatory Factor 5 (IRF5) has been identified to act as a master transcription factor in myeloid cells that controls both acute and chronic inflammation and is protective in pathogen clearance [2,3,4]. IRF5 has been shown to regulate macrophage transcription of pro-inflammatory mediators, monocytes differentiation into pro-inflammatory macrophage phenotype, and more recently, neutrophil effector responses without compromising neutrophil maturation processes or their ability to enter into the tissue [5]. Our preliminary data suggest that IRF5 function in myeloid cells is indeed a key element in controlling the onset of intestinal inflammation and pathology, but its role in resolution of inflammation or cancerogenesis is not well understood. Of relevance, IRF5 has also been identified as a DNA-damage sensor, highlighting a potential beneficial role in CRC [6]. Both tumour-associated macrophages (TAMs) and tumour-associated neutrophils (TANs) appear to play a major role in cancer progression or hinderance [7]. Therefore, dissecting the molecular mechanisms orchestrating neutrophil, monocyte and macrophage function is crucial for identification of treatment options for both inflammatory bowel diseases (IBD) and CRC.



Research objective
First, we will use advanced imaging and spatial transcriptomic analyses to compare myeloid cell heterogeneity in the colon in resolution of inflammation using the *Helicobacter hepaticus* and anti-IL10R (Hh + aIL10R) colitis model [2] with that in microbe driven colitis associated cancer (CAC) models [8], characterised in the Powrie group; as well as during the onset and peak of inflammation, characterised previously in our lab. It was suggested that localisation of macrophages and

neutrophils within the tumour microenvironment might be a crucial determinant of their function [9]. Therefore, using already generated reporter strains (e.g. CX3CR1-GFP x Ly6G-Tomato) we will assess the localisation of different macrophage and neutrophil subsets and their interaction cell-cell contacts, which could also provide further information about their function and potential targeting. This will help identify molecular targets in shaping their phenotype and directing towards resolution rather than progression of inflammation into cancer development.

Second, we will assess the role of IRF5 in myeloid cells. Previous work in the lab has profiled IRF5-dependent inflammatory CD11c+ macrophages at peak of inflammation using scRNA-seq [2]. New data suggest the role for IRF5 in driving neutrophil-dependent inflammation. Based on this work, we aim to utilise already generated targeted mouse models (CX3CR1-cre ER2 IRF5 fl/fl; CCR2-mKate-cre ER2 IRF5 fl/fl; Ly6G-cre IRF5 fl/fl) to understand the beneficial vs pathologic role of IRF5 in resolution of inflammation. We hypothesize that lack of IRF5 in macrophages may be beneficial 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 lack of IRF5 in neutrophils may be detrimental as neutrophils can be involved in priming barrier cell repair and/or regenerating the extra cellular matrix around the perturbances.

Third, will investigate detrimental and beneficial effects of TAMs and TANs in CRC. Investigation of the differences in T cell priming of IRF5-deficient macrophages and/or neutrophils will also offer insight of the interplay of IRF5 in innate immune cells with the adaptive immune system in both resolution and CRC. Thus, in addition to inhibition of IRF5 (as above) we would also consider stimulating IRF5 specifically at tumour sites might improve anti-cancer immunity [10]. This could be achieved by targeted delivery of adenoviral vector expressing IRF5 (overexpression) or inhibition of IRF5 activation through phosphorylating kinase inhibition [11] in CAC models. Of importance, recent work by the Uhlig group identified gain-of-function genetic mutations in this signalling pathway in patients with immune deficiency, multi-organ inflammatory disease such as colitis, arthritis and dermatitis, and diffuse large B cell lymphomas [12], further underlying the possibility of using inhibitors of the pathway for treatment.

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 [13]. 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 also plays a crucial role in mediating monocyte recruitment and their differentiation into pro-inflammatory macrophages, as well as in effector neutrophil functions, during intestinal inflammation and may therefore be central during resolution and cancer development. Moreover, the inhibitor of IRF5 activation pathway may prove beneficial for inflammation-induced cancer.

Training opportunities

The student will be trained in the Hh + aIL10R colitis and CAC models 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 and spatial transcriptomics to define the localisation of macrophage subsets within the tumour microenvironment will be made available.

References:

1. Mantovani, A *et al*. Cancer-related inflammation. *Nature* 454, 436–444 (2008). **2**. Corbin, A. L. *et al.* IRF5 guides monocytes toward an inflammatory CD11c + macrophage phenotype and promotes intestinal inflammation. *Sci. Immunol.* 6085, 1–16 (2020). **3**. Weiss, M. *et al.* IRF5 controls both acute and chronic inflammation. *Proc. Natl. Acad. Sci. U. S. A.* 112, 11001–11006 (2015). **4**. Pandey, S. P., Yan, J., Turner, J. R. & Abraham, C. Reducing IRF5 expression attenuates colitis in mice, but impairs the clearance of intestinal pathogens. *Mucosal Immunol.* 12(4):874-887 (2019). **5**. Khoyratty, T. E. & Ai, Z. Distinct transcription factor networks control neutrophil-driven inflammation. *Nat. Immunol.* 22(9):1093-1106 (2021). **6**. Hu, G. et al. Signaling through IFN regulatory factor-5 sensitizes p53-deficient tumors to DNA damage-induced apoptosis and cell death. *Cancer Res.* 65, 7403–7412 (2005). **7**. Quail, D. F. *et al.* Neutrophil phenotypes and functions in cancer: A consensus statement. *J. Exp. Med.* 219, 39 (2022). **8**. Kirchberger, S. *et al.* Innate lymphoid cells sustain colon cancer through production of interleukin-22 in a mouse model. *J. Exp. Med.* 210, 917–931 (2013). **9**. Caprara, G., Allavena, P. & Erreni, M. Intestinal Macrophages at the Crossroad between Diet, Inflammation, and Cancer. *Int. J. Mol. Sci.* 21, 4825 (2020). **10**. Byrne, A. J. *et al.* A critical role for IRF5 in regulating allergic airway inflammation. *Mucosal Immunol.* 10, 716–726 (2017). 11. Ryzhakov et al. Defactinib inhibits PYK2 phosphorylation of IRF5 and reduces intestinal inflammation. *Nat Commun* 12(1):6702 (2021). **12**. Wang, L. *et al*. Gain-of-function variants in SYK cause immune dysregulation and systemic inflammation in humans and mice. *Nat Genet*. 53(4), 500-510 (2021). **13**. Molgora, M. *et al.* TREM2 Modulation Remodels the Tumor Myeloid Landscape Enhancing Anti-PD-1 Immunotherapy. *Cell* 182(4), 886-900 (2020).

51. Non-Invasive Metabolic Imaging of Liver Cancer[1,2,3,4] – Prof. Valkovič

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

Lay Summary

One of the deadliest cancers around is liver cancer. Currently, the only treatment is a surgical removal of the tumour or a complete liver transplant. Success chance of these procedures depends on how early the cancer is diagnosed. **Therefore, means of early detection of cancerous changes in liver tissue metabolism would be ideal for screening**. Using magnetic Resonance Imaging (MRI) scanners we can measure metabolism of the liver tissue non-invasively.

Here, we will use a modern high filed MRI system to identify the metabolic changes in different stages of liver cancer. Our main goals are:

1. To put together a list of measurements required for liver cancer screening using high field MRI scanner
2. To explore whether metabolism of the healthy liver parts of patients with liver tumours is already metabolically impaired.
3. To compare the status of liver metabolism in cancer patients with people at high risk, e.g., patients with late-stage scarring of the liver.

This will help us better understand the changes in the tumorous livers and allow us to identify people with growing liver tumours even before the cancer becomes visible.

Abstract

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 liver cancer patients 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]. **Changes in PME/PDE could potentially identify early malignant changes in liver tissue that appears normal on conventional imaging.**

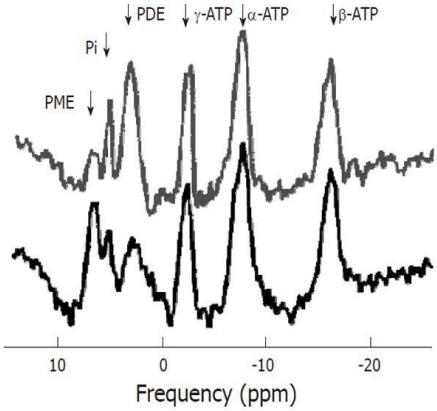


Figure 1 *In vivo ^{31}P MR spectra of human liver tissue obtained from a healthy volunteer (top) and from a patient with HCC (bottom). Note the increased PME/PDE in the HCC patient.[1]*

^{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 metabolites that contribute to PME and PDE peaks[6]. However, due to the inherent inhomogeneities of 7T, especially with the use of surface coils, the quantification is challenging and only a small portion of liver is typically covered. We have recently developed a method for absolute quantification of liver ^{31}P metabolites[7] at 7T and demonstrated that using a whole-body coil can provide metabolic information across the chest[8]. 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 and physicists to answer clinically relevant research questions.

References:

1. Bell JD and Bhakoo KK, NMR Biomed, 1998. **11**(7): p. 354-9.
2. Sayiner M, Golabi P, and Younossi ZM, Dig Dis Sci, 2019. **64**(4): p. 910-917.
3. Mak LY, Cruz-Ramon V, Chinchilla-Lopez P, et al., Am Soc Clin Oncol Educ Book, 2018. **38**: p. 262-279.
4. Glunde K, Bhujwalla ZM, and Ronen SM, Nat Rev Cancer, 2011. **11**(12): p. 835-48.
5. Krikken E, van der Kemp WJM, van Diest PJ, et al., NMR Biomed, 2019. **32**(6): p. e4086.
6. Valkovič L, Chmelík M, and Krššák M, Anal Biochem, 2017. **529**: p. 193-215.
7. Purvis LAB, Valkovič L, Robson MD, and Rodgers CT, Magn Reson Med, 2019. **82**(1): p. 49-61.
8. Valkovič L, Dragonu I, Almujayyaz S, et al., PLoS One, 2017. **12**(10): p. e0187153.

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52. Immune therapies For Acute Myeloid Leukaemia (AML) And Myeloid Blood Cancers[1,2,3] –Prof. Paresh Vyas

Primary Supervisor: Prof. Paresh Vyas
Second Supervisor: Prof Seph Borrow, Prof Ronjon Chakraverty and Prof Andrew McMichael
Eligibility: Track 1, 2 and 3 students only are eligible to apply for this project.

Lay Summary
We aim to improve treatment of Acute Myeloid Leukaemia (AML), an aggressive blood cancer. Unfortunately, most individuals with AML do not show long lasting responses to treatment and few survive more than 1 year. However, a minority of patients (about 1 in 7) are cured. The most potent curative anti-AML treatment is called stem cell transplantation in which donor immune cells are used to reject leukaemia cells in the patient. Although this treatment can be successful, it is associated with significant risk and unwanted side effects. Oxford clinical and scientific experts in AML and immunology will build on their exciting work to develop a new generation of more targeted, less toxic immune therapies for AML, which will be tested in clinical trials within the next three years. These clinical trials will test if specific immune cells, called T cells, can kill and eradicate AML cells with minimal side effects. They will also develop new methods to isolate new antibodies (antibodies are proteins that work with immune cells) to eradicate AML cells. This work will combine with other work in Oxford, developing advanced immune therapy and Oxford's international leading collection of AML clinical trials that treats patients from all over the UK. Finally, this work also has the potential to inform novel therapies for other cancers.

Abstract
AML is the most common aggressive adult blood cancer. It is part of a family of blood cancers called myeloid blood cancers that share common biological features. This project provides the applicant to work with a multi-disciplinary group of AML and myeloid cancer clinical academics/early phase trialists, immunologists and cell therapy experts to transform immune therapy for myeloid cancers. There are three discrete packages of work. First, we will build on our novel observations in isolating HLA class II-restricted antigen-specific anti-AML cells T cells to identify which T cell receptors (TCR) to take forward into first-in-human clinical trials. Second, we will identify TCRs that recognise AML-specific peptides bound to non-polymorphic HLA-E and evaluate their potential for use as 'off the shelf' antigen-specific receptors. Finally, we will isolate antibodies that target AML antigens bound to HLA class II that could be developed for therapeutic and biomarker purposes.

Research objective
Background: Two strands of work have come together in this project. First, we have been working to understand the mechanistic basis of cure mediated by the most established curative cellular immunotherapy, allogeneic stem and immune cell transplantation (allo-SCT). Allo-SCT involves transfer of blood stem and immune cells from a healthy person (donor) to a patient (recipient) (Fig. 1a). Allo-SCT has been in routine clinical practice since the 1960s. ~20,000 allo-SCTs are performed worldwide annually. The most common disease treated by allo-SCT is AML. Allo-SCT is curative because some of the donor immune cells, called T cells, attack and eradicate the patient's cancer cells. This is called Graft-versus-Leukaemia (GvL). However, donor immune cells can also attack the patient's normal healthy tissue, which can cause great harm. This is known as graft versus host disease (GvHD) (Fig 1b).

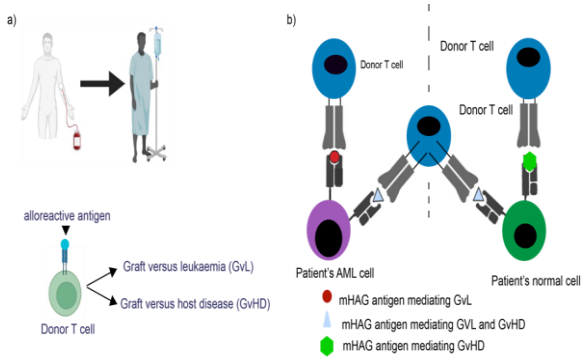

Fig.1. Alloreactive antigen-specific T cells mediate GvL and GVHD in allo-SCT.
a) Donor (left) stem and immune cells are infused into a patient after chemotherapy. b) Alloreactive antigens mainly arise from coding germline SNPs that differ from patient and donor. These are known as minor histocompatibility antigens (mHAG). Some mHAGs are presented only on AML cells (left) and elicit GvL T cell responses. Other mHAGs are expressed only on normal tissue and elicit GvHD (right). Finally, some mHAGs are expressed on both AML and normal cells and elicit both GvL and GvHD.

Remarkably, until our recent work, the field has limited insight into the antigenic specificity of GvL, or how to distinguish T cells that cause beneficial GvL from those that cause harmful GvHD. To address this knowledge deficit, we used an unbiased systematic reverse immunology approach to study elite responders who had been cured by allo-SCT, with minimal GvHD. We identified 24 peptide antigens targeted by alloreactive T cells due to single amino acid differences between patient and donor encoded by **germline** single nucleotide polymorphisms (SNPs) that differed between patient and donor, rather than somatic mutation in the patient's tumour (Fig. 2).

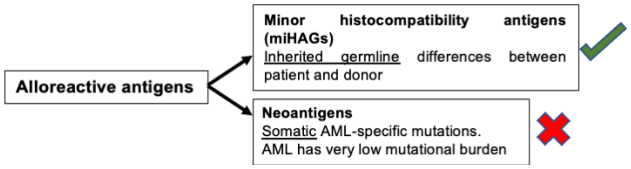

Fig. 2. Alloreactive antigens. In principle these could be either mHAGs (due to coding germline SNPs) or neoantigens (due to somatic AML-specific mutations. Our data shows the antigens identified were mHAGs consistent with the low mutational burden in AML.

Interestingly, 18 of these alloreactive antigens were HLA class II-restricted in patients. Our novel observations are concordant with recent observations. First, AML cells express HLA class II[1]. Second, transcriptional down regulation of HLA class II, but not class I expression, is a common immune evasion mechanism in AML patients[2, 3]. Third, AML cells expressing HLA class II can function as antigen presenting cells[4]. Following antigen identification, we combined with single cell transcriptome and TCR sequencing to identify the cognate TCRs of antigen-reactive T cells. For one TCR we used lentiviral transduction to introduce the TCR into primary human T cells and showed that transduced T cells specifically recognised the alloreactive peptide (recognition was dependent on the amino acid that differs between patient and donor). TCR-transduced T cells could also be activated by primary AML cell lysates expressing the protein containing the SNP, showing that protein is processed and the antigenic peptide is bound to HLA class II and presented to T cells. We are now testing if TCR transduced T cells kill primary AML cells in vitro and in vivo in immunodeficient mice. We wish to develop these observations further to develop new clinical therapies.

The second strand of work involves identifying AML-specific peptides that bind to non-polymorphic and widely expressed HLA-E[5]. The basis for this work is that we have already shown that the relatively non-polymorphic non-classical class I molecule HLA-E on AML cells binds to and presents a peptide from the WT-1 protein. WT-1 is commonly expressed by AML cells. Using recently-established methods[6,7] we isolated antigen-specific T cells and antibodies reactive with WT-1 bound to HLA-E. With the workflow now established we wish to identify other peptide-HLA-E targets for immune therapy.

Specific Project Aims

Pre-clinical data to choose TCRs for TCR-based therapy. *Longitudinal analysis of GVL T cells and AML disease burden*. We have a prospective, protocol-defined collection of sequential pre- and post-transplant bone marrow and peripheral blood samples from 220 AML patients treated by allo-SCT within a clinical trial. We will use multiple approaches to study the kinetics of clonal expansion/contraction of antigen-reactive T cells that recognise the 24 alloreactive antigens we identified. In samples with alloreactive TCRs, we will correlate clone size with sensitive measurement of AML disease burden and clinical data on haematological response, GvHD and immunosuppression. We will also perform single cell RNA sequencing to begin deep phenotypic characterisation of the alloreactive T cells. This correlative data will support inferences on which TCRs may attack AML cells and normal cells. Using this information, we will then prioritise TCRs for further mechanistic work based on their reactivity against AML cells and normal cells.

2. *T cell therapy directed against AML peptides bound to HLA-E* Because HLA-E expression is enhanced in AML and HLA-E is not polymorphic, therapies focused on peptides it presents should have great potential in all patients regardless of HLA type. We will identify new AML-specific HLA-E binding peptides in the same manner we identified WT-1 peptide, using a combination of peptide-HLA-E binding assays and peptide binding affinity measurements. Peptide-HLA-E specific T cells will be primed and generated in vitro and their specificity explored to exclude reactivity to normal tissues. These T cells will be tested for *in vitro* activation and killing of AML cells and *in vivo* in an AML xenograft model for suppression of leukaemic cells. These T cells would have the potential for treatment of relapsing AML in any patient regardless of HLA type.

3. *Developing antibodies against alloreactive peptides bound to common HLA-DR and class I alleles*. We have already made antibodies to HLA-E-WT-1 peptide ((7). We will work with collaborators to make tetramer reagents to screen B cells and phage display libraries for antibodies reactive with mHAGs and new AML-specific HLA-E binding peptides. This challenging work will provide diversify therapeutic options and build diagnostic reagents.

Translational potential

This project aims to identify candidate TCRs (Aim 1 and 2) for TCR cell therapy and antibodies (Aim 3) for chimeric antigen T cell receptor therapy and bi-specific or antibody-drug conjugate therapy.

Training opportunities

The DPhil student will be trained in: (i) basic immunology and specifically T cell biology and isolation of antibodies; (2) molecular biology including single cell analysis: (3) computational biology including analysis of single cell and multi-omic data sets: (4) protein biochemistry and peptide-ligand interactions. The training will be focussed on specific skill sets in developing immune therapies, AML biology and statistics to correlate laboratory data with clinical metadata.

References:

1. Polonen, P., et al.,. Cancer Res, 2019. **79**(10): p. 2466-2479. 2. Christopher, M.J., et al.,. N Engl J Med, 2018. **379**(24): p. 2330-2341. 3. Toffalori, C., et al. Nat Med, 2019. **25**(4): p. 603-611. 4. Hernandez-Malmierca, P., et al.Cell Stem Cell, 2022. **29**(5): p. 760-775 e10. 5. Ogg, G., V. Cerundolo, and A.J. McMichael, Curr Opin Immunol, 2019. **59**: p. 121-129. 6. Yang, H., et al., Sci Immunol, 2021. **6**(57). 7. Li, D., et al., Comms Bio, 2022. **5**(271).

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53. Regulation and Functions of Supermeres in Colorectal Cancer[1,2,3,4] –Prof. Wilson

Primary Supervisor: Prof. Clive Wilson
Second Supervisor: Prof. Adrian Harris and Prof. Chris Cunningham
Eligibility: Track 1, 2, 3 and 4 students only are eligible to apply for this project.

Lay Summary
Tumour growth relies on cancer cells communicating with each other and the normal cells around them. These signals allow the tumour to evolve, while helping it to resist our immune system and therapies. For many years, it was thought that the signals involved were single molecules, which bind to target cells and change their behaviour. However, it now appears that these signals can be much more complex assemblies of protein and other large molecules, which are taken up by cells and completely reprogramme them. These complexes are very challenging to study, because we currently do not know how they are made or what they do. Using a specific cell in fruit flies, in which the assembly sites for these signals are thousands of times larger than in humans, we have worked out how newly discovered signalling complexes called supermeres are made. Informed by these studies, we will look at the control of supermere production in colorectal cancer, then block this process to test how important supermeres are for cancer growth and if they could be targeted in therapies. We will also study supermeres released by cancer cells into patients' blood to find out if they can be used for early detection of cancer, or to predict the severity of the disease or a patient's response to therapy. Overall, this project opens up a new, and previously unexplored field in cancer biology, which should shed light on how cancer cells overcome the immune system and therapies, and how we might stop them from doing this.

Abstract
Intercellular signals and signalling pathways promote cancer formation and progression. Traditionally, such signals are considered to be single molecules like growth factors, but recently, more complex multimolecular assemblies, such as extracellular vesicles (EVs) and protein aggregates, which can harbour multiple activities, have emerged as alternative mediators. Supermeres are recently reported protein:RNA complexes produced by multiple cancer cell types[1]. They are enriched with cargos involved in multiple cancers (glycolytic enzymes, TGFBI, miR-1246, MET, GPC1 and AGO2). Most extracellular RNA is associated with supermeres and not EVs. Cancer-derived supermeres increase lactate secretion, transfer cetuximab resistance and reduce hepatic lipids and glycogen *in vivo*. However, their cellular origin and links with other secreted multimolecular complexes is not known, hindering their detailed analysis. We previously used a *Drosophila* prostate-like cell with highly enlarged endosomal compartments to unpick the biology of exosomes, small EVs generated in these compartments. This work identified a novel, evolutionarily conserved exosome subtype called Rab11a-exosomes made in recycling endosomes, which appear to control cancer progression[2]. We have now identified protein aggregates in these cells with a similar protein signature to human supermeres, shown that their biogenesis is interlinked with Rab11a-exosome formation[3,4], and identified genetic manipulations that block assembly. We hypothesise that human supermeres are also generated in recycling endosomes via similar mechanisms. In this project, the student will test this idea in colorectal cancer (CRC) cells, where we first identified Rab11a-exosomes, and for which molecularly stratified trials are ongoing and serial blood samples from patients can be collected, allowing the specific functions of supermeres and their potential as biomarkers to be tested. The student will spearhead the analysis of this new form of cancer cell signalling, working with basic scientists and clinicians who have diverse skills and expertise.

Research objective
The project has four research objectives and proposed outcomes:

3.1 Determine the role of cellular stress responses in supermere release: In our fly model, supermere-like structures coalesce in Rab11a-positive recycling endosomes to form very large dense-core granules[5]. We have found that like human supermeres, the fly homologue of TGFβ-induced (TGFBI), called MFAS, is the most abundant protein in these complexes (Fig. 1), which are also enriched in other supermere cargos, such as GPI-anchored proteins, proteases[5] and cleaved membrane proteins, like Amyloid Precursor Protein (APP). Complex formation is dependent on MFAS (Fig. 1) and Rab11a-exosomes[3,4]. To determine whether human supermeres are selectively produced in Rab11a-compartments, we will test whether supermere release is affected in CRC cell lines, e.g. HCT116, MDA-MB-231, by stresses that promote Rab11a-exosome secretion, e.g. nutrient depletion, hypoxia, drugs that block growth factor signalling. Supermeres will be isolated by ultracentrifugation; if they are abundant in non-stressed, as well as stressed, cells, their composition will be assessed by western/miRNA analysis, to determine whether like exosomes, their structure is altered by their origin.

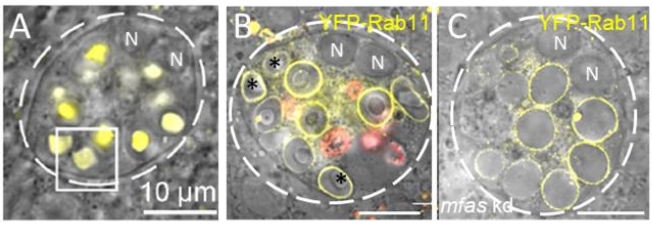

Fig. 1. MFAS/TGFBI is a key regulator of protein aggregation in flies. A. GFP-MFAS fusion protein expressed from the endogenous gene is expressed specifically in dense-core granules (yellow circles) in large compartments (one outlined by square) in a specific secretory cell type (outlined with dashed ellipse). **B.** Compartments containing these cores (some labelled with asterisks) are marked by recycling endosomal Rab11. **C.** *mfas* kd blocks aggregation, but compartments retain Rab11 identity. N = nuclei. Scale bar = 10 µm.

Proposed outcome: Determine the origin and composition of supermeres generated under different stress conditions.

3.2 Identify regulators of supermere biogenesis: Informed by our work in flies, we will genetically manipulate CRC cells under nutrient-depleted and -replete conditions, knocking down genes involved in the formation of supermere-like structures in flies, e.g. *TGFBI*, *ESCRT*s (to suppress exosome biogenesis) and *E-cadherin*, and test how supermere release is affected. Our current data suggest that *TGFBI* kd will block supermere formation without affecting other signalling complexes; the resulting preparations, depleted of supermeres, will be analysed for protein and miRNA content, and if needed, by proteomics and transcriptomics, to more fully determine their composition.

Proposed outcome: Define the mechanisms controlling supermere generation in CRC cells and specific cargos.

3.3 Identify cancer-related functions of supermeres: Cancer supermeres are readily internalised within target cells and have multiple functions in metabolism and drug resistance[1]. We will confirm this using supermere preparations from nutrient-depleted and -replete cells to determine whether supermere signalling changes under stress. We will also test the functions of conditioned medium from cells +/- nutrient-depletion, +/- kd (e.g. *TGFBI* kd) that blocks supermere release to assess supermere function in the presence of other signals, e.g. exosomes. We will include assays for metabolic changes[1], drug resistance[1], including oxaliplatin and 5FU, and effects on endothelial[2] and other stromal cells.

Proposed outcome: The functions of supermeres in the presence or absence of other signals will be defined.

3.4 Assess the therapeutic implications of supermeres in CRC patients: Recent unpublished studies indicate that some Rab11a-exosome markers from CRC cell lines are detected in EV preparations from CRC patients' plasma and these may be differentially expressed in patients that respond differently to neoadjuvant chemoradiotherapy (nCRT). We will isolate supermeres from plasma of these and other patients pre- and post-therapy and from blood-bank controls, confirming integrity after freeze-thaw, testing effects of different anticoagulants, etc; protein and miRNA content will be assessed in relation to therapeutic response.

Proposed outcome: These preliminary studies will assess whether supermeres carry biomarkers that relate to CRC or response to nCRT, and pave the way for more patient-focused studies to block supermere function in the future.

Translational potential

There is increasing evidence that multimolecular complexes, like supermeres, play central roles in cancer signalling, but defining those roles and assessing the translational potential requires advanced cell biological and genetic analysis. Our fly studies have opened up the opportunity to gauge the importance of supermere signalling in CRC and other cancers and determine the potential relevance of supermeres to early detection, patient stratification, prognosis and therapy, guided by the expertise of the collaborating supervisors. This project will provide the proof-of-principle work for future patient-focused, CRUK-funded studies in this area.

Training opportunities

The student will use a range of cancer-relevant techniques, including advanced cell culture and biochemical methods, cell biology, molecular genetics, high-resolution fluorescence imaging and bioinformatics. They will also develop expertise in cancer signalling, working at the interface of the collaborative environment we have established. Overall, this work should open up a new field in cancer biology, relevant to CRC and other cancers.

References:

1. Zhang Q, et al. (2021) Supermeres are functional extracellular nanoparticles replete with disease biomarkers and therapeutic targets. Nat Cell Biol. 23:1240-1254; 2. Fan S-J et al. (2020) Glutamine deprivation alters the origin and function of cancer cell exosomes. EMBO J. 2020, e1030093 and N&V, van Niel G, Théry C. (2020) EMBO J. 2020, 39:e105119; 3. Dar GH, et al. (2021) GAPDH controls extracellular vesicle biogenesis and enhances the therapeutic potential of EV mediated siRNA delivery to the brain. Nat Commun. 12:6666; 4. Marie, P.P., et al. (2020) Accessory ESCRT-III proteins selectively regulate Rab11-exosome biogenesis in *Drosophila* secondary cells. BioRxiv, https://doi.org/10.1101/2020.06.18.158725; 5. Redhai S, et al. (2016) Regulation of dense-core granule replenishment by autocrine BMP signalling in *Drosophila* secondary cells. PLoS Genet. 12:e1006366; 6. Wainwright SM, et al. (2021) *Drosophila* Sex Peptide controls the assembly of lipid microcarriers in seminal fluid. PNAS 118:e2019622118; 7. Fan, S-J et al. (2016) PAT4 levels control amino acid sensitivity of rapamycin-resistant mTORC1 from the Golgi and affect clinical outcome in colorectal cancer. Oncogene 35, 3004-15.

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54. Chronic infection, host immunity and cancer risk[1,2,3,4] – Associate Prof. Yang

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

Lay Summary

Worldwide, chronic infection of certain pathogens (i.e. virus, bacteria and parasite) is responsible for more than 2 million new cancer cases each year, with about a third of these in China. Although the causes of certain cancers from several common pathogens (e.g. *H. pylori*, HBV, HCV, and HPV) are well established, questions remain about their links to other cancers and the relations of other pathogens (e.g. human herpes virus) in cancer development. Moreover, the risk of developing particular cancer and its long-term survival may likely be affected by host (i.e. people) immunity, co-infection with other pathogens, genetic factors from both host and pathogens, and other lifestyle and environmental factors that people living with. However, these have not yet been properly investigated in large studies.

The China Kadoorie Biobank (CKB) included 0.5 million Chinese adults recruited during 2004-08 from 10 different areas across China, with extensive data collected then long-term followed up any disease and death occurred to each participant over years (www.ckbiobank.org). To date, we recorded >70,000 deaths and >1.5 million hospitalization events. We further measured about 20 pathogens among over 30,000 different cancer cases and 10,000 non-cancer CKB participants, and also detected various genetic and a wide range of biological data (e.g. inflammation markers) in subsets of CKB participants.

Based on these enriched data from both host and pathogens, this proposed DPhil project will study and help to develop cutting-edge research of whether infection of specific or multiple pathogens could cause different cancers development and/or affect their survival. It will inform policy makers to develop and implement suitable strategies for cancer prevention and control locally and globally.

Abstract

Chronic infections are estimated to account for ~15% of all cancers (>2 million) worldwide.[1] Previous studies have, however, been constrained by small sample sizes and investigation of a single pathogen with a single cancer. Consequently, uncertainty remains about the roles of common chronic infections in cancer development and progression.[2] It is also unclear as to whether there are important interactions between infections and other lifestyle/behavioural risk factors for cancer, and how genetic variants in both host (e.g. human leukocyte antigen; HLA) and pathogen and their interactions influence host immune response and susceptibility to and progression of infection, as well as subsequent cancer risk. For example, variability in HLA class I or II type antigens that affect the cellular immune response to HPV may partially explain why only a minority of infected women develop cervical cancer.[3]

We are conducting a large case-cohort study including all incident cancer cases in CKB (>30,000), and a randomly selected sub-cohort of participants (~10,000) to assess and quantify the role of different infectious pathogens in the aetiology of various cancers. In collaboration with UK Biobank, IARC/WHO and the German Cancer Research Centre (DKFZ), a CKB custom-designed multiplex serology panel has been developed and will be used to detect and quantify 46 antibodies for about 20 infectious pathogens (Table), selected for their cancer relevance and will enable population-specific variation in infections to be assessed in relation to different cancers.

Table Infectious pathogens and antigens in the CKB Multiplex Serology Panel

Infectious agent	Antigen
Background marker	GST
HSV1	gG
HSV2	mgG unique
VZV	gE / gI
EBV	VCA p18, EBNA-1, ZEBRA, EA-D
CMV	pp150 Nter, pp 52, pp 28
HHV7	U14
HHV6	IE1B trunc, IE1A trunc
HBV	HBc, HBe
HCV	Core, NS3
T. gondii	p22, sag-1
HTLV-1	gag, env
HIV-1	gag, env
HPyV	BK VP1, JC VP1, MCV VP1
HPV	HPV 16 L1, HPV 16 E6, HPV 16 E7, HPV 18 L1
C. trachomatis	pGP3
H. pylori	CagA-N, CagA-C, GroEL, OMP, VacA-N, VacA-C, HP0305, HpaA, HyuA-C, Cad, Catalase, HcpC, NapA, UreA

HBV infection is particularly prevalent in Chinese populations and reported have a 16-fold relative risk for incident liver cancer and 1.5-2.0 fold relative risks for several other cancers. Furthermore, HLA genetic variants associated with chronic HBV infection in

previous GWAS of East Asians were shown to affect HBV infection and liver cancer risk. In collaboration with the Wellcome Centre for Human Genetics, we will sequence HBV and HCV viral genomes from ~4,000 CKB HBV/HCV infected participants, half of whom who developed incident HCC during follow-up and the other half who did not. Genome-wide association studies (GWAS) and genome-to-genome analysis will be used to investigate host-virus interactions and genotype-phenotype relationships.[5] These analyses will improve our understanding of patterns of infection with HBV/HCV subtypes, and interactions of viral polymorphisms with host genetic variations, in influencing disease pathogenesis and progression.

Research objectives

The specific lines of investigation covered by this DPhil project will depend on the student's interests and previous training, and may include the following areas of work: (1) to examine the associations of chronic infection of particular pathogens with risks of different types of cancers; (2) to explore the role of host immune system genetics (e.g. HLA) in susceptibility to specific types of chronic infection and cancer; (3) to establish the value of serological markers, in combination with other lifestyle and genetic risk factors, in predicting the risk of infection-related cancers. By the end of the DPhil, the student will be competent to review the literature, to plan, undertake and interpret analyses of large-scale data, and to report research findings, including 3-5 publications in peer-reviewed journals and presentation at conferences.

Translational potential

This project will lead to improved understanding about the role of infectious pathogens in cancer aetiology, help identify high risk individuals for early detection/mass-screening and targeted cancer treatment and inform policy makers to develop and implement suitable strategies for cancer prevention locally and globally.

Training opportunities

The student will be based within the CKB research group in Nuffield Department of Population Health. There are excellent facilities and a world-class community of population health, data science and genomic medicine researchers. There will be in-house training opportunities in epidemiology, statistics, genetics, and bioinformatics and if necessary attendance at relevant courses.

References:

1. Plummer M, de Martel C, Vignat J, Ferlay J, Bray F, Franceschi S, et al. Global burden of cancers attributable to infections in 2012: a synthetic analysis. Lancet Glob Health. 2016;4:e609-16.
2. IARC. Biological agents. Volume 100 B. A review of human carcinogens. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans 2012; 100(Pt B):1-441
3. Zoodsma M, Nolte IM, Schipper M, Oosterom E, van der Steege G, de Vries EG, et al. Analysis of the entire HLA region in susceptibility for cervical cancer: a comprehensive study. J Med Genet. 2005;42(8):e49.
4. Waterboer T, Sehr P, Michael KM, Franceschi S, Nieland JD, Joos TO, et al. Multiplex human papillomavirus serology based on in situ-purified glutathione s-transferase fusion proteins. Clin Chem. 2005;51(10):1845-1853.
5. Ansari MA, Pedergnana V, L C Ip C, Magri A, Von Delft A, Bonsall D, et al. Genome-to-genome analysis highlights the effect of the human innate and adaptive immune systems on the hepatitis C virus. Nat. Genet. 2017; 49:666–673.